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Evidence for the absence of mutagenic activity of furfuryl alcohol in tests of germ cells in *Drosophila melanogaster*

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Summary

Furfuryl alcohol was evaluated for mutagenic activity in *D. melanogaster* by means of the sex-linked recessive lethal test and the sex-chromosome loss test. Brooding was employed in order to test different stages of spermatogenesis. No evidence was found of a mutagenic effect after adult injection and larval feeding.

Furfuryl alcohol is a chemical belonging to the furan group. It is employed in the manufacture of wetting agents and resins (Merck, 1976) and is also found in peanuts, coffee and popcorn (Shibamato, 1977). The compound is metabolized to furoic acid (Norton, 1975) and induces chromosomal aberrations and sister-chromatid exchanges (SCEs) in Chinese hamster ovary cells (Stich et al., 1981). However, it has been reported not to produce SCEs in vitro or in occupationally exposed humans (Gómez-Arroyo and Sousa, 1985).

In the present paper we report the results of experiments carried out to determine the mutagenic activity of furfuryl alcohol in Drosophila using the sex-linked recessive lethal test (SLRLT) and the sex-chromosome loss test (SCLT).

Materials and methods

Chemicals and treatment procedures

Furfuryl alcohol (2-furan methanol; Baker) was dissolved in 5% sucrose solution at 1300, 3250 and 6500 ppm to yield LD values of 10, 25 and 50 respectively. Two routes of administration were used: adult injection and larval feeding.

For adult treatment, the test solutions were injected intra-abdominally into males 0–2 days old. As a parallel control, a solution of 5% sucrose was used.

For larval feeding, eggs were collected after 2-h egg laying periods and placed in 250-ml bottles (100 eggs/bottle) containing 50 ml of standard food medium. After 24 ± 2 h, 1 ml of furfuryl

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TABLE 1

SEX-LINKED RECESSIVE LETHAL MUTATIONS INDUCED BY FURFURYL ALCOHOL IN ADULT MALES OF *Drosophila melanogaster* AFTER INJECTION

Concentration (ppm)	A		B		C		A+B+C [a]	
	0–2 days l/fertile ♀♀	%	3–5 days l/fertile ♀♀	%	6–7 days l/fertile ♀♀	%	0–7 days l/fertile ♀♀	%
Control	1/1050	0.09	2/1618	0.12	2/1468	0.13	5/4136	0.12
1300	2/1432	0.13	3/1813	0.16	2/1664	0.12	7/4909	0.14
3250	2/1420	0.14	3/2073	0.14	2/1432	0.14	7/4925	0.14
6500	2/1286	0.15	3/1778	0.17	2/1384	0.14	7/4448	0.16

[a] Sum of 2 experiments.

alcohol was dropped on the surface of the food in each bottle. Controls were treated with 5% sucrose only (Velázquez et al., 1984).

Mutagenicity test

For adult treatment, 0–2-day-old Oregon R males, treated as adults, were crossed individually for 2 days with 3 *Basc* virgin females (brood A). After the first brood, 2 additional broods were carried out by transferring the males to fresh virgin females at intervals of 2–3 days. Brood A represents principally the recovery of mature sperm, brood B early sperm and late spermatids and brood C early spermatids and probably late spermatocytes. The same brooding procedure was carried out after larval treatment. The progeny of each male were kept separate to identify possible clusters of lethals.

F_1 progeny were intercrossed and cultures scored for sex-linked recessive lethals in the F_2 generation. All presumptive lethal mutations were rechecked by mating F_2 *Basc*/+ females with *Basc* males. It was judged that a recessive lethal mutation had occurred if no wild-type males and more than 15 *Basc*/+ females were recovered or if the culture had 1 wild-type male and 20 *Basc*/+ females.

For the purpose of testing for sex-chromosome loss (SCLT), 0–2 day-old y/B^S Y y^+ males, treated or not, were mass-mated for 2 days in bottles in a ratio of 1 ♂ to 3 ♀♀, the latter of the composition y^2 w^a. Two additional broods of 2–3 days followed. The F_1 offspring were scored for exceptional XO males and partial loss of Y markers.

Statistical calculations

For each exposure, 100 males were tested in a treatment and a control series. Results were treated statistically with the Poisson test to identify clusters (Owen, 1962) and the Kastenbaum–Bowman test (1970) to determine the significance level of the difference between control and treated series.

TABLE 2

SEX-LINKED RECESSIVE LETHAL MUTATIONS INDUCED BY FURFURYL ALCOHOL IN MALES OF *Drosophila melanogaster* TREATED AS LARVAE

Concentration (ppm)	A		B		C		A+B+C [a]	
	0–2 days l/fertile ♀♀	%	3–5 days l/fertile ♀♀	%	6–7 days l/fertile ♀♀	%	0–7 days l/fertile ♀♀	%
Control	0/1778		0/2173		0/1737		0/5688	
1300	0/2212		2/1795	0.11	3/1828	0.16	5/5835	0.09
3250	5/2300	0.22	2/1418	0.14	0/2210		7/5928	0.12
6500	0/2019		3/1656	0.18	2/2352	0.08	5/6027	0.08

[a] Sum of 3 experiments.

Results and discussion

The results obtained from the SLRLT with treated adult males are summarized in Table 1. No significant differences were obtained between control and treated series.

Table 2 shows the SLRL results from treated larvae. There is no evidence that furfuryl alcohol induces sex-linked recessive lethals in germ cells of developing larvae.

Table 3 shows the negative results obtained from experiments of SCLT. There is no evidence that furfuryl alcohol induces total sex-chromosome loss; no partial losses of Y markers were recovered.

We conclude that furfuryl alcohol is not mutagenic in *Drosophila melanogaster* in the conditions, concentrations, routes of administration and stages tested in the present report.

TABLE 3

FREQUENCIES OF TOTAL SEX-CHROMOSOME LOSS INDUCED BY FURFURYL ALCOHOL IN *Drosophila melanogaster* FOLLOWING INJECTION OF ADULT MALES OF THE COMPOSITION y/B^S Y y^+

Concentration (ppm)	Brood	Total	Chromosome loss	
			XO males	%
Control	A	2887	2	0.07
	B	3400	3	0.09
	C	3143	4	0.13
	A+B+C [a]	9430	9	0.09
1300	A	2588	1	0.04
	B	2989	2	0.07
	C	2855	2	0.07
	A+B+C	8432	5	0.06
3250	A	2742	4	0.15
	B	3003	2	0.07
	C	2336	6	0.26
	A+B+C	8081	12	0.15
6500	A	2649	3	0.11
	B	3134	2	0.06
	C	2360	2	0.08
	A+B+C	8143	7	0.09

[a] Sum of 2 experiments.

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