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Evaluation in *Drosophila melanogaster* of the mutagenic potential of furfural in the *mei-9*[a] test for chromosome loss in germ-line cells and the wing spot test for mutational activity in somatic cells

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Summary

The mutagenic potential of furfural was evaluated by means of the chromosome loss test in germ cells and the wing spot test in somatic cells of *Drosophila melanogaster*. The chromosome loss test was carried out employing repair-proficient as well as repair-deficient females. Males carried the compound Y chromosome, $B^{S}Yy^{+}$. Two routes of administration were used: injection and feeding of adult males. Genetic damage was demonstrable after matings of treated males with females carrying the excision repair-deficient mutant *mei-9*[a]. The somatic mutation and recombination test was carried out treating 72-h transheterozygous *mwh* + / + *flr*[3] larvae. Acute treatment of larvae was chosen as the method of exposure. Evidence indicates that furfural induces somatic damage as measured in the wing spot test.

Furfural is a widely used chemical in the plastics industry. It serves as a solvent for nitrated cotton, cellulose acetate and gums, and is used in the manufacture of varnishes. In addition, furfural is employed as an insecticide, fungicide and germicide (Merck, 1989). It is metabolized by two pathways principally to furoic acid (Flek and Sedivec, 1978). The chemical is also found in several foods and spirits of alcohol beverages (Shibamato, 1977; Sessa and Platter, 1979; Shimizu and Watanabe, 1979; Alfonso et al., 1980; Jeuring and Kuppers, 1980; Loquet et al., 1981; Stich et al., 1981; Marcy and Rouseff, 1984).

Furfural was found to be mutagenic in strain TA100 of *Salmonella typhimurium* by Zdzienicka et al. (1978) but not in tests in the US National Toxicology Program (Mortelmans et al., 1986). It is co-carcinogenic in Syrian golden hamsters in the presence of benzo[*a*]pyrene (Feron, 1972). Further, it has been reported to induce SCEs in human lymphocytes in vitro (Gómez-Arroyo and Sousa, 1985). At the molecular level, furfural has been shown to react with double-strand DNA leading to the formation of single-strand breaks;

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the degradation primarily occurs in AT sequences (Hadi et al., 1990). Further, it has been reported to be carcinogenic as seen by an increase in the frequency of mouse liver tumors by means of the induction of novel mutations, for example, in the *ras* oncogenes (Reynolds et al., 1987). Finally, Woodruff et al. (1985) reported the compound to be positive in the sex-linked recessive lethal test in post-meiotic stages of *Drosophila melanogaster* after adult injection. In connection with this finding, the present paper deals with results from other tests in Drosophila assaying the mutagenic potential of furfural.

Materials and methods

Two systems testing the mutagenic potential of furfural were employed: (1) the *mei-9*^{a} test for chromosome loss to measure the induction of chromosome breaks in germ cells and (2) the wing spot test in order to evaluate effects in somatic tissue.

In carrying out the *mei-9*^{a} test, $y/B^S Y y^+$ males were treated and mated with repair-proficient *y w* females or repair-deficient *y ac sc w*^{a} *mei-9* females. B^S and y^+ are terminal markers on the long arm of the Y, Y^L, and the short arm of Y, Y^S, respectively (Brosseau, 1965). *mei-9*^{a} is an excision repair-deficient mutant (Baker and Carpenter, 1972; Boyd et al., 1976); use of *mei-9*^{a} females has been demonstrated to confer considerable sensitivity on the test for chromosome loss, at least at doses ordinarily used in mutagenicity screening (Zimmering, 1983). Males were injected or fed with the compound at 3750 and 5000 ppm; respective induced LD values at these experimental concentrations were approximately 25% and 33%. Controls were treated with a solution of 5% ethanol. Males were mated with the above described females in a ratio of 1 male:3 females for 2.5 days (brood A), transferred to bottles with virgin females for an additional 3.5 days (brood B); inseminated females in broods A and B bottles were permitted to remain for further egg laying. The procedure was repeated again to make up brood C. Males of brood C were discarded 2.5 days later and all females were discarded 5 days after inception of a culture. Statistical analysis was performed using the Kastenbaum–Bowman tables (1970).

The wing spot assay of Drosophila as described by Graf et al. (1984) was employed. Females of the genotype *mwh* were mated with *flr*^{3}*/TM3*; *Ser* males. Second-instar larvae were collected and placed in vials containing a Whatman glass filter saturated with solutions of fur-

TABLE 1

SEX CHROMOSOME LOSS AFTER INJECTION OF $y/B^S Y y^+$ ADULT MALES OF *Drosophila melanogaster* WITH FURFURAL AND MATING WITH REPAIR-PROFICIENT *y w* FEMALES

Concentration (ppm)	Brood	Total F_1	Regular F_1		CL	%	PL	%
			Females	Males				
Control	A	1981	1098	882	1			
	B	2122	1226	894	1		1	
	C	1745	943	801	1			
	A+B+C	5848	3276	2577	3	0.05	1	0.02
3750	A	1963	1053	907	2		1	
	B	2058	1191	866			1	
	C	1612	798	811	3			
	A+B+C	5633	3042	2584	5	0.09	2	0.03
5000	A	1511	830	681				
	B	2008	1311	875	1		1	
	C	1603	851	747	4		1	
	A+B+C	5122	2812	2303	5	0.01	2	0.04

CL, chromosome loss; PL, partial loss of the Y chromosome (loss of y^+ or B^S).

TABLE 2

SEX CHROMOSOME LOSS AFTER INJECTION OF $y/B^{S}Yy^{+}$ ADULT *Drosophila melanogaster* MALES WITH FURFURAL AND MATING WITH REPAIR-DEFICIENT *y ac sc w*[a] *mei-9*[a] FEMALES

Concentration (ppm)	Brood	Total F_1	Regular F_1		CL	%	PL	%
			Females	Males				
Control	A	920	578	341	1			
	B	1226	625	598	1		2	
	C	660	376	283	1			
	A+B+C	2806	1579	1222	3	0.11	2	0.07
3750	A	990	615	370	5			
	B	482	330	146	4		2	
	C	261	162	97	2			
	A+B+C	1733	1107	613	11*	0.64	2	0.12
5000	A	1242	600	632	10			
	B	489	326	158	5			
	C	448	277	159	4		8[a]	1.8
	A+B+C	2179	1203	949	19*	0.88	8*	0.37

[a] Does not represent a cluster.
* $P = 0.01$.

fural in 5% ethanol. The concentrations of furfural used were 3750, 5000 and 7500 ppm. After 6 h the larvae were removed and placed on regular food to complete development. Upon hatching wings from *mwh* + / + *flr*^{3} (non-Ser) flies were mounted on slides. Scoring was carried out at 400 × magnification for the presence of small single spots (1–2 cells), large single spots (more than 2 cells) and *mwh-flr* twin spots. Briefly, (1) single *mwh* spots were inferred to arise from mutation/deletion at the *mwh*$^+$ locus or an interchange between *mwh* and *flr*^{3}, (2) single *flr*^{3} spots from mutation/deletion at the *flr*$^{3+}$ locus, and (3) twin spots following interchange between *flr*^{3} and the centromere. Virtually all induced single spots were *mwh* in phenotype, suggesting that

TABLE 3

SEX CHROMOSOME LOSS AFTER FEEDING $y/B^{S}Yy^{+}$ ADULT *Drosophila melanogaster* MALES WITH FURFURAL AND MATING WITH REPAIR-PROFICIENT *y w* FEMALES

Concentration (ppm)	Brood	Total F_1	Regular F_1		CL	%	PL	%
			Females	Males				
Control	A	1360	800	557	3			
	B	718	415	303				
	C	272	159	113	3	0.13		
3750	A	1424	822	602				
	B	1402	759	643				
	C	721	411	310	1			
	A+B+C	3548	1992	1555	1	0.03		
5000	A	860	508	351	1			
	B	643	349	292	2			
	C	340	167	173				
	A+B+C	1844	1024	816	3	0.16		

TABLE 4

SEX CHROMOSOME LOSS AFTER FEEDING $y/B^{S}Yy^{+}$ ADULT *Drosophila melanogaster* MALES WITH FURFURAL AND MATING WITH REPAIR-DEFICIENT *y ac sc w*^{a} *mei-9*^{a} FEMALES

Concentration (ppm)	Brood	Total F_1	Regular F_1		CL	%	PL	%
			Females	Males				
Control	A	2282	1335	941	4		2	
	B	2538	1468	1064	5		1	
	C	1690	917	767	1		1	
	A+B+C	6506	3720	2772	10	0.15	4	0.06
3750	A	2310	1306	999	4		1	
	B	1935	1110	815	7		3	
	C	1985	1066	911	4		4	
	A+B+C	6230	3482	2725	15	0.24	8	0.13
5000	A	2046	1151	887	7		1	
	B	2200	1268	922	8		2	
	C	1291	704	581	4		2	
	A+B+C	5537	3123	2390	19	0.34 *	5	0.09

* $P = 0.05$.

mutational events at the *flr*$^{3+}$ locus are relatively rare in that *flr*^{3} does not express itself in clones smaller than a certain size (see Szabad et al., 1983). Results were analyzed by means of the computer program for SMART kindly provided by Dr. F. Würgler.

Results and discussion

Results of the test for chromosome loss in male germ cells are presented in Tables 1–4 and those for the wing spot assay in Table 5. It is clear on inspection of Tables 1 and 2 that following injection the test involving repair-proficient females is negative at both experimental doses whereas the test employing *mei-9*^{a} females is positive for apparent complete loss (CL) and partial loss (PL) of the X and Y chromosomes at the lower concentration and positive for both apparent complete loss and partial loss of the Y chromosome at the higher concentration. Further it may be noted that the positive effect with *mei-9*^{a} was achieved in an average sample size only some 40% of that in the non-*mei-9*^{a} test. There was no

TABLE 5

SUMMARY OF RESULTS OBTAINED FROM TWO EXPERIMENTS IN THE DROSOPHILA WING SPOT TEST WITH FURFURAL

Concentration (ppm)	Number of wings	Spots per wing (Number of spots) Statistical analysis [a]			
		Small single spots (1–2 cells)	Large single spots (> 2 cells)	Twin spots	Total spots
Control (ethanol 5%)	80	0.17 (14)	0.01 (1)	0.00 (0)	0.19 (15)
3750	80	0.24 (19)	0.03 (2)	0.00 (0)	0.26 (21)
5000	84	0.33 (28)+	0.05 (4)	0.01 (1)	0.39 (33)+
7500	80	0.37 (30)+	0.05 (4)	0.00 (0)	0.43 (34)+

[a] Statistical analysis according to Frei and Würgler (1988).
Probability levels: $\alpha = \beta = 0.05$, one-sided statistical test.

evidence from scoring of female progeny that partial loss of the Y chromosome came as a result of pre-meiotic exchange between X and Y chromosomes. Results from feeding tests using *mei-9*^{a} females are shown in Table 4 and confirm the positive results following injection for chromosome loss. Results with repair-proficient females provide no suggestion of a positive effect; however, the sample size was considerably smaller than that with *mei-9*^{a}. Finally, in view of the cytological findings of Traut et al. (1970) that some 90% or so apparent complete losses induced by X-rays in ordinary XY males were in fact partial losses as evidenced by the presence of sex chromosome fragments in ganglial preparations in apparent XO male larvae, it is possible that apparent CL male progeny produced after treatment with furfural are also partial losses.

In the wing spot test furfural was assayed in 2 different experiments employing 3 different concentrations (Table 5). Controls were treated with a solution of 5% ethanol; the frequency of total spots per wing was not significantly different from controls using a solution of 5% sucrose (Graf et al., 1984). In the treated series, significant increases in small single spots and total spots were observed, and suggested a linear relationship with dose. No significant increases were observed for large single spots or twin spots.

Briefly, the evidence indicates that furfural is mutagenic/clastogenic in Drosophila as demonstrated in the *mei-9*^{a} test for chromosome breakage in sperm. Further, treatment with the compound raises significantly the frequency of small single spots and total spots in the somatic wing spot test. The source(s) of these spots, i.e., point mutation/small deletion and/or recombination, is unknown; tests with appropriate inversion heterozygotes would be desirable in this connection.

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