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LACK OF GENOTOXICITY OF SACCHARIN AND SODIUM SACCHARIN STUDIED IN THE *DROSOPHILA* WING SPOT AND SEX-LINKED RECESSIVE LETHAL TESTS

Niraj K. TRIPATHY[1], Rajkishore MISHRA[2], A. ANANDKUMAR[1] and Gupta P. SAHU[1]

[1] Gene-Tox Laboratory, Department of Zoology, Berhampur University, Berhampur-760 007, Orissa, India
[2] Stewart Science College, Cuttack-753 001, Orissa, India

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ABSTRACT

Two non-nutritive sweeteners, saccharin and sodium saccharin, were tested for their genotoxicity in the *Drosophila* wing spot assay and the sex-linked recessive lethal test. 72-hours and 48-hours-old larvae, transheterozygous for the recessive wing cell markers **mwh** (multiple wing hairs) and **flr^3** (flare) from high bioactivation strains (**ORR**) of *Drosophila melanogaster* were exposed to various concentrations of the test compounds for the rest of the larval life. The sex-linked recessive lethal test was performed following the standard technique using the **Basc** strain. Both compounds were non-genotoxic in the wing primordia and in the male germ line.

RESUMEN

Fue probada la genotoxicidad de dos endulzantes no nutritivos, sacarina y sacarina de sodio, en los ensayos de la mancha alar y de los letales recesivos ligados al sexo en *Drosophila*. Larvas transheterocigóticas para los marcadores recesivos de las células del ala, **mwh** (pelos múltiples) y **flr^3** (en flama) de líneas de alta bioactivación (**ORR**) de *Drosophila melanogaster*, de 48 y 72 h fueron expuestas a varias concentraciones de los compuestos mencionados a través de la vida larvaria que les restaba. La prueba de letales recesivos ligados al sexo se realizó siguiendo la técnica estándar para la línea **Basc**. Ambos compuestos no fueron genotóxicos para los primordios del ala ni tampoco para la línea germinal masculina.

INTRODUCTION

After 1970, when cyclamates were banned for use in food, saccharin and its sodium and calcium salts gained importance as non-nutritive sweeteners. Saccharin (CAS No. 81-07-2) and sodium saccharin (CAS No. 128-44-9) are white crystalline powders with an intensely sweet taste. Prior to their use in food, saccharin was used as an antiseptic and preservative to retard fermentation of food, in sweetening pharmaceutical tablets and processing tobacco. The industrial grade of sodium saccharin is used as a brightener in nickel-plating baths and as an antistatic agent in the manufacture of plastics and textiles. Both the compounds are reported to induce bladder carcinoma in rodents (Tisdell *et al.* 1974, IARC 1980, Taylor *et al.* 1980). There is, however, no definite report on the teratogenicity of these compounds. **Table I** summarizes the studies on the genotoxicity of these two compounds in different test systems. The present paper describes the results obtained on the genotoxicity of the artificial sweeteners in somatic (wing primordia) and male germ line cells of *Drosophila melanogaster* following chronic larval exposures.

MATERIALS AND METHODS

For the wing spot assay, two high bioactivation strains of *Drosophila melanogaster:* **ORR**; **mwh** females and **ORR**; **flr^3/TM3** males were outcrossed. The 3rd chromosome markers **mwh** (multiple wing hairs, 3-0.3) and **flr^3** (flare,

TABLE I. SUMMARY OF RESULTS ON THE GENOTOXICITY OF SACCHARIN AND SODIUM SACCHARIN IN DIFFERENT TEST SYSTEMS

Test organism	Test type	Compund tested	Result*	Reference
Salmonella typhimurium	Mutation	Saccharin Sodium saccharin	+ –	Mortelmans *et al.* (1986) Stolz *et al.* (1977) Ashby *et al.* (1978) Pool (1978) Eckhardt *et al.* (1980) De Flora (1981) Imamura *et al.* (1983) Ishidate *et al.* (1984)
Bacillus subtilis	Rec assay	Saccharin	–	Kawachi *et al.* (1980)
E. coli	Mutation/disc diffusion	Sodium saccharin	–	Rosenkranz (1977)
Saccharomyces cerevisiae	Reverse mutation/Aneuploidy	Saccharin/Sodium saccharin	+	Moore and Schmick (1979) Sora *et al.* (1982) Persic (1986)
	Homozygous test/Recombination	Saccharin/Sodium saccharin	–	Mc Cann (1977) Parry *et al.* (1981) Persic (1986)
Schizosaccharomyces pombe	Mutagenicity	Saccharin	–	Ibrahim *et al.* (1981)
Allium cepa	Radiomimetic effect	Saccharin	+	Sax and Sax (1986)
Drosophila melanogaster	SLRL test	Saccharin/Sodium saccharin	–	Samuel and Sanjeeva Rao (1972) Kramers (1977) Eckhardt *et al.* (1980) Wild *et al.* (1980) Present study
	Heritable translocation	Saccharin/Sodium saccharin	–	Sanjeeva Rao *et al.* (1971) Samuel and Sanjeeva Rao (1972)
	Chromosome mutation	Sodium saccharin	inc	Sanjeeva Rao *et al.* (1971)
	Wing spot test	Saccharin/Sodium saccharin	–	Present study
Mouse	Dominant letal test	Saccharin/Sodium saccharin	+	Sanjeeva Rao and Qureshi (1972) Sram and Zudova (1974) Tezabwala and Gothskar (1977)
			–	Machemer and Lorke (1975) Leonard and Leonard (1979)
	Spot test	Sodium saccharin	+ –	Mahon and Dawson (1982) Leonard and Leonard (1979) Fahrig (1982)
	Micronucleus test	Saccharin/Sodium saccharin	–	Leonard and Leonard (1979) Eckhardt *et al.* (1980)
	Sperm morphology	Sodium saccharin	–	Topham (1980)
	Clastogenicity in bone marrow	Sodium saccharin	+	Prasad and Rai (1987)
Rat	Clastogenicity in fibroblast	Saccharin	–	Ishidate *et al.* (1984) Ashby and Ishidate (1986)
Chinese hamster	Clastogenicity in fibroblast	Sodium saccharin	+	Ishidate *et al.* (1984) Ashby and Ishidate (1986)
	in vivo clastogenecity and SCE	Sodium saccharin	+	Abe and Sasaki (1977) Ishidate and Odashima (1977) Wolff and Rodin (1978) Ray-Choudhury *et al.* (1982) Ashby and Ishidate (1986)
Muntiacus muntjak	SCE in lymphocyte	Sodium saccharin	–	Deng *et al.* (1980)
Human cell lines	Clastogenicity and SCE	Saccharin	–	Saxholm *et al.* (1979) Broeger *et al.* (1979)
		Sodium saccharin	+	Chang and Stacey (1974) Wolff and Rodin (1978)
	Ouabain resistance	Saccharin/Sodium saccharin	+	Suzuki and Suzuki (1988)

* + = positive, – = negative, inc = inconclusive

TABLE II. COMPOUNDS TESTED IN THE *DROSOPHILA* WING SPOT AND SLRL TESTS

Compound tested (CAS No.)	Solvent	Larval age (h)	Treatment (h)	Concentration range (mM)
Saccharin (81-07-2)	3% Ethanol + 1% Tween 80	48 72	72 48	50-300
Sodium saccharin (128-44-9)	Distilled water	48 72	72 48	50-300

3-38.8) are expressed phenotypically as multiple trichomes (wing hairs) or short and thick trichomes, respectively, in contrast to the single trichome per normal wing cell. For descriptions of the genetic symbols, see Frölich and Würgler (1989), Lindsley and Zimm (1992) and Graf and Singer (1992). In the sex-linked recessive lethal test, the stocks of the wild type strain **Oregon R** and the homozygous **Basc** were used.

The test compounds, saccharin and sodium saccharin, were obtained from Boots Company (India) Limited, Bombay, as gift samples. The concentrations tested, the durations of treatment and the solvents used are given in **Table II.**

For the wing spot test, eggs from optimally fertile parents were collected on standard *Drosophila* food for 8 h. After 2 and 3 days, i. e., at 48 ± 4 hours and 72 ± 4 hours of age, the larvae were washed out from the food with 20% NaCl solution. Approximately 100 larvae of each age group were transferred into vials containing 1.5 g instant medium (Carolina Biological Supply Company, USA), rehydrated with 5 ml of the solvent containing a known concentration of the test compound. Since both compounds were nontoxic to the larvae, concentrations as high as 300 mM could be tested. The wings of the eclosing flies were mounted in Faure´s solution (Graf *et al.* 1984) and screened under a compound microscope at 400 X magnification. The wing spots (clones) were classified either as singles (**mwh** or **flr**^{3}) or twins (**mwh**// **flr**^{3}). The single spots were further classified as small single spots, 1-2 cells in size (s = 1-2), and large single spots, larger than 2 cells in size (s > 2). The frequency of the different categories of spots were evaluated statistically with the conditional binomial test (Frei and Würgler 1988).

For the sex-linked recessive lethal test, **Oregon R** adult males, fed at the same larval age and with similar concentrations of the test compounds as in the wing spot test, were crossed to 3 **Basc** homozygous females for 3 days. Single F_1 **Basc** heterozygous females were mated to their **Basc/Y** sibs. The absence of males with wild type eyes in the F_2 progeny was the criterion for the presence of sex-linked lethal mutations. The data on the frequency of lethal induction were evaluated statistically with the conditional binomial test (Kastenbaum and Bowman 1970).

TABLE III. SUMMARY OF DATA OBTAINED IN THE WING SPOT TEST

Chemical tested	Larval age (hours)	Duration of treatment (hours)	Concentration (mM)	Wings tested	Spots per wing (No. of spots) Statistical Diagn.*			
					Small singles (s = 1-2) m = 2.0	Large singles (s > 2) m = 5.0	Twins (t) m = 5.0	Total (t) m = 2.0
Saccharin	72	48	0	80	0.39 (31)	0.04 (3)	0.00 (0)	0.43 (34)
			50	80	0.36 (29)-	0.04 (3)i	0.00 (0)i	0.40 (32)-
			100	80	0.35 (28)-	0.04 (3)i	0.00 (0)i	0.39 (31)-
			300	80	0.35 (28)-	0.05 (4)i	0.01 (1)i	0.41 (33)-
	48	72	0	80	0.40 (32)	0.01 (1)	0.00 (0)	0.41 (33)
			50	80	0.39 (31)-	0.04 (3)i	0.01 (1)i	0.44 (35)-
			100	80	0.38 (30)-	0.05 (4)i	0.00 (0)i	0.43 (34)-
			300	80	0.41 (33)-	0.05 (4)i	0.03 (2)i	0.49 (39)-
Sodium saccharin	72	48	0	80	0.40 (32)	0.04 (3)	0.00 (0)	0.44 (35)
			50	80	0.36 (29)-	0.04 (3)i	0.00 (0)i	0.40 (32)-
			100	80	0.36 (29)-	0.03 (2)-	0.00 (0)i	0.39 (31)-
			300	80	0.41 (33)-	0.06 (5)i	0.00 (0)i	0.48 (38)-
	48	72	0	80	0.41 (33)	0.06 (5)	0.00 (0)	0.48 (38)
			50	80	0.41 (33)-	0.04 (3)-	0.00 (0)i	0.45 (36)-
			100	80	0.39 (31)-	0.05 (4)-	0.00 (0)i	0.44 (35)-
			300	80	0.39 (31)-	0.08 (6)i	0.03 (2)i	0.49 (39)-

* Statistical diagnoses according to Frei and Würgler (1988): – = negative, i = inconclusive, m = multiplication factor. Probability level: $\alpha = \beta = 0.05$, one sided statistical tests

TABLE IV. SUMMARY OF DATA OBTAINED IN THE SEX-LINKED RECESSIVE LETHAL TEST

Chemical tested	Larval age (hours)	Duration of treatment (hours)	Concentration (mM)	Males tested	X chromosome			Concl.*	Lethals per male		
					Total	Lethal	%		0	1	2
Saccharim	Pooled control		0	130	2621	2	0.08		128	2	0
	72	48	50	61	1243	3	0.24	NS	58	3	0
			100	60	1209	3	0.25	NS	57	3	0
			300	70	1415	4	0.28	NS	66	4	0
	48	72	50	52	1079	2	0.19	NS	50	2	0
			100	60	1224	3	0.25	NS	57	3	0
			300	65	1327	4	0.30	NS	61	4	0
Sodium saccharin	Pooled control		0	140	2859	3	0.10	NS	137	3	0
	72	48	50	48	910	2	0.22	NS	46	2	0
			100	60	1248	3	0.24	NS	57	3	0
			300	63	1346	4	0.30	NS	59	4	0
	48	72	50	41	836	2	0.24	NS	39	2	0
			100	56	1090	3	0.28	NS	53	3	0
			300	79	1597	6	0.38	NS	73	6	0

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

RESULTS

In the wing spot test, two independent experiments were conducted for each compound and for each larval stage. For each experiment a concurrent control was run where the larvae of the same age were treated with the solvent alone. Because of a lack of statistically significant difference between the spot frequencies in the repeated experiments, the data were pooled and are summarized in **Table III.**

The larvae of both ages were treated with three different concentrations (50, 100 and 300 mM) of the test compounds. There was no significant increase above control level in the spot frequency at any of the test concentrations of both saccharin and sodium saccharin. **Figs. 1-4** show the spot size distribution for both the compounds at the highest concentration following exposures of 48 hours and 72 hours old larvae.

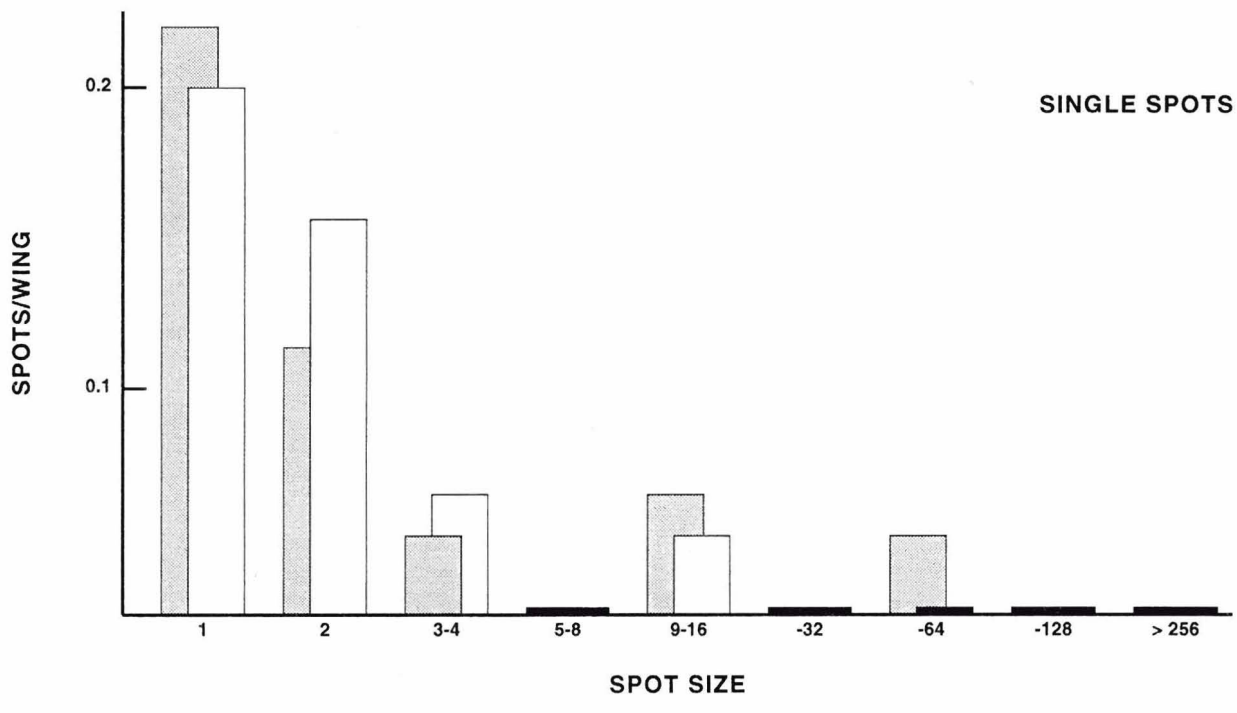
The data of the sex-linked recessive lethal test are summarized in **Table IV.** There was no significant increase in the frequency of sex-linked recessive lethals in the treated series following larval exposures to both saccharin and sodium saccharin.

DISCUSSION

The wing spot test, a one-generation short-term test in *Drosophila* involving the somatic cells (wing primordia), assays for several genetic end points (Graf *et al.* 1984, Würgler and Vogel 1986). The principle of this test involves the exposure of **mwh** + / + **flr**^{3} transheterozygous larvae to a chemical mutagen. Induced genetic changes in the wing disc cells following mitotic division may get expressed as mutant mosaic spots on an otherwise normal wing.

Mutation of the **mwh** $^+$ or **flr**$^+$ genes will lead to a homozygous mutant genotype expressed either as a **mwh** or a **flr**^{3} spot (Graf *et al.* 1984). The induction of small deletions of chromosome parts carrying the wild type alleles of the marker mutations leads to similar spots (Haynie and Bryant 1977). Further, **mwh** single spots may also arise due to induction of mitotic recombination in the chromosome region between the two marker *loci* (Graf *et al.* 1984). Twin spots with **mwh** and **flr**^{3} subclones stem from the induction of mitotic recombination in the chromosome region between the centromere and the **flr**^{3} *locus* (Becker 1976). In the present experiments, since the frequency of single and twin spots were not higher than controls, it is concluded that saccharin and its sodium salt are nongenotoxic in the primordial wing cells of *Drosophila.*

The sex-linked recessive lethal test is considered the best validated mutagenicity test in *Drosophila.* On the other hand, it is laborious as it involves at least two fly generations. Sex-linked recessive lethals represent gene mutations, small deletions or other types of chromosome aberrations (Lee *et al.* 1983). Following adult feeding and injection, Kramers (1977) obtained weak positive results with an impure sample and clear negative results with pure samples of saccharin. This compound was also reported non-genotoxic by Samuel and Sanjeeva Rao (1972). Following similar routes of administration, sodium saccharin was reported non-mutagenic in the same test (Eckhardt *et al.* 1980, Wild *et al.* 1980). Our results obtained following larval feeding, exposing premeiotic spermatogonial cells to the two sweeteners, confirm the findings following adult exposures made by the above-mentioned authors. It is thus concluded that even very high doses of saccharin and sodium saccharin are non-genotoxic in male germ line cells of *Drosophila.*



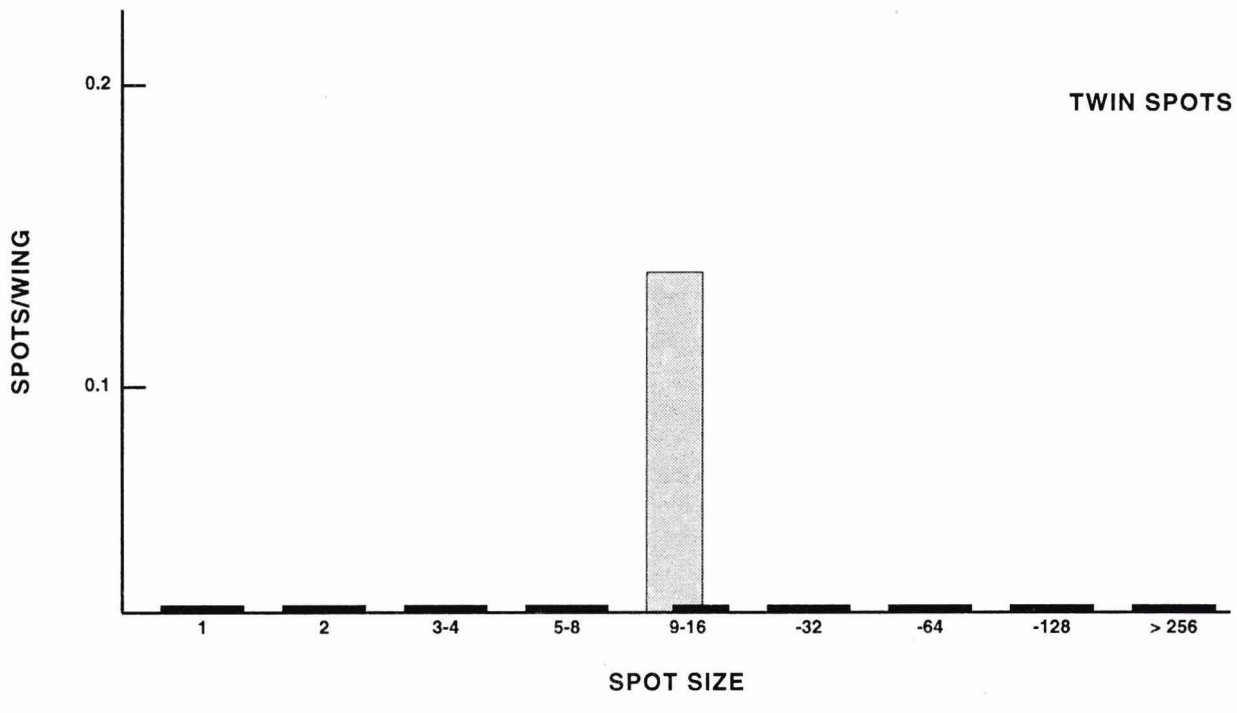


Fig. 1. Spot frequencies in 80 wings, shown as size class distributions, induced in 48 h larvae by 300 mM of saccharin □, control □

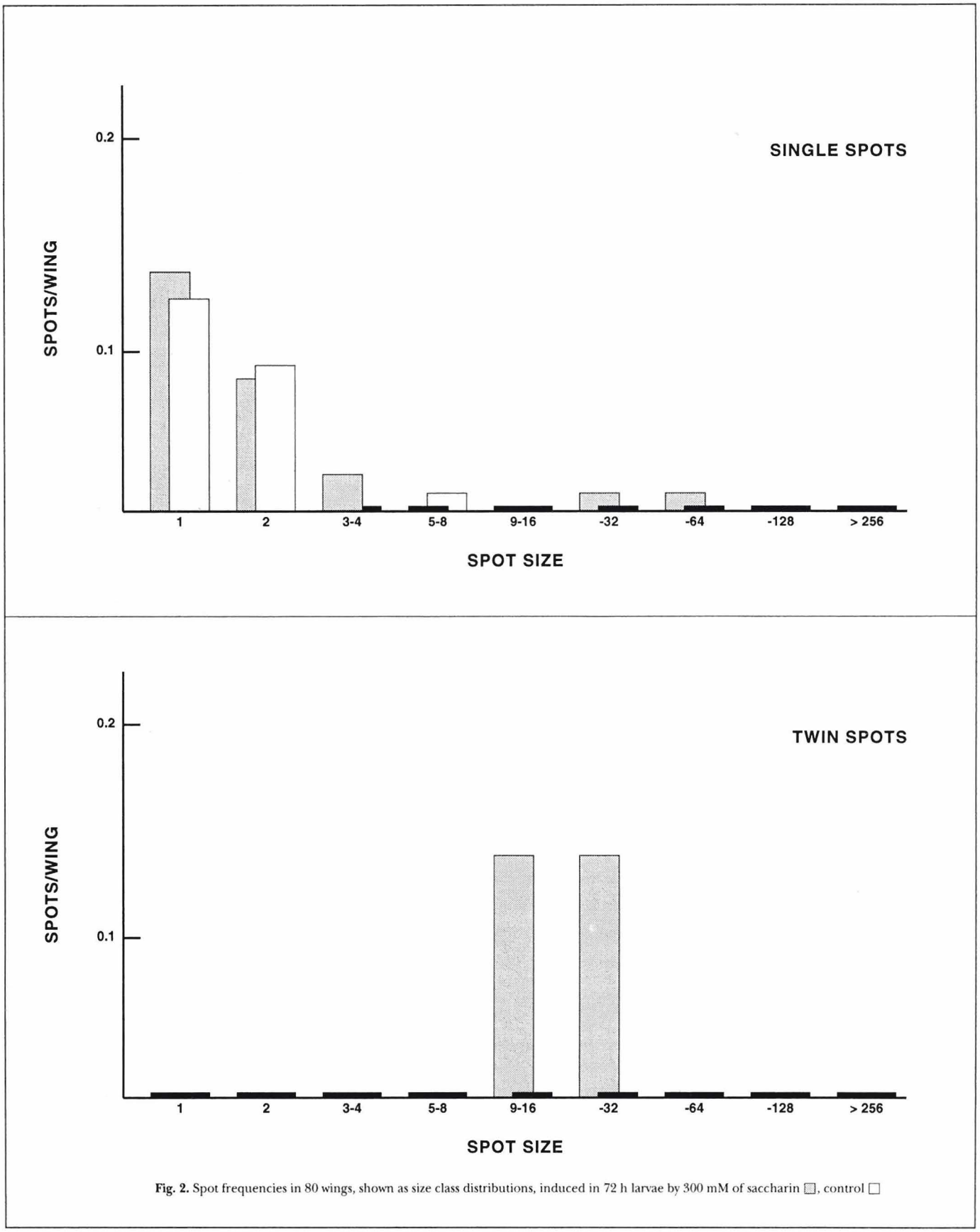
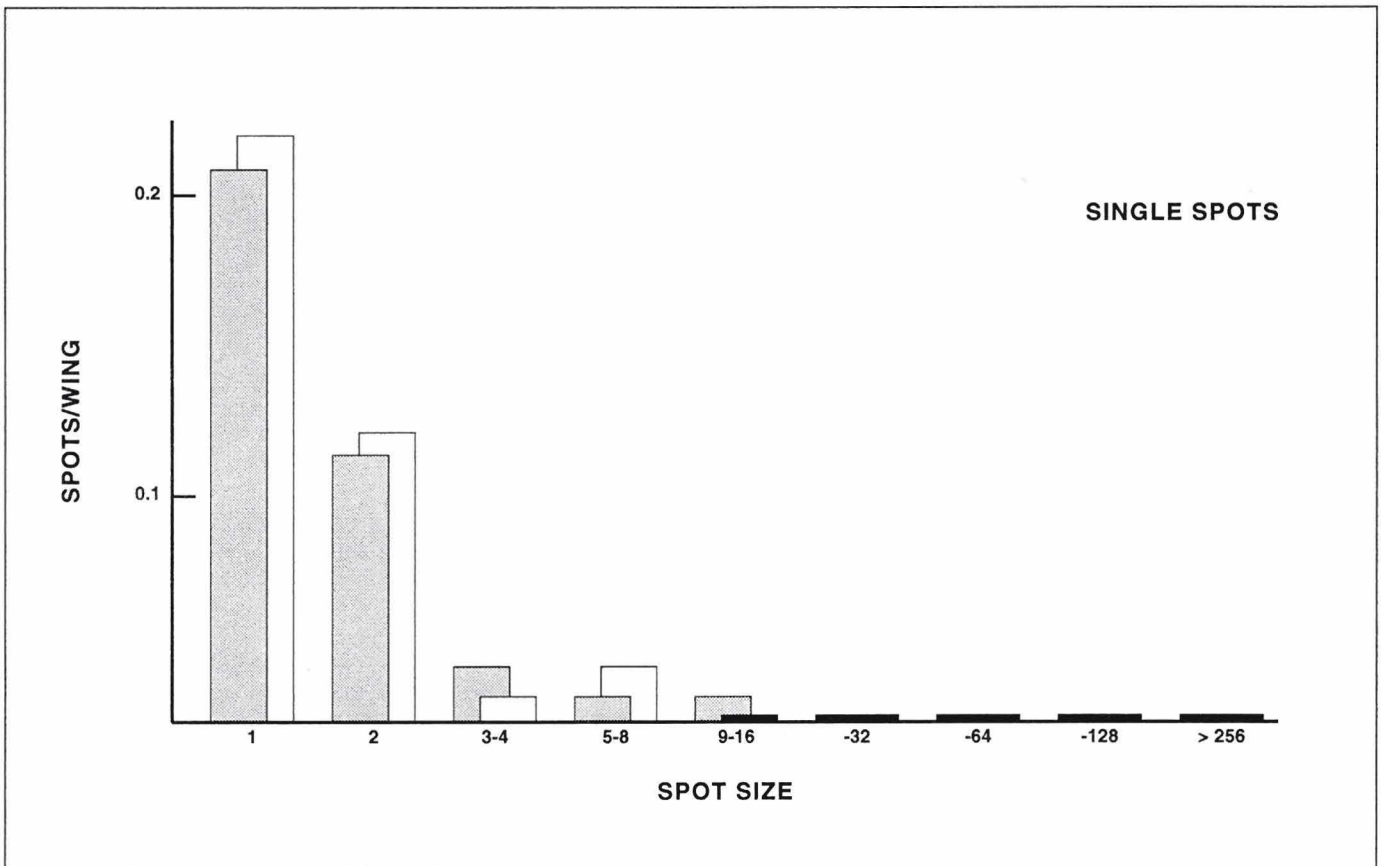


Fig. 2. Spot frequencies in 80 wings, shown as size class distributions, induced in 72 h larvae by 300 mM of saccharin ▨, control □



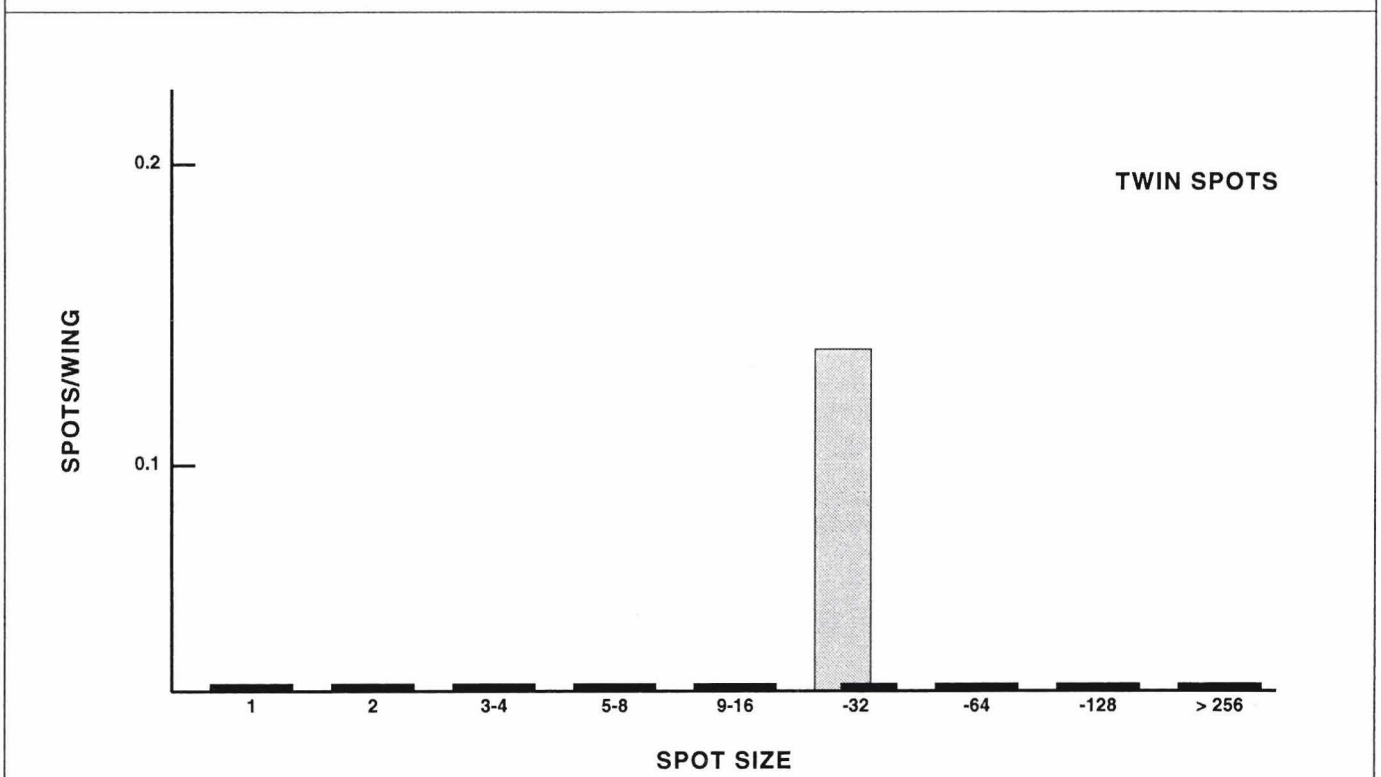


Fig. 3. Spot frequencies in 80 wings, shown as size class distributions, induced in 48 h larvae by 300 mM of sodium saccharin ▨, control □

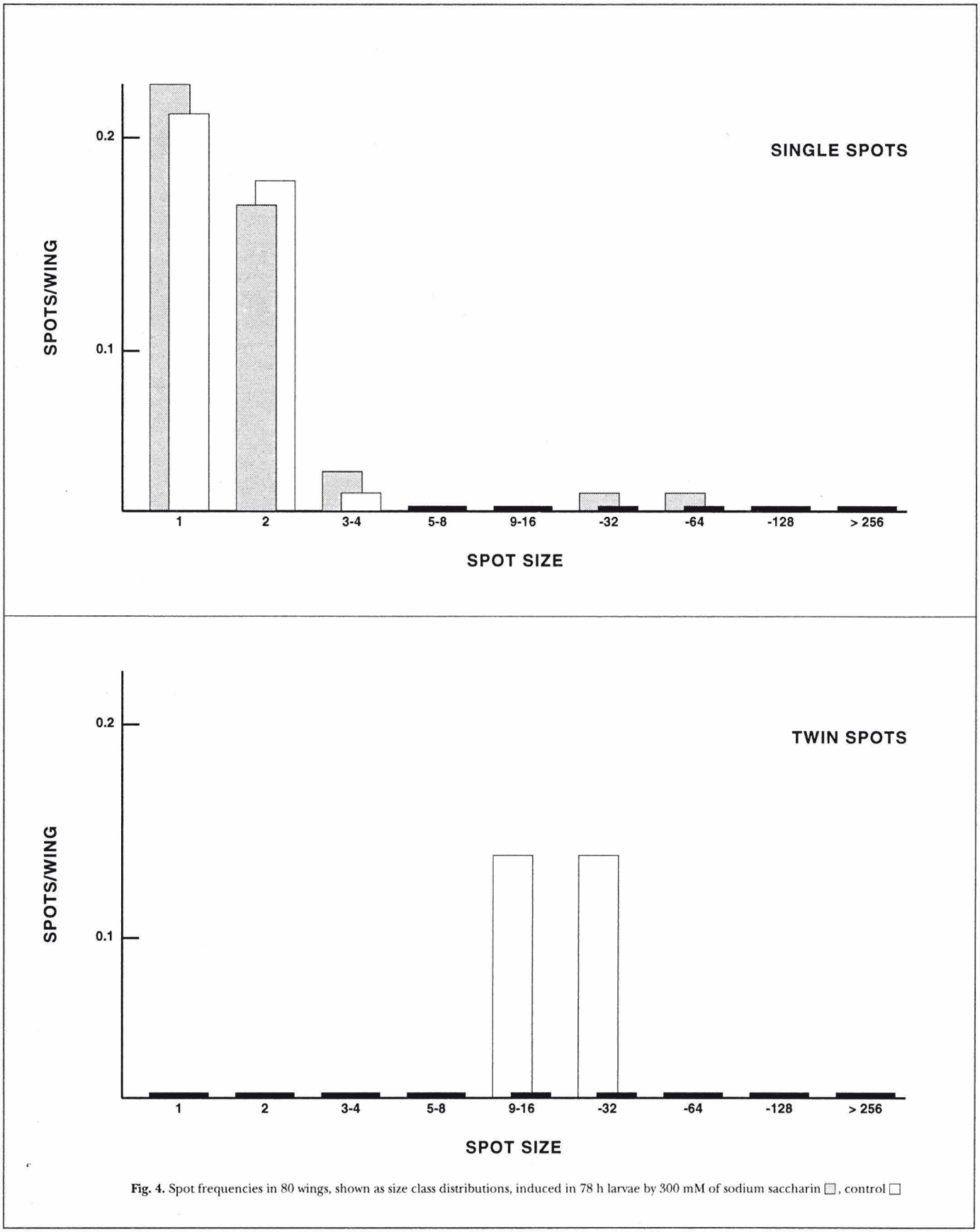


Fig. 4. Spot frequencies in 80 wings, shown as size class distributions, induced in 78 h larvae by 300 mM of sodium saccharin ▨, control □

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REFERENCES

Abe S. and Sasaki M. (1977). Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. J. Natl. Cancer Inst. *58*, 1635-1641.

Ashby J., Styles J.A., Anderson D. and Paton D. (1978). Saccharin: an epigenetic carcinogen/ mutagen? Food Cosmet. Toxicol. *16*, 95- 103.

Ashby J. and Ishidate M. Jr. (1986). Clastogenicity *in vitro* of the Na, K, Ca and Mg salts of saccharin; and of magnesium chloride; consideration of significance. Mutat. Res. *163*, 63-73.

Becker H.J. (1976). Mitotic Recombination. In: *Genetics and Biology of Drosophila* (M. Ashburner and E. Novitski, Eds.), Academic Press, New York, Vol. 1C, pp. 1020-1087.

Broegger A., Ardito G. and Waksvik H. (1979). No synergism between caffeine and saccharin in the induction of sister chromatid exchange in human lymphocytes. Hereditas *91*, 135-138.

Chang P. and Stacey T. (1974). Sodium saccharin: cytogenetic effect on human lymphocytes *in vitro*. Proc. Pa. Acad. Sci. *48*, 50-51.

De Flora S. (1981). Study of 106 organic and inorganic compounds in the *Salmonella*/microsome test. Carcinogenesis *2*, 283-298.

Deng C., Liu A. and Zhu B. (1980). Effect of sodium saccharin upon sister chromatid exchanges in cultured human lymphocytes. Chunghua I Hsueh Tsa Chih (Beizing) *60*, 676-678.

Eckhardt K., King M.T., Gocke E. and Wild D. (1980). Mutagenicity study of Ramsen-Fahlberg saccharin and contaminants. Toxicol. Lett. *7*, 51-60.

Fahrig R. (1982). Effects in the mammalian spot test: cyclamates versus saccharin. Mutat. Res. *103*, 43-47.

Frei H. and Würgler F.E. (1988). Statistical methods to decide whether mutagenicity test data from *Drosophila* assays indicate a positive, negative, or inconclusive result. Mutat. Res. *203*, 297-308.

Frölich A. and Würgler F.E. (1989). New tester strains with improved bioactivation capacity for the *Drosophila* wing spot test. Mutat. Res. *216*, 179-187.

Graf U., Würger F.E., Katz A.J., Frei H., Juon H., Hall C.B. and Kale P.G. (1984). Somatic mutation and recombination test in *Drosophila melanogaster*. Environ. Mutagen. *6*, 153-188.

Graf U. and Singer D. (1992). Genotoxicity testing of promutagens in the wing somatic mutation and recombination test in *Drosophila melanogaster*. Rev. Int. Contam. Ambient. *8*, 15-27.

Haynie J.L. and Bryant P.J. (1977). The effect of X-rays on the proliferation dynamics in the imaginal wing disc of *Drosophila melanogaster*. Wilhelm Roux´ Archiv. *183*, 85-100.

IARC (1980). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. Vol. 22, pp. 111-170.

Ibrahim M.A.K., Al-Zaidy H. and Jabra M.S. (1981). The mutagenic activity of saccharin in *Schizosaccharomyces pombe*. Heredity *46*, 289.

Imamura A., Kurumi Y., Dansuka T., Kodama M., Kawachi T. and Nagao M. (1983). Classification of compounds by cluster analysis of Ames test data. Gann *74*, 196-204.

Ishidate M. Jr. and Odashima S. (1977). Chromosome tests with 134 compounds on Chinese hamster cells *in vitro*-screening for chemical carcinogens. Mutat. Res. *48*, 337-354.

Ishidate M. Jr., Sofuni T., Yoshikawa K., Hayashi M., Nohmi T., Sawada M. and Matasuoka A. (1984). Primary mutagenicity screening of food additives currently used in Japan. Food Chem. Toxicol. *8*, 623-636.

Kastenbaum M.A. and Bowman K.O. (1970). Tables for determining the statistical significance of mutation frequencies. Mutat. Res. *9*, 527-549.

Kawachi T., Yahagi T., Kada T., Tazima Y., Ishidate M., Sasaki M. and Sugiyama T. (1980). Cooperative programme on short-term assays for carcinogenicity in Japan. In: *Molecular and Cellular Aspects of Carcinogen Screening Tests* (R. Montesano, H. Bartsch and L. Tomatis, Eds.). IARC Sci. Publ. No. 27, pp. 323-330.

Kramers P.G.N. (1977). Mutagenicity of saccharin in *Drosophila*: the possible role of contaminants. Mutat. Res. *56*, 163-167.

Lee W.R., Abrahamson S., Valencia R., von Halle E.S., Würgler F.E. and Zimmering S. (1983). The sex-linked recessive lethal test for mutagenesis in *Drosophila melanogaster*. A report of the U.S.EPA. Gene-Tox Program. Mutat. Res. *123*, 183-279.

Leonard A. and Leonard E.D. (1979). Mutagenicity test with saccharin in the male mouse. J. Environ. Pathol. Toxicol *2*, 1047-1054.

Lindsley D.L. and Zimm G.G. (Eds.) (1992). *The Genome* of *Drosophila melanogaster*. Academic Press, San Diego, 1133 p.

Machemer L. and Lorke D. (1975). Methods for testing mutagenic effects of chemicals on spermatogenesis of Chinese hamster, results obtained with cyclophosphamide, saccharin and cyclamates. Arz. Forsch. *25*, 1889-1896.

Mahon G.A.T. and Dawson G.W.P. (1982). Saccharin and the induction of presumed somatic mutations in the mouse. Mutat. Res. *103*, 49-52.

McCann J. (1977). Short-term tests. In: *Cancer Testing Technology and Saccharin*. Washington DC, Office of Technology Assessment, Congress of the United States, pp. 91-100.

Moore C.W. and Schmick A. (1979). Genetic effects of impure and pure saccharin in yeast. Science *205*, 1007-1010.

Mortelmans K., Haworth S., Lowler T., Speck W., Tainer B. and Zeiger E. (1986). *Salmonella* mutagenicity test: II. Results from the testing of 270 chemicals. Environ. Mol. Mutagen. *8*, 1-119.

Parry J.M., Parry E.M. and Barrett J.G. (1981). Tumor promoters induce mitotic aneuploidy in yeast. Nature *294*, 263-265.

Persic L. (1986). Effect of saccharin on the meiotic division of *Saccharomyces cerevisiae*. Mutat. Res. *174*, 195-197.

Pool B. (1978). Nonmutagenicity of saccharin. Toxicology *11*, 95-97.

Prasad O. and Rai G. (1987). Induction of chromosomal aberration by pre feeding saccharin in albino mice. Ind. J. Exp. Biol. *25*, 124-128.

Ray-Choudhuri R., Currens M. and Lype P.T. (1982). Enhancement of sister chromatid exchanges by tumour promoters. Br. J. Cancer *45*, 769-777.

Rosenkranz H. (1977). Short-term tests. In: *Cancer Testing Technology and Saccharin*. Washington DC, Office of Technology Assessment, Congress of the United States, pp. 101 - 103.

Samuel B.C. and Sanjeeva Rao M. (1972). Induction of mutations in *D. melanogaster* with O-sulphobenzoic imide. *Drosophila* Inform Serv. *48*, 47.

Sanjeeva Rao M., Samuel B.C. and Qureshi A.B. (1971). Genetic effects of sodium saccharin in *Drosophila melanogaster*. J. Hered. *3*, 57-60.

Sanjeeva Rao M. and Qureshi A.B. (1972). Induction of dominant lethals in mice by sodium saccharin. Ind. J. Med. Res. *60*, 599-603.

Sax K. and Sax H.J. (1968). Possible mutagenic hazards of some food additives, beverages and insecticides. Jap. J. Genet. *43*, 89-94.

Saxholm H.J.K., Iversen O.H., Reitz A. and Broegger A. (1979). Carcinogenesis testing of saccharin. No transformation or increased sister chromatid exchange observed in two mammalian cell systems. J. Cancer *15*, 509-513.

Sora S., Lucchini G. and Magni G.E. (1982). Meiotic diploid progeny and meiotic nondisjunction in *Saccharomyces cerevisiae*. Genetics *101*, 17-33.

Sram R.J. and Zudova Z. (1974). Mutagenicity studies of saccharin in mice. Bull. Environ. Cont. Toxicol. *12*, 186-192.

Stoltz D.R., Stavic B., Bendall R.D. and Craig J. (1977). The mutagenicity of saccharin impurities. I. Detection of mutagenic activity. J. Environ. Pathol. Toxicol. *1*, 139-146.

Suzuki H. and Suzuki N. (1988). Mutagenicity of saccharin in human cell strain. Mutat. Res. *209*, 13-16.

Taylor J.E., Weinberger M.A. and Friedman L. (1980). Chronic toxicity and carcinogenicity to the urinary bladder of sodium saccharin in the in-utero exposed rats. Toxicol. Appl. Pharmacol. *54*, 57-75.

Tezabwala B.U. and Gotheskar S.V. (1977). Preliminary studies on mutagenicity of saccharin by induction of dominant lethals. Ind. J. Cancer *14*, 232-234.

Tisdell M.O., Nees P.O., Harris D.L. and Derse P.H. (1974). Long term feeding of saccharin in rats. In: *Sweeteners* (G.E. Inglett, Ed.), Avi Publishing, Westport, pp. 145-158.

Topham J.C. (1980). Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? Mutat. Res. *74*, 379-387.

Wild D., Eckhardt K. and King M.T. (1980). Comparative results of short-term *in vitro* and *in vivo* mutagenicity tests obtained with selected environmental chemicals. In: *Short-Term Mutagenicity Test Systems for Detecting Carcinogens* (K. Norpoth and R.C. Graner, Eds.), Springer, Berlin, pp. 170-178.

Wolff S. and Rodin S. (1978). Saccharin-induced sister chromatid exchanges in Chinese hamster and human cells. Science *200*, 543-545.

Würgler F.E. and Vogel E.W. (1986). *In vivo* mutagenicity testing using somatic cells of *Drosophila melanogaster*. In: *Chemical Mutagens, Principles and Methods for their Detection* (F.J. de Serres, Ed.), Vol. 10, Plenum Press, New York, pp. 1-72.