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THE HERBICIDES DALAPON AND DIURON TESTED FOR GENOTOXICITY IN *Drosophila melanogaster*

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ABSTRACT

The herbicides dalapon and diuron, used in large quantities in Mexico, were evaluated for genotoxicity in *Drosophila melanogaster*. Tests for the induction of sex-linked recessive lethals were carried out with both compounds. With diuron only, a test for sex chromosome loss using a repair deficient system was carried out in addition. Under the conditions of experiments, dalapon proved negative and diuron positive in the recessive lethal test. The results obtained for the sex chromosome loss test are largely negative. There is no indication of a true genotoxic effect of diuron in this assay.

RESUMEN

Se evaluaron los efectos genotóxicos de los herbicidas dalapón y diurón, ampliamente usados en México. Para ambos compuestos se analizó la inducción de mutaciones letales recesivas ligadas al sexo. La prueba de pérdida de cromosomas sexuales, utilizando un sistema deficiente en reparación, se corrió solamente para el diurón. Bajo las condiciones experimentales el dalapón resultó negativo y el diurón positivo en el estudio de letales recesivos. Los resultados obtenidos en la prueba que mide la pérdida de los cromosomas sexuales son negativos. No hay indicación de efectos verdaderamente genotóxicos del diurón en este ensayo.

INTRODUCTION

Among the environmental chemicals with possible hazards to man, the pesticides play an important role since residues of these substancies may remain in food and be ingested

by humans (Duggan and Weatherwax 1967). With the dispersal of hundreds of millions of kilograms of pesticides on hundred of millions of hectares of land around the world, prudence requires that the mutagenic properties of these chemicals be determined and that a variety of bioassay systems be used in the process (Plewa *et al.* 1984).

Dalapon (2, 2 dichloropropionic acid sodium salt), a permeation-spreading herbicide, is employed in orchards and fields (Tsukioka and Shimizu 1985) to erradicate deep-rooted perennial grasses (Weeraratna 1980). The herbicide is soluble in water at ambient temperature and neither photodecomposes nor evaporates; nevertheless, it is biodegradable by dehalogenation producing inactive pyruvate (Matsumara 1973, Audus 1976, Barbera 1976). About 228, 821 tons of dalapon were imported into Mexico during the 1985-1986 period (Productora Nacional de Semillas, personal communication). Diuron (3-(3, 4-dichlorophenyl)-, 1-dimethylurea), a ureic herbicide, attacks roots of growing plants (Majka and Levis 1977). Herbicidal uses of diuron are numerous on both crops and noncrop land; it is used primarily to control annual grass and broadleaf weeds before emergence in alfalfa, artichokes, asparagus, barley, blueberries, corn, cotton, grapes, pineapple, sorghum, sugarcane and nut tree crops(Klingman *et al.* 1975). Diuron has low water solubility and is extremely persistent in soils although it can ultimately be degraded by microbial action (Attaway *et al.* 1982). It acts by strongly reducing the photosynthetic activity of plants by inhibition of the Hill reaction in the chloroplast (Brown 1978). The production of diuron in Mexico was about 320 tons (Productora Nacional de Semillas, personal communication).

The present paper deals with the genotoxic effects of dalapon and diuron in the sex-linked recessive lethal test and of diuron additionally in a sex chromosome loss test in *Drosophila melanogaster*.

MATERIAL AND METHODS

Stocks

In the test to screen for sex-linked recessive lethals (SLRLT), Oregon-R wild type males and females carrying a balancer X-chromosome, *Basc*, with the genotype In(1) $sc^{S1L}sc^{8R}$ + S, $sc^{S1}sc^{8}w^{a}B$ were used. For the sex chromosome loss test (SCLT) the males carried a ring X-chromosome (X^{c2}) marked with the recessive mutants *y f bb*$^{-}$ and a special Y chromosome, B^{S} Y y^{+}, bearing the dominant marker B^{S} on the long arm of the Y chromosome, Y^{L}, and the dominant alleles of *y* and *bb*, i.e. y^{+} and bb^{+}, on the short arm, Y^{S} (Brosseau 1958). Males were mated with repair-deficient females of the constitution *y ac sc* w^{a} *mei*-9^{a}. It is known that *mei*-9^{a} is a meiotic mutant as well as a repair-deficient mutant that strongly affects excision repair (Baker and Carpenter 1972, Boyd *et al.* 1976). It sensitizes the sex chromosome loss test to the genetic effects of many chemical mutagens (Zimmering 1983). For an explanation of genetic symbols see Lindsley and Grell (1968).

Experimental procedures

Test for recessive lethals: Treated and control males were mated individually with three virgin females to produce three broods: Brood A (two days), Brood B (three days) and Brood C (two days); thus primarily postmeiotic and late meiotic germ cells were tested (Chandley and Bateman 1962). F_1 females were mated individually to sibs and F_2 progeny were scored for the presence or absence of wild type males. A lethal mutation is taken to have occurred if no wild type males were recovered in F_2 among at least 16 *Basc*/+ females or if wild type males were less than 5 % of the total *Basc*/+ females. Suspected lethals were retested in F_3.

Test for chromosome loss: Treated and control males were mass mated in bottles with virgin females and transferred so as to produce three broods of 2-3-2 days.

Regular F_1 females are phenotypically *y* and F_1 males w^a B^S. Complete loss (CL) of X or Y generates a phenotypically *y ac* w^a male, while partial loss (PL) of the Y chromosome in the case of loss of the y^+ marker from the Y-chromosome produces a *y ac* w^a B^S male and a w^a male in the case of loss of the B^S marker.

Chemicals and solvents: Dalapon (trade names Basfpon, Dowpon) was obtained from BASF (Mexico City), diuron (trade names Karmex, Telvar, Urox D) from Du Pont (Mexico City). The solvent for dalapon was a solution of 5 % sucrose, and for diuron a solution of 1.5 % DMSO.

Treatment: For the SLRLT experiments, adult males 0-48 hours old, were injected with 0.2-2.3 μl of fresh solution. For the SCLT experiments adult males were fed during 24 hours on a filter disc placed at the bottom of vials saturated with 0.5-0.7 ml of the solution for 24 hours and then transferred to vials with fresh solutions for an additional 24 hours.

Statistical analysis: The cumulative Poisson (Owen 1962) was employed to identify clusters. After correction for clusters (if any) the data were analyzed with the Kastenbaum-Bowman test (1970) to determine the significance of the difference between control and treated values.

RESULTS AND DISCUSSION

Results of the *Basc* test for sex-linked recessive lethal mutations after treatment with the herbicides by injection of adult Oregon-R males are shown in Table I and II. Table I shows the results obtained after treatment with dalapon. It can be seen from this table that there is no significant difference between control and treated values at 5 % level in any of the broods at any of the concentrations. These results are in agreement with the negative findings reported by Siebert and Lemperle (1974) for mitotic conversion test in *Saccharomyces cereviseae* and with those by Moriya *et al.* (1983), who found no evidence that dalapon significantly increases the frequency of reversions in the Salmonel-

TABLE I. RESULTS OF THE SEX-LINKED RECESSIVE LETHAL TEST IN *Drosophila melanogaster* AFTER INJECTION OF THE HERBICIDE DALAPON INTO ADULT OREGON-R WILD TYPE MALES. THE SYMBOLS # 1/# tests SIGNIFY THE NUMBER OF LETHALS IDENTIFIED IN A TEST SAMPLE OF X-CHROMOSOMES. THE DATA REPRESENT THE SUM OF TWO EXPERIMENTS

Concentration (ppm)	*Brood A (0–2 days)* # *1/# tests*	%	*Brood B (3–5 days)* # *1/# tests*	%	*Brood C (6–7 days)* # *1/# tests*	%	*Broods A+B+C (0–7 days)* # *1/# tests*	%
Control								
5% sucrose	0/1175		0/1616		0/1233		0/4024	
1	0/1294		0/1413		1/927	0.11	1/3634	0.03
10	0/1426		2/1744	0.11	0/904		2/4074	0.05
25	1/1235	0.08	1/1756	0.06	1/1300	0.08	3/4291	0.07

TABLE II. RESULTS OF THE SEX-LINKED RECESSIVE LETHAL TEST IN *Drosophila melanogaster* AFTER INJECTION OF THE HERBICIDE DIURON INTO ADULT OREGON-R WILD TYPE MALES. THE SYMBOLS # 1/# tests SIGNIFY THE NUMBER OF LETHALS IDENTIFIED IN A TEST SAMPLE OF X-CHROMOSOMES. THE DATA REPRESENT THE SUM OF THREE EXPERIMENTS

Concentration (ppm)	*Brood A (0–2 days)* # *1/# tests*	%	*Brood B (3–5 days)* # *1/# tests*	%	*Brood C (6–7 days)* # *1/# tests*	%	*Broods A+B+C (0–7 days)* # *1/# tests*	%
Control								
DMSO 1.5%	1/1662	0.06	0/1324		2/1289	0.15	3/2475	0.07
10	3/1251	0.24	3/1793	0.17	4/1932	0.21	10/4976	0.20
20	2/1450	0.14	1/1575	0.06	4/1467	0.27	7/4492	0.16
40	6/1081	0.55*	8/1738	0.46*	2/1359	0.15	16/4178	0.38*

*p=0.05

la/microsome test. The frequencies of lethals recovered after diuron treatment are given in Table II. This herbicide induced significant increases in the frequency of recessive lethals in Broods A and B at a concentration of 40 ppm. It may be recalled that Broods A and B represent postmeiotic stages, principally mature sperm and late spermatids. The mean frequency over the three broods is 0.38 % a value some 5 to 6 times greater than the control (0.07 %). Results of experiments testing for sex chromosomes loss with

diuron are shown in Table III. The results obtained are largely negative. It was not possible to test the ring-X males at 40 ppm because of the toxic effects of the chemical at that concentration.

Briefly, the herbicide dalapon was negative in the test for sex-linked recessive lethals in *Drosophila* whereas the herbicide diuron was positive in that test and negative in the test for sex chromosome loss using repair-deficient females *mei*-9^a.

TABLE III. COMPLETE CHROMOSOME LOSS (CL), PARTIAL CHROMOSOME LOSS (PL) AND MOSAICS FOUND IN THE PROGENY OF DIURON-TREATED MALES WITH REPAIR-DEFICIENT *mei*$^{-9a}$ FEMALES OF *Drosophila melanogaster*. THE DATA REPRESENT THE SUM OF THREE EXPERIMENTS

Concentration (ppm)	*Brood*	*Total F_1*	*Regular females*	*Regular males*	*Chromosome Loss*				*Mosaics*	
					CL	%	*PL*	%		%
Control										
DMSO 1.5%	A	3144	2033	1085	20	0.64	5	0.16	1	0.03
	B	2163	1141	1008	12	0.55	2	0.09		
	C	1820	1033	770	15	0.82	2	0.11		
	A+B+C	7127	4207	2863	47	0.66	9	0.13	1	0.01
10	A	4185	2416	1744	19	0.45	5	0.12	1	0.02
	B	3371	1861	1489	18	0.53	2	0.06	1	0.03
	C	2962	1628	1047	13	0.48	3	0.11	1	0.04
	A+B+C	10248	5905	4280	50	0.49	10	0.10	3	0.03
20	A	2245	1260	972	66	0.27	7	0.31*		
	B	2143	1210	918	12	0.56	3	0.14		
	C	2361	1331	1018	9	0.38	2	0.08	1	0.04
	A+B+C	6749	3801	2908	27	0.40	12	0.18	1	0.01

*$p=0.05$

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REFERENCES

Attaway H. H., Camper N. D. and Paynter M. J. B. (1982). Anaerobic microbial degradation of diuron by pond sediment. Pest. Biochem. *17*, 96-101.

Audus L. J. (1976). *Herbicides*. Academic Press, London, 2nd. Ed., 608 p.

Baker B. and Carpenter A. (1972). Genetic analysis of sex chromosomal mutants in *Drosophila melanogaster*. Genetics *71*, 255-286.

Barbera C. (1976). *Pesticidas Agricolas*. Ediciones Omega, Barcelona. 3rd. Ed., 569 p.

Boyd J., Golino M. and Setlow R. (1976). The *mei-9*^{a} mutant of *Drosophila melanogaster* increases mutagen sesitivity and decreases excision repair. Genetics *84*, 527-544.

Brosseau G. (1958). Crossingover between Y chromosomes. D.I.S. *32*, 115.

Brown A. W. A. (1978). *Ecology of Pesticides*. Wiley, New York, 438 p.

Chandley A. C. and Bateman A. J. (1962). Timing of spermatogenesis in *Drosophila melanogaster* using tritiated thymidine. Nature *20*, 299-300.

Duggan R. E. and Weatherwax J. R. (1967). Dietary intake of pesticide chemicals. Science *157*, 1006-1010.

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

Klingman G. C., Aston F. M. and Noordhoff L. J. (1975). *Weed science: Principles and practice*. Wiley, New York, 431 p.

Linsdley D. L. and Grell E. H. (1968). Genetic variations of *Drosophila melanogaster*. Carnegie Institution of Washington, Wash., D.C., 471 p.

Majka J. T. and Levis T. L. (1977). Absorption, mobility and degradation of cyanacine and diuron in soils. Weed Sci. *25*, 401-406.

Matsumara F. (1973). *Environmental pollution by pesticides* (C. A. Edward, Ed.), Plenum Press, New York, pp. 501-506.

Moriya M., Otha T., Watanabe K., Miyazawa T., Kato K. and Shirazu Y. (1983). Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat. Res. *116*, 185-216.

Owen D. B. (1962). *Handbook of statistical tables*. Addison-Wesley, London.

Plewa M. J., Wagner E. D., Gentile G. J. and Gentile J. M. (1984). An evaluation of the genotoxic properties of herbicides following plant and animal activation. Mutat. Res. *136*, 233-245.

Siebert D. and Lemperle E. (1974). Genetic effects of herbicides: induction of mitotic gene conversion in *Saccharomyces cerevisae*. Mutat. Res. *22*, 111-120.

Tsukioka T. and Shimizu S. (1985). Determination of the herbicides frenock and dalapon in soil and river water by mass fragmentation. Analyst *110*, 39-42.

Weeraratna C. S. (1980). Effect of dalapon sodium on nitrification and denitrification in a tropical loam soil. Weed Res. *20*, 291-293.

Zimmering S. (1983). The *mei-9*^{a} test for chromosome loss in *Drosophila*: a review of assays of 21 chemicals for chromosome breakage. Environ. Mutagen. *5*, 907-921.