2 Kim, Y.S., Tsao, D., Siddiqui, B., Whitehead, J.S., Amstein, P., Bennett, J., and Hicks, J., Cancer *45* (1980) 1185.
3 Leavit, J., Barrett, J.C., Crawford, B.D., and Ts'o, P.O.P., Nature *271* (1978) 262.
4 Lotan, R., Biochim. biophys. Acta *605* (1980) 33.
5 Siddiqui, B., and Kim, Y.S., Cancer Res. *44* (1984) 1648.
6 Tsao, D., Morita, A., Bella, A., Luu, P., and Kim, Y.S., Cancer Res. *41* (1982) 1052.
7 Collins, S.J., Ruscetti, F.W., Gallagher, R.E., and Gallo, R.C., Proc. natn. Acad. Sci. USA *75* (1978) 2458.
8 Gusella, J., Geller, R., Clarke, B., Weeks, V., and Housman, D., Cell *9* (1976) 221.
9 Nordenberg, J., Wasserman, L., Beery, E., Aloni, D., Malik, H., Stenzel, K.H., and Novogrodsky, A., Exp. Cell Res. *162* (1986) 77.
10 Augeron, C., and Laboisse, L., Cancer Res. *44* (1984) 3961.
11 Go, V.L.W., and Zamcheck, N., Cancer *50* suppl. (1982) 2618.
12 Fogh, J., and Trempe, G., in: Human Tumor Cells in vitro, p.115. Ed. J. Fogh. Plenum Press, New York 1975.
13 Shi, Z.R., Tsao, D., and Kim, Y.S., Cancer Res. *43* (1983) 4045.
14 Huberman, E., and Callaham, M.F., Proc. natn. Acad. Sci. USA *76* (1979) 1293.
15 Bauer, G., Hofler, P., and Zur Hausen, H., Virology *121* (1982) 184.
16 Riggs, A.D., and Jones, P.A., Adv. Cancer Res. *40* (1983) 1.
17 Colburn, N.H., Ozanne, S., Licht, U., Yuspa, S., Wendel, E., Jardini, E., and Abruzzo, G., Ann. N.Y. Acad. Sci. *359* (1981) 251.
18 Taffet, S.M., Greenfield, A.R.L., and Haddox, M.K., Biochem. biophys. Res. Commun. *114* (1983) 1194.
19 Collins, S.J., Bodner, A., Tinge, R., and Gallo, R.C., Int. J. Cancer *25* (1980) 213.
20 Rabson, A.S., Stern, R., Tralka, T.S., Costa, J., and Wilczek, J., Proc. natn. Acad. Sci. USA *74* (1977) 5060.
21 Goslin, R., O'Brien, M.J., Steele, G., Mayer, R., Wilson, R., Carson, J.M., and Zamcheck, N., Am. J. Med. *71* (1981) 246.
22 Primus, F.J., Clark, C.A., and Goldenberg, D.M., in: Diagnostic Immunohistochemistry, p.263. Ed. R.A. Delellis. Masson Publishing USA, 1981.
23 Drewinko, B., Yang, L.Y., Leibovitz, A., Bariogie, B., Lutz, D., Jansson, B., Stragand, J.J., and Trujillo, J.M., Cancer Res. *44* (1984) 4241.
24 Wagener, C., Muller-Wallraf, R., Nisson, S., Groner, J., and Breuer, H., J. natn. Cancer Inst. *67* (1981) 539.

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Accumulation of drastic mutants in selection lines for resistance to the insecticides dichlorvos and malathion in *Drosophila melanogaster**

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Summary. Viability tests were performed on second and third chromosomes from lines of *Drosophila melanogaster* selected for increased resistance to the organophosphorus insecticides dichlorvos and malathion, in order to evaluate the accumulation of drastic alleles. Our results show that malathion reduces significantly the relative viability of chromosome 3 and also increases the frequency of drastic alleles in this chromosome, while dichlorvos increases significantly the frequency of drastic alleles in chromosome 2.
Key words. Drosophila melanogaster; dichlorvos; malathion; viability tests; drastic mutants.

Lethal alleles have repeatedly been found at high frequencies in experimental populations subjected to selection for quantitative characters[1,2]. In these laboratory populations, lethals arise by mutation and could increase its frequency by different means, for example, by heterotic effects of lethal heterozygotes[3] or by epistatic interactions between linked lethal genes[4]. When the selected character is the resistance to a potentially mutagenic compound, the response must be higher due to the induced increase in the mutation frequency. Therefore, studies on the occurrence of drastic mutants in these selected lines could provide useful information on the genotoxicity of tested compounds.

There is no doubt that natural insect populations contain many pesticide-resistant variants and, when challenged by selection, quickly develop resistance. In this way, positive responses in selection experiments for increased resistance to several insecticides have been reported by different authors[5-7] but, up to now, no data have been published on the genetic load induced in the selected lines by analyzing quantitatively and qualitatively the effects of detrimental alleles accumulated.

Organophosphorus compounds generally show alkylating properties[8] and, consequently, they are potentially mutagenic agents. Dichlorvos and malathion are two organophosphorus insecticides fairly well studied from the mutagenic point of view but, unfortunately, no general agreement has been reached on their genotoxicity because both positive and negative results were found using different organisms and mutagenicity assays[9-13].

The present report deals with the occurrence of drastic mutants in lines of *Drosophila melanogaster* selected, during the adult stage, for increased resistance to dichlorvos and to malathion. The question whether the results of this kind of experiment would be interesting from the point of view of mutagenicity studies is considered.

Materials and methods. 1) Chemicals and treatment procedure. Dichlorvos, or 0,0-dimethyl-0-(2,2-dichlorovinyl) phosphate, with a purity of 97%, was supplied by Productos Cruz Verde S.A. (Barcelona) and was diluted in an aqueous solution which contained 5% sucrose. Malathion, or S-[1,2-di(ethoxycarbonil) ethyl] dimethyl phosphorothiolothionate, 50% emulsifiable concentrate (50% active ingredient, 50% xylol, dispersing and emulsifying agents), obtained from Agrocrós S.A. (Barcelona), was first dissolved in dimethyl sulfoxide (DMSO, Panreac) and then diluted with a 5% sucrose solution to give a final DMSO concentration of 1%. In both cases, the insecticide concentrations used for treatment were based on the active ingredient. For adult feeding treatment, 3–4-day-old flies were starved for 4 h and then fed the test solution in special glass filter feeding units[14].

2) Selection experiments. Selection experiments were performed on a population of *Drosophila melanogaster* designated MRA, previously selected for increased resistance to malathion[15]. The selection procedure was simple. In the line selected for increased resistance to malathion, flies surviving after adult treatment were used as parents of the next generation. The selection was practised during 8 generations, increasing the concentration of malathion from 50 to 100 ppm. In the line selected for increased resistance to dichlorvos the experiment lasted for 20 generations, but the selection was applied in alternate generations because of the high toxicity of dichlorvos. In this line, the concentrations applied ranged

Table 1. Relative viability of homozygous chromosomes 2 and 3 including and excluding lethals and semilethals

Population	Chromosome 2 No. chromosomes tested	Relative viability % ± SE	Chromosome 3 No. chromosomes tested	Relative viability % ± SE	No. flies scored
Control	42	28.64 ± 0.86	42	28.22 ± 1.86	4547
	(41)	(29.01 ± 0.80)	(35)	(28.22 ± 1.86)	(3707)
Dichlorvos	18	26.36 ± 2.79	18	25.71 ± 3.12	1684
	(15)	(29.23 ± 2.75)	(14)	(30.73 ± 2.66)	(1367)
Malathion	21	27.03 ± 2.84	21	14.19 ± 2.38[a]	1603
	(18)	(31.54 ± 1.65)	(10)	(24.07 ± 1.64)[a]	(960)

Data in parentheses correspond to counts excluding drastic alleles. [a]Significant at 0.01 level (based on t-test).

Table 2. Viability classes for chromosomes 2 and 3

Population	Chromosome 2 Quasi-normal	Drastic	Chromosome 3 Quasi-normal	Drastic
Control	97.62	2.38	83.34	16.66
Dichlorvos	83.34	16.66[a]	77.78	22.22
Malathion	85.72	14.28	47.62	52.38[b]

[a] Significant at 0.05 level, [b] significant at 0.01 level (based on chi-square test).

from 1 to 5 ppm. At the end of the experiment, both selected lines showed a significant level of resistance with respect to the original population.

3) Viability tests. Viability measurements were done just when the selection experiments were finished. Males from the selected lines, as well as from the base population, were mass-mated with virgin females of the tester strain *Cy 0/Pm; TM2 Ubx/Sb*. F_1 males of the phenotype *Cy; Ubx* were singly mated with 3 virgin females of the tester stock. The number of males used was 50 for control and 25 for each treatment. Males and virgin females of the F_2 generation with phenotype *Cy; Ubx* were mated to obtain progeny homozygous for specific second or third chromosomes.

4) Culture conditions. A standard food medium enriched with living yeast was used for raising the flies and for the tests, and all experiments were performed in a controlled temperature at about 25 °C.

Results and discussion. Table 1 shows the number of chromosomes tested, the number of flies scored at F_3 and the relative viability of homozygous chromosomes 2 and 3 for control and treatments. These results are expressed including and excluding lethal and semilethal mutations. We consider that a chromosome carries a lethal when only heterozygous offspring is observed in F_3, while a chromosome carries a semilethal when the frequency of normal offspring is less than 50% of the expected frequency. From our data it appears that only the treatment with malathion is able to reduce significantly the relative viability of chromosome 3.

When we analyzed the percentage of chromosomes carrying quasi-normal alleles vs those carrying drastic alleles (lethals and semilethals) we found (table 2) that in both selection lines there is an apparent increase in the proportion of chromosomes with drastic alleles. Nevertheless, the chi-square test indicates that only the increases of drastic mutants induced by dichlorvos in chromosome 2 and by malathion in chromosome 3 are significant.

Our data indicate that chronic treatment with the organophosphorus insecticides used in this study induces, in addition to the significant increase in the resistance level in front of them, an accumulation of genotoxic effects that can be evaluated by means of the decrease of relative viability and/or by the increase in the number of chromosomes carrying drastic alleles. The difference observed between chromosomes in their ability to accumulate drastic alleles has also been found in natural populations[16] as well as in populations exposed to chemicals[17], and it has been postulated to be due to the existence of different linkage complexes in the two chromosomes.

The increase in the number of chromosomes 2 carrying drastic alleles found in the dichlorvos selection line can be related to the positive results of Hanna and Dyer[10] in an experiment on the accumulation of recessive lethal mutations after 40 generations of exposure to dichlorvos. The lack of published data makes it impossible to compare our results for malathion selection with those of other workers.

We conclude that prolonged treatment with compounds suspected of mutagenicity can lead to an accumulation of genetic alterations, decreasing the relative viability and/or increasing the number of chromosomes with drastic alleles. Therefore, selection procedures may be used to obtain valuable complementary data in the mutagenic screening of chemicals, principally with those showing high toxicity.

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1 Yoo, B.H., Genet. Res. *35* (1980) 19.
2 Skibinski, D.O.F., J. Hered. *77* (1986) 31.
3 Salceda, V.M., Genetics *57* (1967) 691.
4 Nei, M., Jap. J. Genet. *39* (1964) 7.
5 Crow, J.F., A. Rev. Ent. *2* (1957) 227.
6 Georghiou, G.P., A. Rev. Ecol. Syst. *3* (1972) 133.
7 Singh, R.S., and Morton, R.A., Can. J. Genet. Cytol. *23* (1981) 355.
8 Bedford, C.T., and Robinson, J., Xenobiotica *2* (1972) 307.
9 Ashwood-Smith, M.J., Trevino, J., and Ring, R., Nature *240* (1972) 418.
10 Hanna, P.J., and Dyer, K.F., Mutation Res. *28* (1975) 405.
11 Chen, H.H., Hsueh, J.L., Sirianni, S.R., and Huang, C.C., Mutation Res. *88* (1981) 307.
12 Wildemauwe, C., Lontie, J.F., Schoofs, L., and Van Larebeke, N., Residue Rev. *89* (1983) 129.
13 Degraeve, N., and Moutschen, J., Envir. Res. *34* (1984) 170.
14 Vogel, E., and Lüers, H., Drosophila Inf. Serv. *51* (1974) 113.
15 Velázquez, A., Tesina de Licenciatura, p. 130. Universidad Autónoma de Barcelona, 1983.
16 Allen, A.C., Genetics *63* (1969) 629.
17 Mohamed, A.H., and Stern, M.S., Envir. Res. *35* (1984) 327.

0014-4754/87/101122-02$1.50 + 0.20/0