

EVIDENCE FOR NON-MUTAGENICITY OF EPICHLOROHYDRIN IN *Drosophila melanogaster*

A. Velázquez, N. Xamena, A. Creus and R. Marcos

ABSTRACT

The possible mutagenic effect of epichlorohydrin (ECH) on male germ cells of *Drosophila melanogaster* was studied.

Feeding of adults and injection with ECH were unable to induce a significant increase in the frequency of sex-linked recessive lethal mutations in an insecticide-resistant strain. Adult injection in a sensitive strain was also ineffective.

Moreover, negative results were obtained when ECH was tested for its ability to induce sex-chromosome loss and non-disjunction.

INTRODUCTION

Epichlorohydrin (1-chloro-2,3-epoxypropane, ECH) is an organochlorine compound usually used as an intermediate, solvent or stabilizer in the production of a wide variety of chemicals. Its applications include the manufacture of paper, textiles, pharmaceuticals and pesticides (IARC, 1976).

The epoxide ring structure of the ECH molecule and its alkylating properties have been of particular concern to genetic toxicologists (Bridges, 1981). Mutagenic effects of ECH were reported in bacteria (McCann *et al.*, 1975; Wade *et al.*, 1978, 1979; Voogd *et al.*, 1981; Knaap *et al.*, 1982), *Neurospora* (Kølmær and Giles, 1955), *Schizosaccharomyces* (Migliore *et al.*, 1982), and mammalian cells *in vitro* (Dean and Hodson-Walker, 1979; White, 1980; Srám *et al.*, 1981; Knaap *et al.*, 1982). However, the results from *in vivo* studies are conflicting (Kučerová *et al.*, 1977; Picciano, 1979; Rossi *et al.*, 1983 a,b).

In *Drosophila*, the mutagenic activity of ECH was first documented by Rapoport (1948). Later Knaap *et al.* (1982) reported that ECH was mutagenic in the sex-linked recessive lethal test only after treatment of adult males by injection. To provide more data for *Drosophila*, we present here the results recently obtained by us in the sex-linked recessive lethal (SLRL), sex-chromosome loss (SCL) and non-disjunction (ND) assays.

MATERIALS AND METHODS

All relevant data concerning materials and methods are presented in tabular form (Table I). For a complete explanation of genetic symbols, see Lindsley and Grell (1968).

RESULTS AND DISCUSSION

The results presented here constitute part of a more extensive study on the mutagenicity of several insecticides in *Drosophila melanogaster*. In this study the use of the *MRA* insecticide-resistant strain was proven to be effective (Velázquez *et al.*, 1984, 1986, 1987; Batiste-Alentorn *et al.*, 1986) because it allows the testing of higher doses.

Among the different mutagenicity assays with *Drosophila*, the sex-linked recessive lethal test deserves high priority due to its sensitivity. We started the mutagenic evaluation of ECH by testing for its ability to induce SLRL mutations in the *MRA* strain, through treatment of adult males by feeding and injection. The results obtained are summarized in Table II and indicate that feeding of 5 and 10 mM ECH for a period of 48 h was ineffective in producing a significant increase in the mutation frequency in post-meiotic male germ cells. These results reinforce and extend the previous negative results found by Knaap *et al.* (1982) in feeding experiments with the wild-type *Oregon-K* strain, with exposure of adult males to concentrations of 2.6 and 5.1 mM for a 24-h period.

In contrast with the significantly enhanced SLRL mutation frequencies found by these investigators in injection experiments at concentrations of 5.1 and 25.5 mM, no mutagenic effect was observed in our injection experiment with 35 mM. Taking into account this discrepancy together with the fact that we used an insecticide-resistant strain, we decided to perform an additional test, in which *Berlin-K* (wild-type) males were used, to check whether our negative results may be attributed to a particular characteristic of the *MRA* strain. As the *Berlin-K* standard strain is more sensitive than the *MRA* strain to the toxic effects of ECH, the concentration tested in our experiment was 25 mM. Due to the effects on survival and/or fertility, principally in the third brood, the number of chromosomes tested is not in keeping with the

Table I - Materials and Methods for sex-linked recessive lethals, sex-chromosome loss and non-disjunction tests.

Strains	MRA: Wild-type strain selected for resistance to malathion Basic: In(1) sc ^{S1L} sc ^{8R} + S, sc ^{S1} sc ⁸ w ^a B Ring-X: males with genotype R(1)2, y B / B ^S Y y ⁺ y sp : y w spl sn ³ ; bw sp ²
Culture medium	Standard food medium enriched with living yeast
Culturing temperature	Standard temperature (23-25°C)
Compound	Epichlorohydrin (1-chloro-2,3-epoxypropane, ECH) with a label purity of 97% was kindly provided by Productos Cruz Verde, S.A., Barcelona.
Preparation of the test solution	For ingestion treatments, ECH was dissolved in a 5% sucrose solution. For injection treatments ECH was dissolved in 0.7% NaCl
Route of administration	
Adult feeding	Males were treated for 48h in glass filter feeding units after 4h of starvation.
Adult injection	About 0.2 µl of solution was injected into the abdomen of the males. Flies were allowed to recover for 24h before mating.
Larval feeding	Third instar larvae were treated in 125-ml bottles containing 25 ml of culture medium; 1 ml of solution was added to the surface of the medium.
Mating scheme	
Lethal mutation test	Standard Basic scheme (Würgler <i>et al.</i> , 1984).
Sex-chromosome loss and non-disjunction tests	Standard Ring-X scheme (Valencia <i>et al.</i> , 1984).
Brooding scheme	
Lethal mutation test	For adult treatment, males were mated with three new virgin females for each of three broods (3, 2 and 2 days).
Sex-chromosome loss	For adult treatment, males were mated with new virgin females (5 ♂♂/7 ♀♀) for each of two broods (3 and 2 days). For larval treatment only one brood was used.
Non-disjunction test	As we treated larvae, only one brood was performed.

Table II - Frequencies of sex-linked recessive lethals in *Drosophila* male germ cells of the *MRA* insecticide-resistant strain exposed to epichlorohydrin following adult ingestion and adult injection treatments.

Route of administration	Conc. (mM)	Time (h)	Expt	Number of lethals/number of chromosomes tested (%)				Total ^a
				Brood 1 (3 days)	Brood 2 (2 days)	Brood 3 (2 days)		
Adult ingestion	0	48	-	3/1571 (0.19)	6/1768 (0.34)	2/1818 (0.11)	11/5157 (0.21)	
	5	48	1	1/ 859 (0.12)	3/ 865 (0.35)	1/ 851 (0.12)	5/2575 (0.19)	
			2	1/ 578 (0.17)	1/ 449 (0.20)	2/ 464 (0.43)	4/1491 (0.27)	
		1 + 2	2/1437 (0.14)	4/1314 (0.30)	3/1315 (0.23)	9/4066 (0.22)		
Adult injection	10	48	1	2/ 608 (0.33)	1/ 677 (0.15)	2/ 707 (0.28)	5/1992 (0.25)	
	35	48	2	2/ 658 (0.30)	3/ 557 (0.54)	2/ 551 (0.36)	7/1766 (0.40)	
			1 + 2	4/1266 (0.32)	4/1234 (0.32)	4/1258 (0.34)	12/3758 (0.32)	
	0	-	-	5/2775 (0.18)	4/1960 (0.20)	2/2315 (0.09)	11/7050 (0.16)	
Adult injection	35	-	1	2/ 677 (0.29)	0/ 484 (-)	0/ 538 (-)	2/1699 (0.12)	
			2	1/ 583 (0.17)	2/ 537 (0.37)	0/ 306 (-)	3/1426 (0.21)	
		1 + 2	3/1260 (0.24)	2/1021 (0.20)	0/ 844 (-)	5/3125 (0.16)		

^a Pooled data from three broods.

Differences between treatments and control are not significant (Kastenbaum and Bowman test).

guidelines of Lee *et al.* (1983). Nevertheless, and in spite of this limitation, the results from Table III constitute another indication of lack of mutagenicity of ECH in the SLRL test. ECH showed no significant mutagenic activity in any of our assays for gene mutations.

Table III - Frequencies of sex-linked recessive lethals in *Drosophila* male germ cells of the *Berlin-K* strain exposed to epichlorohydrin following adult injection treatment.

Conc. (mM)	Expt	Number of lethals/number of chromosomes tested (%)			
		Brood 1 (3 days)	Brood 2 (2 days)	Brood 3 (2 days)	Total ^a
0	-	3/1453 (0.21)	2/1002 (0.20)	2/987 (0.20)	7/3442 (0.20)
25	1	0/ 301 (-)	0/ 269 (-)	1/217 (0.46)	1/ 787 (0.12)
	2	2/ 707 (0.28)	2/ 547 (0.36)	0/265 (-)	4/1519 (0.26)
	1 + 2	2/1008 (0.20)	2/ 816 (0.24)	1/482 (0.21)	5/2306 (0.22)

^a Pooled data from three broods.

Differences between treatment and control are not significant (Kastenbaum and Bowman test).

Although it is scarcely likely that a chemical showing no mutagenicity in tests assaying for point mutations would induce chromosome damage, to complete this study we performed another series of experiments to test whether ECH can induce sex-chromosome loss (SCL) or non-disjunction (ND). In these assays we used the *Ring-X* strain because the detection of chromosome damage is facilitated by the presence of a ring-shaped chromosome. Table IV shows the extent of sex-chromosome loss induction after injection of adult males with 25 mM ECH, while the frequency of SCL and ND after treatment of third instar larvae with 30 and 50 mM ECH are summarized in Table V. Tables IV and V refer to treated post-meiotic and pre-meiotic germ cell stages, respectively. The analysis shows that the results are not significantly different from the controls indicating that ECH is also ineffective in producing total or partial sex-chromosome loss or non-disjunction, regardless of the treatment procedure or the germ cell stage treated.

The repeated negative results obtained in this work do not confirm the mutagenic activity of ECH in *Drosophila* previously reported by Rapoport (1948) and Knaap *et al.* (1982). This discrepancy may be due to differences in sensitivity to

Table IV - Frequencies of offspring resulting from sex-chromosome loss in *Drosophila* male germ cells of the *Ring-X* strain exposed to epychlorohydrin via adult injection.

Conc. (mM)	Brood	Regular offspring			Complete losses		Partial losses		Other ^a	Total
		♀♀	♂♂	S.R.	(No.)	(%)	(No.)	(%)		
0	1	2188	2018	1.08	33	0.77	0	—	8	4247
	2	2767	2284	1.21	70	1.36	1	0.02	7	5129
	1 + 2	4955	4302	1.15	103	1.10	1	0.01	15	9376
25	1	2194	2069	1.06	53	1.22	0	—	9	4325
	2	772	766	1.01	17	1.09	0	—	5	1560
	1 + 2	2966	2835	1.04	70	1.19	0	—	14	5885

^aIncludes phenotypes corresponding to spontaneous non-disjunction and mosaics.

S.R., sex-ratio.

Differences between treatment and control are not significant (Kastenbaum and Bowman test).

the genotoxic activity of ECH of the strains used, probably reflecting some differences in metabolizing activities. It is possible that the *Drosophila* strains used by us are more effective in the detoxification or conversion of ECH to less genotoxic active metabolites. It is interesting to point out that the mutagenicity of ECH can be decreased and even suppressed by the action of mammalian metabolizing systems in *Salmonella* (Andersen *et al.*, 1978; Voogd *et al.*, 1981), *Schizosaccharomyces* (Rossi *et al.*, 1983a) and human lymphocytes (White, 1980). Moreover, the *in vivo* studies on the genotoxicity, metabolism and blood kinetics of ECH in mice conducted by Rossi *et al.* (1983b) demonstrated that the failure of ECH to induce mutagenic effects is due to the rapid metabolic clearance of the compound, which leads to low target doses.

Summing up, in our opinion the negative results presented here cannot be taken to outweigh the earlier positives in *Drosophila* because positive/negative responses will be expected for ECH-type genotoxins under *in vivo* conditions.

Table V - Frequencies of offspring resulting from sex-chromosome loss and non-disjunction in *Drosophila* male germ cells of the Ring-X strain exposed to epichlorohydrin by feeding of third-instar larvae.

Expt.	Conc. (mM)	Regular offspring		Complete losses		Partial losses		Non-disjunction		Other ^a	Total
		♀	♂	(No.)	(%)	(No.)	(%)	(No.)	(%)		
I	0	639	696	16	1.18	0	—	1	0.07	1	1353
	30	1001	903	24	1.24	0	—	2	0.10	2	1932
	0	275	283	7	1.24	0	—	0	—	0	565
	50	377	298	8	1.17	0	—	2	0.29	0	685
II	0	669	721	16	1.14	0	—	1	0.07	1	1408
	30	833	811	24	1.44	0	—	0	—	1	1699
	0	1053	1071	27	1.25	0	—	5	0.23	2	2158
	50	1318	892	29	1.29	0	—	1	0.04	2	2242
I + II	0	1308	1417	32	1.17	0	—	2	0.07	2	2729
	30	1834	1714	48	1.33	0	—	2	0.06	3	3601
	0	1328	1354	34	1.25	0	—	5	0.18	2	2723
	50	1695	1190	37	1.26	0	—	3	0.10	2	2927

^a Includes phenotypes corresponding to spontaneous female non-disjunction and mosaics.

S.R., sex-ratio.

Differences between treatments and control are not significant (Kastenbaum and Bowman test).

ACKNOWLEDGMENTS

We thank Productos Cruz Verde, S.A., Barcelona, for generously supplying the epichlorohydrin batch. This investigation was supported in part by the Spanish Ministry of Education and Science (Grant No. 0577/84 CAICYT). One of us (A. Velázquez) was supported during this work by a fellowship (F.P.I.) from the same Ministry.

RESUMO

Foi estudado o possível efeito mutagênico do epichlorohydrin (ECH) sobre as células germinativas de machos de *Drosophila melanogaster*.

A alimentação e a injeção de adultos com ECH não foram capazes de induzir um aumento significativo na frequência de mutações letais recessivas ligadas ao sexo em uma linhagem resistente a inseticida. A injeção dos adultos de uma linhagem sensível também não teve efeito.

Além do mais, resultados negativos foram obtidos quando ECH foi testado para a capacidade de induzir a perda do cromossomo do sexo e a não-disjunção.

REFERENCES

- Andersen, M., Kiel, P., Larsen, H. and Maxild, J. (1978). Mutagenic action of aromatic epoxy resins. *Nature* 276: 391-392.
- Batiste-Alentorn, M., Xamena, N., Velázquez, A., Creus, A. and Marcos, R. (1986). Mutagenicity testing of the pyrethroid insecticide cypermethrin in *Drosophila*. *Mutagenesis* 1: 343-346.
- Bridges, B.A. (1981). Comparative mutagenicity of epichlorohydrin and cadmium. In: *Comparative Chemical Mutagenesis* (F.J. de Serres and M.D. Selby, eds.). Plenum Press, New York, pp. 1015-1037.
- Dean, B.J. and Hodson-Walker, G. (1979). An *in vitro* chromosome assay using cultured rat-liver cells. *Mutation Res.* 64: 329-337.
- IARC (1976). Epichlorohydrin. *IARC Monographs on the evaluation of carcinogenic risk of chemicals to humans* 11: 131-139.
- Knaap, A.G.A.C., Voogd, C.E. and Kramers, P.G.N. (1982). Comparison of the mutagenic potency of 2-chloroethanol, 2-bromoethanol, 1,2-epoxybutane, epichlorohydrin and glycidaldehyde in *Klebsiella pneumoniae*, *Drosophila melanogaster* and L5178Y mouse lymphoma cells. *Mutation Res.* 101: 199-208.
- Køhlmark, G. and Giles, N.H. (1955). Comparative studies of monoepoxides as inducers of reverse mutations in *Neurospora*. *Genetics* 40: 890-902.
- Kucerová, M., Zhurkov, V.S., Polivková, Z. and Ivanova, J.E. (1977). Mutagenic effect of epichlorohydrin. II. Analysis of chromosomal aberrations in lymphocytes of persons occupationally exposed to epichlorohydrin. *Mutation Res.* 48: 355-360.
- Lee, W.R., Abrahamson, S., Valencia, R., Von Halle, E.S., Wurgler, F.E. and Zimmering, S. (1983).

- The sex-linked recessive lethal test for mutagenesis in *Drosophila melanogaster*. *Mutation Res.* 123:183-279.
- Lindsley, D.L. and Grell, E.H. (1968). Genetic variations of *Drosophila melanogaster*. *Carnegie Institution of Washington Publ.* 627, Washington DC, 472 pp.
- McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. *Proc. Natl. Acad. Sci.* 72: 5135-5139.
- Migliore, L., Rossi, A.M. and Loprieno, N. (1982). Mutagenic action and metabolic conversion of a series of alkene-oxides in the yeast *S. pombe* assay. *Mutation Res.* 102:425-437.
- Picciano, D. (1979). Cytogenetic investigation of occupational exposure to epichlorohydrin. *Mutation Res.* 66: 169-173.
- Rapoport, I.A. (1948). Mutagenic effects of ethylene-glycol, glycidol and epichlorohydrin. *Genetika* 60: 469-472.
- Rossi, A.M., Migliore, L., Loprieno, N., Romano, M. and Salmona, M. (1983a). Evaluation of epichlorohydrin (ECH) genotoxicity. Microsomal epoxide hydrolase-dependent deactivation of ECH mutagenicity in *Schizosaccharomyces pombe* in vitro. *Mutation Res.* 109:41-52.
- Rossi, A.M., Migliore, L., Lascialfari, D., Sbrana, I., Loprieno, N., Tortoretto, M., Biololi, F. and Pantarotto, C. (1983b). Genotoxicity, metabolism and blood kinetics of epichlorohydrin in mice. *Mutation Res.* 118: 213-226.
- Srám, R.J., Tomatis, L., Clemmensen, J. and Bridges, B.A. (1981). An evaluation of the genetic toxicity of epichlorohydrin. *Mutation Res.* 87: 299-319.
- Valencia, R., Abrahamson, S., Lee, W.R., Von Halle, E.S., Woodruff, R.C., Würgler, F.E. and Zimmering, S. (1984). Chromosome mutation test for mutagenesis in *Drosophila melanogaster*. *Mutation Res.* 134: 61-88.
- Velázquez, A., Creus, A., Xamena, N. and Marcos, R. (1984). Mutagenicity of the insecticide endosulfan in *Drosophila melanogaster*. *Mutation Res.* 136: 115-118.
- Velázquez, A., Xamena, N., Creus, A. and Marcos, R. (1986). Indication for weak mutagenicity of the organophosphorus insecticide dimethoate in *Drosophila melanogaster*. *Mutation Res.* 172: 237-243.
- Velázquez, A., Creus, A., Xamena, N. and Marcos, R. (1987). Lack of mutagenicity of the organophosphorus insecticide malathion in *Drosophila melanogaster*. *Environ. Mutagen.* 9: 343-348.
- Voogd, C.E., van der Stel, J.J. and Jacobs, J.J.J.A.A. (1981). The mutagenic action of aliphatic epoxides. *Mutation Res.* 89: 269-282.
- Wade, D.R., Airy, S.C. and Sinsheimer, J.E. (1978). Mutagenicity of aliphatic epoxides. *Mutation Res.* 58: 217-223.
- Wade, M.J., Moyer, J.W. and Hine, C.H. (1979). Mutagenic action of a series of epoxides. *Mutation Res.* 66: 367-371.
- White, A.D. (1980). In vitro induction of SCE in human lymphocytes by epichlorohydrin with and without metabolic activation. *Mutation Res.* 78: 171-176.
- Würgler, F.E., Sobels, F.H. and Vogel, E. (1984). *Drosophila* as assay system for detecting genetic

changes. In: *Handbook of Mutagenicity Test Procedures* (B.J. Kilbey, M.S. Legator, W. Nichols and C. Ramel, eds.). 2nd Edn., Elsevier, Amsterdam, pp. 555-641.

(Received November 29, 1988)