Bull. Environm. Contam. Toxicol. 25, 513-517 (1980)

Mutagenic and Embryotoxic Effects of Paraquat and Diquat

A. Selypes, L. Nagymajtényi, and G. Berencsi
Institute of Public Health and Epidemiology, University Medical School, Szeged, H-6720 Hungary

In our previous report on the in vivo cytogenic effect of paraquat, it was concluded that the compound had no mutagenic effect on the bone marrow cells of the mouse (SELYPES et al. 1978). In the first part of this study we investigated whether diquat would have a mutagenic effect under similar experimental circumstances, and in the second part, we wanted to establish whether the two herbicides, paraquat and diquat, would cause congenital malformations and damage the chromosomes of the embryonic liver cells because, according to published literature data (KHERA et al. 1968, BUS et al. 1975) in the presence of certain parameters they have an embryotoxic effect through the placenta.

MATERIAL AND METHOD

1) For the mutagenic experiments, CFLP strain male mice with an average weight of 35 g were used. The diquat-containing herbicide Reglone (20% diquat content) was administered per os and also i.p. to groups of 20 animals each.

a) As for the i.p. dosage, the following process was maintained:

In the first group a dose of i.p. LD50, that is 22 mg/kg of herbicide, was injected into each animal, and after 24 h the preparation of the bone marrow was performed. In the other groups, doses of 1/3 (7.3 mg/kg), 1/6 (3.6 mg/kg) and 1/30 of LD50 (0.73 mg/kg) were injected every other day on 2, 2 and 5 occasions, respectively 24 h after the last injection the preparation of the bone marrow was performed.

b) The per os treated animals, were given half of the LD50 dose, that is 90.0 mg/kg of diquat, and the preparation of the bone marrow was performed after 48 h.

c) For control, 10 non-treated animals were used.

Preparation of the bone marrow, both in the treated and the non-treated groups, was performed according to the method described by VOGEL & RÖHRBORN (1970). 10-10 mitoses, that is 100-100 cells per group, were evaluated according to the Edinburgh classification.

2) During the teratogenic experiments CFLP strain, nullipara female mice were used in groups of 20.

a) On the 9th day of gravidity, the animals were once given i.p. half of the LD50 dose, that is 8 mg/kg of paraquat-containing Gramoxone.

0007-4861/80/0025-0513 $01.00

b) In this group, on the 9th day of gravidity, the animals were once given i.p. half of the LD50 dose, that is 11 mg/kg of diquat-containing Reglone.

c) In this group, on the 9th, 10th, 11th and 12th days of gravidity, the animals were given once each time i.p. the 1/8 of the LD50 dose, that is 2 mg/kg paraquat.

d) In this group, on the same days of gravidity as in group c), the animals were given once each time i.p. 1/8 of the LD50 dose, that is 2.7 mg/kg diquat.

e) In this control group, on the same days of gravidity as in groups c) and d), the animals were given i.p. a physiological saline solution. The preparation of the embryos was performed on the 18th day of gravidity. Besides counting both the live and dead foetuses, the postimplantation values of DL were also determined, according to the formula of SEARLE (1963). After weighing, each embryo was examined. Half of the embryos of each mother animal were fixed in Bouin solution and then dissected under stereomicroscope. The other half were dyed with Alizarin red-S by the method of STAPLES & SCHNELL (1963) for skeletal examination. Of the livers of these embryos chromosome preparations were made by the method of DATTA et al. (1970). Of each group 10-10 mitoses of 10-10 embryos were evaluated and 10% of the mitoses were karyotypified.

RESULTS AND DISCUSSION

In Table 1 the effect of the diquat containing herbicide is shown on the chromosomes of the bone marrow of the mouse. Altogether 5 to 6% chromosome changes were found in the cells of the mice treated with various doses, and this finding, compared to the values obtained from the control animals ($p>0.05$), is not significant. All the aberrations were chromatid types and restricted to a gap not symptomatic.

TABLE 1. Chromosome Aberrations in Mouse Bone-Marrow Cells After Diquat Treatment.

Diquat i.p. mg/kg	Number of Evaluated Cells	Cells with Aberrations %	Type of Aberrations %
1 x 22.0	100	6	6 gap
2 x 7.3	100	6	6 gap
2 x 3.6	100	5	5 gap
5 x 0.73	100	5	5 gap
Diquat p.o. mg/kg			
1 x 90.0	100	6	6 gap
Control	100	8	8 gap

Tables 2 and 3 show the data of the teratogenic examinations. In Table 2 it can be seen that in two paraquat treated groups the number of dead and reabsorbed foetuses and the average embryonic weight agrees with those in the control group, while in the diquat treated groups the number of dead foetuses increased as well as the postimplantation DL value. The average embryonic weight, however, decreased as the number of embryos retarded in weight increased. In the course of the outward and the dissective examinations, congenital malformations were not observed. When examining the skeleton of the embryos dyed with Alizarin red-S, the following retardation symptoms were sought: large fontanelles, wider cerebral sutures, flat and dumbbell shaped ventral nuclei of the vertebrae, missing one or more ossification points in the sternum, missing ossification of the nuclei in the metatarsals and the phalanges. Paraquat did not cause any change in the treated groups compared to the control. Diquat caused retardation in the embryos of females treated repeatedly with smaller doses; changes in the skull, vertebrae, sternum and the limbs were all observed. This can be explained with the embryotoxic effect of diquat as reported in the literature. It is significant because it is thought to be less toxic and so less dangerous than paraquat. Table 4 shows the data obtained during the examination of the embryonic liver cells. It can be seen that in the embryos of both the paraquat and diquat treated females, the ratio of changed cells increased, though not significantly. Except in one case, the changes were less symptomatic chromatid type gaps.

In our experiments it was found that in the liver cells of the embryos paraquat and diquat was not mutagenic, however diquat had an embryotoxic effect on the bone structure of mice.

TABLE 2. Effect of Paraquat and Diquat Treatment on the Embryos of Mice.

Pesticide i.p. mg/kg	Day of Gravidity at Treatment	No. of Embryos		Postimplant Domin.Lethal. %	Avg. Embryon. weight,g
		Living	Dead or Resorped		
Paraquat 1 x 8.0	9	50	3	0	1.27±0.03
Paraquat 4 x 2.0	9-12	50	4	2	1.25±0.02
Diquat 1 x 11.0	9	50	8	9	1.08±0.04
Diquat 4 x 2.7	9-12	50	9	11	0.90±0.07
Control	9 9-12	50	3	-	1.30±0.02

TABLE 3. Data Obtained During Examination of Alizarin Red-S Dyed Embryos (Embryotoxicity).

Pesticide i.p. mg/kg	Number of Examined Embryos	Number of embryos with Changes	Retardation Symptoms			
			Sternum	Vertebra	Skull	Limbs
Paraquat 1 x 8.0	50	1	1	-	-	0
Paraquat 4 x 2.0	50	1	1	-	-	1
Diquat 1 x 11.0	50	6	2	-	-	6
Diquat 4 x 2.7	50	13	4	2	5	13
Control	50	1	1	-	-	0

TABLE 4. Chromosome Aberrations in the F_1 Embryos After the i.p. Treatment of the Female Parent with Pesticide.

Pesticide i.p. mg/kg	Number of Embryos	Number of cells Examined	Cells with Aberrations %	Aberrations in Number %	Chromatid Type Aberrations %
Paraquat 1 x 8.0	10	100	5	-	5
Paraquat 4 x 2.0	10	100	7	-	7
Diquat 1 x 11.0	10	100	9	-	9
Diquat 4 x 2.7	10	100	10	1	9
Control	10	100	2	-	2

REFERENCES

ANDERSON, D.: Mutat. Res. 31, 349 (1976).

BUS, J.S., M.M. PREACHE, S.Z. CAGEN, H.S. POSNER, B.C. ELIASON, C.W. SHARP, J.E. GIBSON: Toxicol. Appl. Pharmacol. 33, 450 (1975).

DATTA, P.K., H. FRIGGER, E. SCHLEIERMACHER: Chemical Mutagenesis in Mammals and Man, Springer-Verlag, Berlin-Heidelberg-New York (1970) p. 194-213.

EDWARDS, R.G., A.G. SEARLE: Genet. Res. 4, 389 (1963).

FENNELLY, J.J., J.T. GALLAGHER, R.J. CARROL: Brit. Med. J. 3, 722 (1968).

KHERA, K.S., L.L. WHITTA, D.J. CLEGG: Ind. Med. Surg. 37, 257 (1968).

SELYPES, A., M. NEHÉZ, A. PÁLDY, L. NAGYMAJTÉNYI, G. BERENCSI: Egészségtud. 22, 277 (1978).

VOGEL, F., G. RÖHRBORN: Chemical Mutagenesis in Mammals and Man, Springer-Verlag, Berlin-Heidelberg-New York (1970).