Rev. Int. Contam. Ambient. 6, 69-74, 1990.

CYTOGENETIC EFFECTS PRODUCED BY THE UREIC HERBICIDES DIURON AND LINURON IN *Vicia faba* AND HUMAN LYMPHOCYTES CULTURES

Sandra GÓMEZ - ARROYO, Josefina CORTÉS - ESLAVA, Armando RAMÍREZ - DOMÍNGUEZ, Alfredo GUTIÉRREZ - ABAD and Rafael VILLALOBOS - PIETRINI

Laboratorio de Citogenética y Mutagénesis Ambientales, Centro de Ciencias de la Atmósfera, UNAM, Coyoacán 04510 D.F., México

(Recibido julio 1990, aceptado octubre 1990)

Keywords: sister chromatid exchanges, ureic herbicides, diuron, linuron, *Vicia faba*, human lymphocytes

ABSTRACT

In order to evaluate the cytogenetic effect of diuron and linuron, sister chromatid exchanges (*SCE*) in cells of *Vicia faba* root tips and in human lymphocytes in culture were used as test systems. Both herbicides did not induce *SCE* in these biological materials except at the lowest concentration of linuron (10 ppm) which produced a significant response only in *Vicia*.

RESUMEN

Se verificó el efecto citogenético de los herbicidas uréicos diurón y linurón, mediante el análisis de intercambio de cromátidas hermanas (*ICH*) en las células de las puntas de las raíces de *Vicia faba* y en el cultivo de linfocitos humanos, usados como sistemas de prueba. Ambos herbicidas no indujeron *ICH* en los materiales biológicos empleados excepto el linurón a la concentración menor (10 ppm) que produjo respuesta significativa solamente en *Vicia*.

INTRODUCTION

The ureic herbicides are derived from urea ($H_2NC(=O)NH_2$). They are solid crystals with low solubility in water (Metcalf 1971, Barberá 1976). It seems that the herbicide activity is a general property of the phenyl-N-N-dimethylurea structure. The phenyl nuclei can be substituted by chloro or nitro groups and its activity increased with ortho or para substitution (Metcalf 1971).

It is considered that the ureic herbicides disturb photosynthesis in which the primary site of action is located in the photosystem II within, or near to, the phase envolving oxygen (Asthon and Alden 1973).

Diuron (3-(3, 4-dichlorophenyl)-1-1-dimethylurea) and linuron (3-(3, 4-dichlorophenyl)-1-methoxi-1-methylurea) are ureic herbicides.

Diuron is also known by its trade names karmex, marmer, DCMU, di-on, karmex-DL and sup'rflo. It is a compound of substituted ureas used as a herbicide in pre- and post-emergent applications for such crops as cotton, apples, papaw, peas, alfalfa, bananas,

pineapples, etc. In mice it caused irritability of the eyes and skin and the LD_{50} was 3400 mg/kg (Thomson 1975, Barberá 1976). In protein deficient rats, diuron caused cholinergic stimulation, depression of the central nervous system, local gastroenteritis, organ inflammation and degenerative changes in the kidney, liver and pancreas (Boyd and Krupa 1970).

In relation to genetic effects, diuron did not induce mitotic gene conversion in yeast (Siebert and Lemperle 1974), however it produced diuron resistant mutants in *Anacystis nidulans R2* (Golden and Haselkorn 1985), depressed thymidine incorporation in testicular DNA of mice and in bacterial test demonstrated weak mutagenic activity (Seiler 1978). In some microbial systems (*Salmonella* and *Escherichia*) diuron did not induce mutations (Moriya *et al.* 1983) and in the micronucleus test in mice it was inactive also (Seiler 1978).

Linuron, with the trade names of lorox, afalon, prefalon, and sarklex, is a substituted urea, used for pre- and post-emergent applications in crops of potatoes, cereals, sorghum, cotton, celery, etc. At higher concentrations linuron is employed as soil sterilizer. In mice, linuron causes irritation in the eyes, nose and skin and its LD_{50} is 1500 mg/kg (Thomson 1975, Barberá 1976).

Linuron is a compound that cytologically prevented cell wall formation (Grant 1973), in maize induced chlorophyll mutations (Morgun 1982), in mice inhibited thymidine incorporation into testicular DNA (Seiler 1978), and chomosome clumping (Grant 1973). However in bacterial systems linuron did not induce mutations (Andersen *et al.* 1972, Marshall *et al.* 1976, Seiler 1978, Moriya *et al.* 1983) nor induced micronuclei in mice (Seiler 1978).

Due to the fact that these herbicides are widely used in México and because of the few studies and the contradictory results obtained on their effect on the genetic material, in this study the cytogenetic damage of these two herbicides is analysed by means of the sister chromatid exchange (*SCE*) assay which is very sensitive in evaluating the genetic activity of chemicals (Perry and Evans 1975). For this purpose root tip meristems of *Vicia faba* and human lymphocytes in culture have been used, as the *SCE* assay has been shown to be most useful for this type of study (Gómez-Arroyo *et al.* 1987, 1988).

MATERIAL AND METHODS

Vicia faba

Seeds were germinated between two cotton layers wetted with tap water. The primary roots that reached 2-3 cm in length were introduced into a solution containing 100 μM 5-bromo-2'deoxyuridine (BrdUrd), 0.1 μM 5-fluorodeoxyuridine (FdUrd) and 5 μM of uridine (Urd) for 20 h. Then they were treated for 2 h in solutions containing the following concentrations of diuron (Dupont) and linuron (Helios): 10, 20, 40, 50, 75, 100, 150 and 300 ppm. Fresh solutions of BrdUrd, FdUrd and Urd were applied for a second replicate cycle (20 h). The treatments were carried out in the dark at 19°C. Controls were exposed to distilled water and underwent the same procedure.

After this, the meristems were cut and treated with colchicine 0.05% for 3 h and stained using the Feulgen differential technique described by Tempelaar *et al.* (1982)

modified as follows: cuttings were fixed with glacial acetic acid for 1 h, then put in ethanol-acetic acid (3:1) for 2 days at −20°C and later in ethanol 70% for 15 min and after hydrolysis in 5N HCl for 80 min at 28°C. They were then washed 3 times with distilled water and stained with the Schiff reagent (Feulgen staining) for 12 min in the dark. Then cuttings were treated with pectinase 2% disolved in 0.01M citrate buffer (pH 4.7) for 15 min at 28°C, followed by acetic acid 45% for 10 min and then finally transferred to cold ethanol 70% for 30 min.

The squash was done in 45% acetic acid and the slides were made permanent by the dry-ice technique (Conger and Fairchild 1953), dehydrated by means of two changes in absolute butanol and then mounted in Canada balsam.

Human lymphocytes

Cultures of peripheral blood of healthy individuals were made using the same donor for each experiment and its replicate.

Eight drops of blood were put on a culture glass having 3 ml of McCoy's 5A (Microlab) plus 0.2 ml of phytohemagglutinin (Gibco). This was incubated at 37°C for 24 h then BrdUrd was added to the culture medium at a final concentration of 5 μg/ml simultaneously with the corresponding concentrations of diuron and linuron (10, 20, 40, 50, 75, 100, 150 and 300 ppm). 70 h after the begining of the culture, 0.04 μg/ml of colchicine (Merck) was added and two hours later the cells were harvested by centrifugation and the pellet resuspended in hypotonic solution of 0.75M KCl for 20 min. Cells were again centrifuged and finally fixed in methanol-acetic acid (3:1). The slides were made by placing a drop of cell suspension onto the slide and allowing this to dry in air and were immersed for 20 min in a solution of 0.05 μg/ml of Hoechst-33258 in deionized water in the dark, then rinsed and mounted in phosphate buffer of 6.8 pH with a coverslip, then irradiated with UV light for one hour, after removal of the cover-glass, slides were stained in Giemsa distilled water (1:50) for 30 min.

The scoring of *SCE* was made on 25 metaphase cells for each experiment and its replicate in both *Vicia faba* and human lymphocytes. Statistical analysis was applied by means of the Student *t* test. Slides were manipulated in code so that it was not known to which group they belonged.

RESULTS AND DISCUSSION

There were no significant differences in the data obtained with diuron in *SCE* frequencies in *Vicia faba* (Table I) as well as in human lymphocytes (Table III). These negative results were in agreement with those reported for mitotic gene conversion using the *loci ade* 2 *trp* 5 of *Saccharomyces cerevisiae* (Siebert and Lemperle 1974), the mutants *rII* of the T_4 bacteriophage (Anderson *et al.* 1972), mutations in *Salmonella typhimurium* and *Escherichia coli* (Moriya *et al.* 1983) and micronuclei data from mice erythrocytes (Seiler 1978). However, diuron was also reported to strongly inhibit testicular DNA synthesis in mice (Seiler 1978), induce sex-linked recessive lethal mutations in *Drosophila melanogaster* (Rodríguez-Arnaiz *et al.* 1989), mutation to resistance in *Anacystis nidulans*, mutation of *Salmonella typhimurium* in the presence of mammalian microsomes (Seiler

TABLE I. SISTER CHROMATID EXCHANGES (*SCEs*) INDUCED BY DIURON IN *Vicia faba*†

Treatment (ppm)	*SCEs/cell* $\bar{X} \pm SE$	*t value*
0	28.62 ± 0.61	
20	29.34 ± 0.67	0.560 NS
40	28.68 ± 0.75	0.044 NS
50	29.12 ± 0.70	0.370 NS
100	31.22 ± 0.86	1.768 NS
150	26.82 ± 0.73	1.340 NS
300	28.46 ± 0.47	0.088 NS

† n = 50 metaphases in 2 experiments
NS not significant

TABLE II. SISTER CHROMATID EXCHANGES (*SCEs*) INDUCED BY LINURON IN *Vicia faba*†

Treatment (ppm)	*SCEs/cell* $\bar{X} \pm SE$	*t value*
0	28.36 ± 0.53	
10	33.07 ± 0.59	4.20*
20	Cellular death	

† n = 50 metaphases in 2 experiments
* $p < 0.001$

TABLE III. SISTER CHROMATID EXCHANGES (*SCEs*) INDUCED BY DIURON IN HUMAN LYMPHOCYTES *in vitro*†

Treatment (ppm)	*SCEs/cell* $\bar{X} \pm SE$	*t value*
0	5.56 ± 0.27	
20	5.96 ± 0.31	0.69 NS
40	7.10 ± 0.43	2.19 NS
50	7.78 ± 0.43	3.15 NS
75	7.48 ± 0.38	2.93 NS
100	5.64 ± 0.27	0.16 NS
150	6.24 ± 0.39	1.02 NS
300	6.30 ± 0.20	1.52 NS

† n = 50 metaphases in 2 experiments
NS not significant

TABLE IV. SISTER CHROMATID EXCHANGES (*SCEs*) INDUCED BY LINURON IN HUMAN LYMPHOCYTES *in vitro*†

Treatment (ppm)	*SCEs/cell* $\bar{X} \pm SE$	*t value*
0	6.78 ± 0.34	
10	6.88 ± 0.31	0.19 NS
20	Cellular death	

† n = 50 metaphases in 2 experiments
NS not significant

1978, Golden and Haselkorn 1985), and mutation for resistance in *Saccharomyces cerevisiae* (Meunier and Colson 1989).

Linuron was tested from a low of 10 ppm up to 300 ppm, but it was found that 20 ppm was very toxic and induced cell death. In *Vicia faba*, 10 ppm produced a significant level of *SCE* response (Table II). However, the same concentration did not induce *SCE* in human lymphocytes *in vitro*.

Andersen *et al.* (1972), Marshall *et al.* (1976) and Moriya *et al.* (1983) found negative results in *Salmonella typhimurium*, but on the other hand, Seiler (1978) described inhibition of testicular DNA synthesis in mice, Wuu and Grant (1966) observed mutations in *Hordeum vulgare* and Morgun (1982) induced chlorophyll mutations in maize.

The fact that linuron produced genetic alterations only in plants is possibly due to promutagen activations caused by plant metabolic processes (Plewa *et al.* 1988). This could originate from the microsomal fraction *S-10* described in *Vicia faba*, which acted in a similar way to mammalian liver *S-9* (Takehisa and Kanaya 1983, Takehisa *et al.* 1988).

ACKNOWLEDGEMENTS

We thank Norma Noriega, Biol. Miguel Angel Meneses and M. en C. Ana Rosa Flores for their wortwhile technical collaboration.

REFERENCES

Andersen K.J., Leighty E.G. and Takahashi M.T. (1972). Evaluation of herbicides for possible mutagenic properties. J. Agric. Food Chem. *20*, 649-656.

Asthon F.M. and Alden S.C. (1973). *Mode of action of herbicide*. Wiley, New York. pp. 10-24, 367-393.

Barberá C. (1976). *Pesticidas agrícolas*. Omega, Barcelona, pp. 399-412.

Boyd E.M. and Krupa V. (1970). Protein deficient diet diuron toxicity. J. Agr. Food Chem. *18*, 1104-1107.

Conger A.D. and Fairchild L.M. (1953). A quick-freeze method for making smear slides permanent. Stain Technol. *28*, 281-283.

Golden S.S. and Haselkorn R. (1985). Mutation to herbicide resistance maps within the *psbA* gene of *Anacystis nidulans R2*. Science *229*, 1104-1107.

Gómez-Arroyo S., Noriega-Aldana N., Juárez-Rodríguez D. and Villalobos-Pietrini R. (1987). Sister chromatid exchanges induced by the organophosphorus insecticides methyl parathion, dimethoate, phoxim and methyl azinfos in cultured human lymphocytes. Contam. Ambient. *3*, 63-70.

Gómez-Arroyo S., Castillo-Ruiz P., Cortés-Eslava J. and Villalobos-Pietrini R. (1988). *Vicia faba*-sister chromatid exchanges of the organophosphorus insecticides methyl parathion, dimethoate, oxydemeton methyl, azinphos methyl and phoxim. Cytologia *53*, 627-634.

Grant W.F. (1973). Cytological effects of environmental mutagens-pesticides. Mutat. Res. *21*, 221-222.

Marshall T.C., Dorough H.W. and Swim H.E. (1976). Screening of pesticides for mutagenic potential using *Salmonella typhimurium* mutants. J. Agric. Food Chem. *24*, 560-563.

Metcalf L.R. (1971). The chemistry and biology of pesticides. In: *Pesticides in the environment*. Vol. 1 part. 1, M. Dekker, New York. pp. 49-51.

Meunier B. and Colson A.M. (1989). Increased diuron resistance in the joint expression of mutations located at the *DIV2, DIV3* and *DIV4 loci* of *Saccharomyces cerevisiae*. Curr. Genet. *15*, 121-127.

Morgun V.V. (1982). Cytogenetic and genetic activity of herbicides: atrazin, simazin, prometrin and linuron. Tsitol. Genet. *16*, 33-41.

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

Perry P. and Evans H.J. (1975). Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. Nature *258*, 121-124.

Plewa M.J., Wagner E.D. and Gentile J.M. (1988). The plant/microbe coincubation assay for the analysis of plant-activated promutagens. Mutat. Res. *197*, 207-219.

Rodríguez-Arnaiz R., Ramos Morales P., Gaytán Oyarzun J.C., Rodríguez Zúñiga D.L. and Zimmering S. (1989). The herbicides dalapon and diuron tested for genotoxicity in *Drosophila melanogaster*. Rev. Int. Contam. Ambient. *5*, 59-64.

Seiler J.P. (1978). Herbicidal phenylalkylureas as possible mutagens. I. Mutagenicity tests with some urea herbicides. Mutat. Res. *58*, 353-359.

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

Takehisa S. and Kanaya N. (1983). A comparison of *Vicia faba*-root *S10* and rat-liver *S9* activation of ethanol, maleic hydrazide and cyclophosphamide as measured by sister chromatid exchanges induction in Chinese hamster ovary cells. Mutat. Res. *45*, 263-270.

Takehisa S., Kanaya N. and Rieger R. (1988). Promutagen activation by *Vicia faba*: an assay based on the induction of sister-chromatid exchanges in Chinese hamster ovary cells. Mutat. Res. *197*, 195-205.

Tempelaar M.J., de Both M.T.J. and Versteegh J.E.G. (1982). Measurement of *SCE* frequencies in plants: a simple Feulgen staining procedure for *Vicia faba*. Mutat. Res. *103*, 321-326.

Thomson W.T. (1975). *Agricultural chemicals books. II. Herbicides.* Thomson Ed., New York. pp. 157-190.

Wuu K.D. and Grant W.F. (1966). Morphological and somatic chromosomal aberrations induced by pesticides in barley (*Hordeum vulgare*). Can. J. Genet. Cytol. *8*, 481-501.