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MICRONUCLEI INDUCED IN *Tradescantia* BY ARSENIC

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Arsenic is emited into the environment from the industrial exhaust of factories and agricultural practices and by natural processes from the erosion of the soil, the surface of the earth, and from volcanic rocks.

The main source of human exposure to organic and inorganic arsenic arises from different foods, drinking water and from various medicines (Léonard and Lauwerys 1980), producing hyperkeratosis, anemia, nasal septum perforation, leucoderma, lung cancer and death (Tsuchiya 1977).

The toxicity of arsenic is related to its valency and concentration (Paton and Allison 1972, Savory and Fedor 1977), the most common form in the environment is the pentavalent, although the trivalent is the most toxic.

One of the most valuable biological test system to determine the genetic damage induced by pollutants is the *Tradescantia* micronucleus assay in which micronuclei (*MCN*) are scored in the tetrad state (Villalobos-Pietrini *et al.* 1986). The microsporogenic cycle is well known (Sax and Edmonds 1933, Taylor 1950). In this study the capacity of potassium arsenite to induce *MCN* has been tested at the following concentrations: 1, 10, 1×10^2, 1×10^3, 5×10^3, 1×10^4, 2×10^4 and 3×10^4 mg/L.

For the study, 225 vigorous plants of *Tradescantia* clone 4430 with young inflorescences were selected; the stems were cut 6 cm below the inflorescences and they were grouped into 9 sets with 25 cuttings each.

The treatments were made by exposing the stem cuttings with the young inflorescences to the different concentrations of potassium arsenite for 30 hours, followed by a 6-hour recovery period in tap water. The inflorescences were fixed in ethanol acetic acid (3:1) for 48 h and transferred to 70% ethanol where they could be maintained in a refrigerator indefinitely until required.

Observations were made by using a dissecting microscope. From the inflorescences, buds were selected 2.0 to 3.5 mm in size by means of a micrometer escale. The anthers were removed and dissected on a slide to which a drop of 0.5% acetocarmine was added to liberate the pollen mother cells. When cells in the tetrad stage were found, the debris was removed and a cover slip was placed over the cells. Slides were warmed by means of an

alcohol lamp. To prevent a slide from becoming overheated, the temperature was controlled by placing the slide on the back of the hand.

The slides were stored in the freezer for 7 days and were made permanent following the technique of Conger and Fairchild (1953). In each of the 9 sets, 1500 tetrads were observed, scoring the frequency of *MCN* per 100 tetrads.

The formation of *MCN* involve the induction of acentric fragments, chromosomes with inactivated centromere and isochromosomes which remain out of the normal kinetics of anaphase. These chromosomal entities persist in tetrad cells where they are seen as small nuclei.

The data obtained were the result of an experiment and its replica. The difference of proportions test (Spiegel 1970) was used to compare the frequencies obtained with each concentration and its respective control.

Although it has been shown that arsenic accumulates in higher amounts in roots than in stems and that only a small quantity reaches the reproductive structures (Walsh *et al.* 1977), in these experiments the root barrier was avoided because plants cuttings were used.

Concentrations of 1, 10, 1×10^2, and 1×10^3 mg/L of potassium arsenite induced *MCN* in tetrads beyond twice that of the controls (Table I). Concentrations of 5×10^3, 1×10^4, 2×10^4 and 3×10^4 mg/L resulted in withering of the stems, leaves and inflorescences which were in general greater with higher concentrations. Although the data were significant in all cases, the genetic damage was less than that observed in the former concentrations. It is possible that flaccidity and constrictions of the stems as well as necrotic areas in the bases of the inflorescences caused by arsenic preventing the chemical reaching of the pollen mother cells in the flowers. This damage appeared in the treatments and gradually became more severe with time.

TABLE I. MICRONUCLEI OBSERVED IN TETRADS OF *Tradescantia* AFTER 30 HOURS OF TREATMENT WITH DIFFERENT CONCENTRATIONS OF POTASSIUM ARSENITE AND 6 HOURS OF RECOVERY

Concentrations (mg/L)	*Total of tetrads*	*MCN/100 tetrads* $\bar{X} \pm S.D.$	*"z" values*
0	10410	2.55 ± 0.36	
1	3000	6.43 ± 0.42	10.21*
10	3000	7.09 ± 0.23	11.95*
1×10^2	3000	6.80 ± 0.37	11.18*
1×10^3	3000	5.53 ± 0.75	8.28*
5×10^3	3000	3.30 ± 0.32	2.27*
1×10^4	3000	3.53 ± 0.56	2.88*
2×10^4	3000	3.60 ± 0.56	3.08*
3×10^4	3000	3.33 ± 0.19	2.97*

*Significant difference of the proportions test of arsenite treated specimens as compared to the controls at $P < 0.05$

In this study, long treatment periods were applied to assure as much as possible exposure of the pollen mother cells to the arsenic solution. To maintain the cuttings in good shape during the periods of treatment and post-treatment, air was pumped through the liquid. Thus, it is possible that arsenic (III) was partially converted into arsenic (V), due to the fact that arsenic is a reducing agent, easily oxidized by air especially in solution (Vahter and Norin 1980). Therefore, arsenic is considered to be composed of the (III) and (V) oxidation states. Vahter and Envall (1983) noted that the trivalent form oxidized unless the pH of the solution was low, in this study the arsenite solutions had pH values higher than 7.0 (except the 1 mg/L solution) and increased with the increment of the concentrations to 10.2. This supports the hypothesis that considerable oxidation may have taken place.

Arsenic is known to block sulphydril groups or bind them in proteins, inhibit repair enzymes and substitute the phosphate groups of DNA (Paton and Allison 1972, Savory and Fedor 1977, Pershagen 1981). Several arsenic compounds have been reported to produce chromosomal aberrations in human lymphocytes (Paton and Allison 1972, Bencko 1977, Petres *et al.* 1977, Nordenson *et al.* 1978, Nakamuro and Sayato 1981, Sweins 1983), and sister chromatid exchanges in root tips cells of *Vicia faba* (Gómez-Arroyo *et al.* 1988).

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REFERENCES

Bencko V. (1977). Carcinogenic, teratogenic, and mutagenic effects of arsenic. Environ. Health Perspect. *19*, 179-182.

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

Gómez-Arroyo S., García-Hernández A. and Villalobos-Pietrini R. (1988). Induction of sister-chromatid exchanges in *Vicia faba* by arsenic-contaminated drinking water. Mutat. Res. *208*, 219-224.

Léonard A. and Lauwerys R.R. (1980). Carcinogenicity, teratogenicity and mutagenicity of arsenic. Mutat. Res. *75*, 49-62.

Nakamuro K. and Sayato Y. (1981). Comparative studies of chromosomal aberrations induced by trivalent and pentavalent arsenic. Mutat. Res. *88*, 73-80.

Nordenson I., Beckman G., Beckman L. and Nordström S. (1978). Occupational and environmental risks in and around a smelter in northern Sweden. II. Chromosomal aberrations in workers exposed to arsenic. Hereditas *88*, 47-50.

Paton G.R. and Allison A.C. (1972). Chromosome damage human cell cultures induced by metal salts. Mutat. Res. *16*, 332-336.

Pershagen G. (1981). The carcinogenicity of arsenic. Environ. Health Perspect. *40*, 93-100.

Petres J., Baron D. and Hafedorn M. (1977). Effects of arsenic cell metabolism and cell proliferation: cytogenetic and biochemical studies. Environ. Health Perspect. *19*, 223-227.

Savory J. and Fedor F.A. (1977). Arsenic poisoning. In: *Clinical chemistry and metals* (S.S. Brown, Ed.). Elsevier/North-Holland, Amsterdam, pp. 271-286.

Sax K. and Edmonds H.W. (1933). Development of the male gametophyte in *Tradescantia*. Bot. Gaz. *95*, 156-163.

Spiegel M.R. (1970). *Statistics*. Schaum's outline series. Schaum Publishing, New York, pp. 142-143.

Sweins A. (1983). Protective effects of selenium against arsenic-induced chromosomal damage in cultured human lymphocytes. Hereditas *98*, 249-252.

Taylor J.H. (1950). The duration of differentiation in excised anthers. Amer. J. Bot. *37*, 137-143.

Tsuchiya K. (1977). Various effects of arsenic in Japan depending on type of exposure. Environ. Health Perspect. *19*, 35-42.

Vahter M. and Norin H. (1980). Metabolism of 74 As-labeled trivalent and pentavalent inorganic arsenic in mice. Environ. Res. *24*, 446-457.

Vahter M. and Envall J. (1983). *In vivo* reduction of arsenate in mice and rabbits. Environ. Res. *32*, 14-24.

Villalobos-Pietrini R., Gómez-Arroyo S., Flores-Márquez A.R. and Cisneros A. (1986). *Tradescantia*-micronucleus test on potassium dichromate. Contam. Ambient. *2*, 63-70.

Wals L.M., Summer E. and Kuney D.R. (1977). Occurrence and distribution of arsenic in soil and plants. Environ. Health Perspect. *19*, 67-71.