Contam. Ambient. 2, 63-70, 1986

# *TRADESCANTIA*-MICRONUCLEUS TEST ON POTASSIUM DICHROMATE

RAFAEL VILLALOBOS-PIETRINI, SANDRA GOMEZ-ARROYO, ANA ROSA FLORES-MARQUEZ AND ADRIANA CISNEROS.

Laboratorio de Citogenética y Mutagénesis Ambientales, Centro de Ciencias de la Atmósfera, Universidad Nacional Autónoma de México, Coyoacán 04510, México, D. F. y Centro de Investigación y Reproducción Animal, Universidad Autónoma de Tlaxcala.

## ABSTRACT

Cuttings with inflorescences of *Tradescantia* clone 4430 were allowed to absorb several different concentrations of potassium dichromate during 6 hours and analysed 30 hours later. The frequencies of micronuclei (MCNs) in tetrads were more than twice those of the control. The relationship concentration-frequency was asymptotic.

## RESUMEN

Se permitió que cortes de Tradescantia clone 4430 con inflorescencias absorbieran diversas concentraciones de dicromato de potasio durante seis horas y fueran analizadas 30 horas después. Las frecuencias de los micronúcleos (MCNs) observados en las tétradas rebasaron el doble del registro en los testigos, la relación concentración-respuesta fue asintótica.

## INTRODUCCION

Nowadays and specially due to industrial development, heavy metals have obtained an important place among environmental pollutants and represent a potential health risk due to their acute toxicity.

A common pollutant is chromium, which is considered an essential micronutrient, involved in the normal metabolism of glucose (Mertz 1969, Freund *et al.* 1979), and as a cofactor in the increased activity of some enzymes (Mertz 1969). It provides additional structural configuration and stabilization to nucleic acids and it also serves in the maintenance of protein structure (Wacker and Vallee 1959).

Chromium is never found in the uncombined state. Its is found in several oxidation states, the most common are hexavalent (VI) and trivalent (III) forms.

Although in nature plants contain small amounts of metals (Gruskho 1948,

Gilbert 1957, Starich and Blincoe 1983), the role they play in their metabolism is not known (Gilbert 1957). In alfalfa, an organic chromium complex of about 2600 daltons was found. In other plants this complex has a different molecular weight (Starich and Blincoe 1983).

The extent of contamination depends mainly on soil condition, plant type (Davis 1982) and availability to the plant (Davis 1956).

Some of the effects produced by an excess of chromium in plants are: the prevention of development of oats (Hunter and Vergnano 1953), beans (Walace *et al.* 1976), Rhodesian grass (Soane and Saunder 1959), sugar beets (Pesek and Kolsky 1967), barley (Skeffington *et al.* 1976), and rice (Kamada and Doki 1974); the inhibition of inflorescence development and delay of stem growth in tobacco (Soane and Saunder 1959); the inhibition of seed germination in *Picea abies* (Supuka 1974) and in beans (Mukherji and Kumar 1978); and chlorosis in tomatoes, sugar beets, potatoes (Hewitt 1953), tobacco (Soane and Saunder 1959), oats (Hunter and Vergnano 1953) and rice (Verfaillie 1974).

The heavy metal effects involve several plant tisues. As an example the gametes which develop in the inflorescences have been widely used in genetics toxicology as the mother cell chromosomes of pollen grains are sensitive to physical (Sparrow 1951, Ma *et al.* 1980, 1982, Villalobos-Pietrini and Balderas 1982) and chemical agents (Ma *et al.* 1978, 1983, 1984, 1985, Ma 1979, 1981b, Villalobos-Pietrini and Balderas 1982). The sensitivity of the gametic cells to the mutagens is related to the state of the microsporogenic cycle in which the treatment is applied. There is agreement in the studies made on different plants treated with several agents that the most sensitive state is prophase I (Sparrow 1951, Sparrow *et al.* 1952, Ochlewsa 1964, Ma *et al.* 1978, 1980, Ma 1979, 1981a, b, Villalobos-Pietrini and Balderas 1982).

One of the most used plants to detect the damage produced by pollutants in germ cells is *Tradescantia,* a member of the Commelinaceae, the microsporogenic cycle of which is well known, so for *T. reflexa* it takes 15 days (Sax and Edmonds 1933) and for *T. paludosa* it lasts 17 days (Taylor 1950). Among *Tradescantia* the clone 4430 has been commonly used because of its high sensitivity to chemical mutagens (Van't Hof and Schairer 1982, Villalobos-Pietrini *et al.* 1986). In order to evaluate the damage produced to *Tradescantia,* probably the easiest and most efficient method is the microuclei (MCNs) in tetrads, at the end of the meiosis (Ma *et al.* 1978).

Some responses obtained after exposures are acentric fragments (Sparrow and Singleton 1953, Read 1959, Schmid 1976, Ma 1979, 1981a, b), chromosomes with inactivated centromere and isochromosomes (Gómez-Arroyo and Villalobos-Pietrini 1983, Gómez-Arroyo *et al.* 1985) that are excluded from the daughter nuclei at the end of the cell division and remain in the cytoplasm of the tetrads as MCNs (Ma 1979, 1981a). This test system has also been used to monitor polluted location *in situ* (Ma *et al.* 1980, 1984).

It has been shown that chromium (VI) induces mutations in *Salmonella typhimurium* (Petrilli and De Flora 1977, 1982, Bennicelli *et al.* 1983), in *Escherichia coli* (Venitt and Levy 1974, Nakamuro *et al.* 1978, Petrilli and De Flora 1982) and in *Schizosaccharomyces pombe* (Bonatti *et al.* 1976); it also produces lethality, mitotic gene conversion and reverse mutations in *Saccharomyces cereviceae* (Kharab and Singh 1985); causes lesions to chicken embryo hepatocytes DNA (Tsapakos *et al.* 1983); induces chromosomal aberrations in bone marrow cells of rats

(Bigaliev *et al.* 1976), embryo cells of hamsters (Tsuda and Kato 1977) and in mice cells in culture (Umeda and Nishimura (1979); inhibits DNA synthesis and decreases cell survival in hamster fibroblasts (Levis *et al.* 1977, 1978). It also produces chromosomal aberrations, sister chromatid exchanges (SCE) and mitotic delay in ovary embryos of hamsters (CHO) (Majone and Levis 1979); SCE in human lymphocytes *in vitro* (Gómez-Arroyo *et al.* 1981) and in rodent cell cultures, and transformation of hamster cells *in vitro* (Bianchi *et al.* 1983); C-mitosis in meristematic cells of *Allium cepa* (Levan 1945) and *Vicia faba* root tips (Gómez-Arroyo and Vilallobos-Pietrini 1983); chromosomal aberrations (Gómez-Arroyo and Villalobos-Pietrini 1983) and SCE in *Vicia faba* (Gómez-Arroyo *et al.* in preparation).

Due to the fact that hexavalent forms of the chromium compounds, are the most toxic to living organisms it is worth knowing the effects of potassium dichromate on gametic cells of *Tradescantia* clone 4430 using the micronuclei in tetrads as a test system.

## MATERIAL AND METHODS

The biological material used was *Tradescantia* clone 4430 which originated as a result of the interespecific cross between *T. hirsutiflora* and *T. subacaulis* (Sparow and Sparrow 1976).

The plants were reproduced vegetatively and maintained in plastic trays with a mixture of leaf and moldy sand (2:1) in the greenhouse of the Centro de Ciencias de la Atmósfera, UNAM. They were irrigated twice a week.

210 plants were selected with vigorous stems and young inflorescences. They were cut 6 cm from the top and formed 7 groups.

Cuttings were allowed to absorb through its normal vascular system the potassium dichromate solutions of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0% contained in glasses. The control was exposed to distilled water. All groups were treated for 6 hours.

Later, cuttings were washed with tap water and placed in glasses containing tap water and constant aereation for 30 hours. The inflorescences were fixed in ethanol-acetic acid (3:1) and after 24 hours transfered to 70% ethanol (Ma 1981a).

By means of a dissecting microscope furnished with a micrometric objective, buds of 2.0 — 3.5 cm were selected because in previous observations, tetrads were found in buds of these lengths. Buds were dissected on slides and the anthers were placed in 0.5% acetocarmine and pressed in order to let the pollen mother cells free and to verify the presence of tetrads. The debris were removed before application of the coverglass. Then the slide was heated over an alcohol flame avoiding boiling. Gentle pressure was applied with the palm of the hand over the coverglass and some layers of absorbent paper. Permanent slides were made following Conger and Fairchild technique (1953). In order to avoid bias, slides were handled with a code. For each concentration, 1500 to 3000 tetrads were observed, scoring normal tetrads and tetrads with different numbers of MCNs, expressed at last as number of MCNs per 100 tetrads.

To compare the mean values obtained in the treated and control groups, the difference of proportion test (Spiegel 1961) was applied. The data are the results of one experiment and its replica.

## RESULTS

The *Tradescantia* cuttings exposed to the lower concentrations (0.1, 0.2 and 0.4%) of potassium dichromate did not show physiological damage, but up to 0.6%, necrotic areas in the stem and leaves were observed to reach fading in the cuttings treated with 1.0%. In all cases the inflorescences were not affected. The damage observed at the end of the treatment increased after 30 hours.

Table I shows means and standard devations of MCNs per 100 tetrads for each group. There was not a concentration-response relationship, but by statiscal analysis the frequencies of MCNs were found significantly different from the controls ($P < 0.001$). The frequency of MCNs per 100 tetrads in the groups treated exceeded double that of the control. The response was asymptotic to concentrations ,Table I).

TABLE I. MICRONUCLEI (MCNs) IN TETRADS OF *TRADESCANTIA* INDUCED BY SEVERAL CONCENTRATIONS OF POTASSIUM DICHROMATE

| *Concentration* | *MCNs/100 tetrads* |
|---|---|
| (%) | $\bar{X} \pm$ S. D. |
| Control | 3.38 ± 0.47 |
| 0.1 | 7.15 ± 0.68 * |
| 0.2 | 7.93 ± 0.47 * |
| 0.4 | 6.89 ± 0.40 * |
| 0.6 | 7.83 ± 0.33 * |
| 0.8 | 7.14 ± 1.04 * |
| 1.0 | 7.36 ± 1.00 * |

* $P < 0.001$

## DISCUSSION

As noted in Table I, potassium dichromate can affect the chromosomes of the pollen mother cells in prophase I. The plants showed the damage 30 hours later as MCNs which are the consequences of the production of acentric fragments (Read 1959), chromosomes with inactivated centromere (Gómez-Arroyo y Villalobos-Pietrini 1983, Gómez-Arroyo *et al.* 1986) and isochromosomes (Schmid 1976, Gómez-Arroyo y Villalobos-Pietrini 1983, Gómez-Arroyo *et al.* 1986) that are excluded from the nuclei at the end of the meiotic division (Ma 1979, 1981a). The results agree with those of Gómez-Arroyo and Villalobos-Pietrini (1983) who found that the same compound induced MCNs in the meristematic root tips cells of *Vicia faba* and that there was not a concentration-response relationship. However, Ma *et al.* (1984) treating *Tradescantia paludosa* Sax clone 03 with potassium chromate, obtained negative results in the induction of MCNs.

The effectiveness of potassium dichromate in damaging the genetic material is shown by the production of point mutations (Bonnatti *et al.* 1981), chromosomal aberrations (Bigaliev *et al.* 1976, Tsuda and Kato 1977, Nakamuro *et al.* 1978, Nishimura and Umeda 1978, Majone and Levis 1979, Umeda and Nishimura 1979, Gómez-Arroyo and Villalobos-Pietrini 1983), cell transformation (Tsuda and Kato 1977), DNA synthesis inhibition (Levis *et al.* 1977, 1978) and mitotic delay (Majone and Levis 1979).

Plants take up chromium from the soil, accummulate it mainly in roots (90%) and of the remainder only 2% is translocated to leaf structures (Huffman and Allaway 1973). In spite of the fact that the cuttings treated in this work do not have roots, the whole amount of chromium probably did not reach the inflorescences, because they did not show physiologic alterations even with the highest concentrations.

Chromium (VI), the form contained by potassium dichromate, has an increased biological activity because it was able to cross the cell membranes (Mertz 1969, Taylor and Parr 1978), using the same transport mechanisms to enter the cells as other oxyanions like phosphate and sulphate (Jennette 1981). In the cytoplasm it is reduced by the enzymes composing the electron-transport cytochrome P-450 and by NADPH (nicotinamide and adenine reduced dinucleotide phosphate) to form Cr III (Jennette 1981) which binds nucleic acids and nucleoproteins (Herrmann and Speck 1954, Levis *et al.* 1977) and thus can lead to mutagenic and carcinogenic activity (Tsapakos and Wetterhan 1983).

When Cr (VI) comes into contact with micromosomal fraction before crossing the *Salmonella* cell membrane it is reduced to Cr (III) and thus mutagenic activity is decreased (Löfroth 1978, Bennicelli *et al.* 1983. Chelators also reduced Cr (VI) to Cr (III) preventing entrance into the cell and decreasing or eliminating its mutagenicity (Gentile *et al.* 1981). Chromium is one of the carcinogenic heavy metals which is consistently detected as beig mutagenic (Babich *et al.* 1985).

In rats injected intravenously with potassium dichromate, the reduction of Cr (VI) to Cr (III) is made in the blood (Cavalleri *et al.* 1985) and the only way the latter penetrated liver cells was by binding to a low-molecular-weight substance (Yamamoto *et al.* 1981). Ormos and Mányai (1977) found that higher chromate concentrations than normal inhibited chromate uptake in the red blood cells.

Tsapakos and Wetterhahn (1983) found little Cr (III) bound to DNA in the absence of the complete microsomal reducing system. Meanwhile the binding of Cr (VI) to DNA in the presence of microsomes and NADPH was caused by a labile intermediate chromium oxidation state, Cr (V) was produced and required for the maximum binding (Jennette 1982).

Within the cell Cr (VI) also damages the lysosomes, leaving free among other enzimes, lipases which hydrolyse tissue lipids producing epoxyaldehides that have been related to cancer (Schoental 1975).

As can be seen in Table I, the frequency of MCNs in all the groups treated with chromium goes beyond double the control value and is significantly different ($P < 0.001$). These data point out that potassium dichromate is a good inducer of MCNs, although there was not a concentration-frequency relationship. Instead the response was asymptotic to concentration. This was probably due to inhibition of entry of chromium into the cell when high concentrations were reached (Ormos and Mányai 1977), some extracellular reduction of Cr (VI) occurred (Jennette

1981), low translocation of Cr occurred in the upper part of the plants (Huffman and Allaway 1973), or there was a saturation of sensitive sites of the chromosomes.

Acknowledgements

This work was performed under contract PCCBBNA-021988 of CONACyT.

## REFERENCES

Babich H., Devanas M. A. and Stotzky G. (1985). The mediation of mutagenicity and clastogenicty of heavy metals by physicochemical factors. Environ. Res. *37,* 253-280.

Bennicelli C., Camoirano A., Petruzzelli S., Zanachi P. and De Flora S. (1983). High sensitivity of *Salmonella* TA102 in detecting hexavalent chromium mutagenicity and its reversal by liver and lung preparations. Mutat. Res. *122,* 1-5.

Bianchi V., Celotti L., Lanfranchi G., Majone F., Marin G., Montaldi A., Sponza G., Tamino G., Venier P., Zanteschi A. and Levis A. G. (1983). Genetics effects of chromium compounds. Mutat. Res. *117,* 279-300.

Bigaliev A. B., Elemesova M. S. H. and Bigaleiva R. K. (1976). Chromosome aberrations in the somatic cells of mammals evoked by chromium compounds. Tsitol. Genet. *10,* 222-224.

Bonatti S., Meini H. and Abbondandolo A. (1976). Genetic effects of potassium dichromate in *Schizosaccharomyces pombe.* Mutat. Res. *38,* 147-150.

Cavalleri A., Minoia C., Richelmi P., Baldi C. and Micoli G. (1985). Determination of total and hexavalent chromium in bile after intravenous administration of potassium dichromate in rats. Environ. Res. *37,* 490-496.

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

Davis G. K. (1956). Chromium in soils and animals. In: *Chromium, chemistry of chromium and its compounds.* (M. J. Udy, ed.) Reinhold, New York, pp. 105-109.

Davis R. D. (1982). Influence of micropollutants on vegetation. Wat. Sci. Tech. *14,* 31-44.

Freund H., Atamian S. and Fisher J. E. (1979). Chromium deficiency during total parenteral nutrition. JAMA *241,* 496-498.

Gentile J. M., Hyde K. and Shubert J. (1981). Chromium genotoxicity as influenced by complexation and rate effects. Toxicol. Lett. *7,* 439-448.

Gilbert F. (1957). Mineral nutrition and balance of life. Univ. Oklahoma Press.

Gómez-Arroyo S. and Villalobos-Pietrini R. (1983). Chromosomal alterations induced by some chromium salts. Cytologia *48,* 185-193.

———, Altamirano M. and Villalobos-Pietrini R. (1981). Sister-chromatid exchanges induced by some chromium compounds in human lymphocytes *in vitro.* Mutat. Res. *90,* 425-431.

———, Castillo-Ruiz P. and Villalobos-Pietrini R. (1986). Chromosomal alterations induced in *Vicia faba* by different industrial solvents: thinner, toluene, benzene, n-hexane, n-heptane and ethyl acetate. Cytologia *51,* 555-564.

———, Baíza A. M., López G. and Villalobos-Pietrini R. (1985). A comparative study of the cytogenetic effects of the insecticides heptachlor, malathion, and methyl parathion in *Vicia faba.* Contam. Ambient. *1,* 7-16.

Gruskho Y. M. (1948). Chromium as bioelement. Biokhimiya *13,* 124-126.

Herrmann H. and Speck L. B. (1954). Interaction of chromate with nucleics acids in tissues. Science *104,* 426-427.

Hewitt E. J. 1953. Metal interrelationships in plant nutrition. I. Effects of some metal toxicities on sugar beet, tomato, oat, potato and marrowstem kale grown in sand culture. J. Exp. Bot. *4,* 59-64.

Huffman E. and Allaway W. (1973). Chromium in plants: distribution in tissues organelles, and extracts, and availability of bean leaf Cr to animals. J. Agric. Food Chem. *21,* 982-985.

Hunter J. G. and Vergnano O. (1953). Trace-element toxicities in oat plants. Appl. Biol. *40,* 761-777.

Jennette K. W. (1981). The role of metals in carcinogenesis: biochemistry and metabolism. Environ. Health Perspec. *40,* 223-252.

——— (1982). Microsomal reduction of the carcinogen chromate produces chromium (V). J. Am. Chem. Soc. *104,* 874.

Kamada K. and Doki K. (1974). Reduction of chromium (VI) in soil and the determination of chromium by direct atomic absorption spectrophotometry. Nippon Dojo-Heryogaku Zasshi *45,* 597-599. Chem. Abstr. *83,* 8253, 1975.

Kharab P. and Singh I. (1985). Genotoxic effects of potassium dichromate, sodium arsenite, cobalt, chloride and lead nitrate in diploid yeast. Mutat. Res. *155,* 117-120.

Levan A. (1945). Cytological reactions induced by inorganic salt solutions. Nature *156,* 751-752.

Levis A., Buttignol M. and Vettorato L. (1977). Inhibition of DNA synthesis in BHK fibroblast treated *in vitro* with potassium dichromate. Experientia *33,* 82-84.

———, Bianchi V., Tamino G. and Pergoraro B. (1978). Cytotoxic effects of mammalian cells *in vitro*. Br. J. Cancer *37,* 386-396.

Löfroth G. (1978). The mutagenicity of hexavalent chromium is decreased by microsomal metabolism. Naturwissenschaften *65,* 207.

Ma T. H. (1979). Micronuclei induced by X-rays and chemical mutagens in meiotic pollen mother cells of *Tradescantia* a promising mutagen test system. Mutat. Res. *64,* 307-313.

——— (1981a). *Tradescantia* micronucleus bioassay and pollen tube chromatid aberration test for *in situ* monitoring and mutagen screening. Environ. Health Perspec. *37,* 85-90.

——— (1981b). *Tradescantia* MCN-in-tetrad mutagen test for on-site monitoring and further validation. EPA-600/S1.

———, Anderson V. A. and Harris M. M. (1985). Mutagenicity of drinking water detected by the *Tradescantia* micronucleus test. Can. J. Genet. Cytol. *27,* 143-150.

———, ———, ——— and Bare J. L. (1983). *Tradescantia*-micronucleus (Trad-MCN) test on the genotoxicity of malathion. Environ. Mutagenesis *5,* 127-137.

———, Kontos G. J. and Anderson V. A. (1980). Stage sensitivity and dose response of meiotic pollen mother cells of *Tradescantia* to X-rays. Environ. Expt. Bot. *20,* 169.

———, Sparrow A. H., Schairer L. A. and Nauman A. F. (1978). Effect of 1,2-dibromoethane (DBE) on meiotic chromosomes of *Tradescantia*. Mutat. Res. *58,* 251-258.

———, Haris M. M., Anderson V. A., Ahmed I., Mohammad K., Bare J. L. and Lin G. 1984). *Tradescantia*-micronucleus (Trad-MCN) test on 140 health-related agents. Mutat. Res. *138,* 157-167.

———, Tsungci F., Ho J., Chen D., Zhou R., Lin G., Dai J. and Li J. (1982). Extraordinary high micronucleus frequency induced by X-rays in a special clone of *Tradescantia reflexa*. Mutat. Res. *104,* 101-103.

Majone F. and Levis A. G. (1979). Chromosomal aberrations and sister chromatid exchanges in Chinese hamster cells treated *in vitro* with hexavalent chromium compounds. Mutat. Res. *67,* 231-238.

Mertz W. (1969). Chromium occurrence and function in biological systems. Physiol. Rev. *49,* 163-239.

Mukherji S. and Kumar B. R. (1978). Characterization of chromium toxicity in different plant materials. Ind. J. Exp. Biol. *16,* 1017-1019.

Nakamuro K., Yoshikawa K., Sayato Y. and Kurata H. (1978). Comparative studies of chromosomal aberration and mutagenicity of trivalent and hexavalent chromium. Mutat. Res. *58,* 175-181.

Nishimura M. and Umeda M. (1978). Mutagenic effect of some metal compounds on cultured mammalian cells. Mutat. Res. *54,* 246-247.

Ochlewsa M. (1974). Influence of ionizing radiation on microsporogenesis in rye *(Secale cereale* L.) Hodwla Rosl, Aklm. Nasienn. *18,* 151-162.

Ormos G. and Mányai S. (1977). Chemical modification of erithrocytes. Effect on the velocity of chromate uptake. Acta Biochim. et Biophys. Acad. Sci. Hung. *2,* 343-352.

Pesek F. and Kolsky V. (1967). Content of elements contaminating the edible parts of agricultural crops. Content of heavy metals and trace elements in sugar beets from the region of the Lovosice chemical plant. Rostl. Vyroba *13,* 445-462. Chem. Abstr. *67,* 52789.

Petrilli F. L. and De Flora S. (1977). Toxicity and mutagenicity of hexavalent chromium on *Salmonella typhimurium*. Appl. Environ. Microbiol. *33,* 805-809.

——— and ——— (1982). Interpretations on chromium mutagenicity and carcinogenicity. *Mutagens in Our Environment.* Alan R. Liss, New York pp. 453-464.

Read J. (1959). Mitosis, and inhibition of mitosis by radiation. In: *Radiation biology of Vicia faba in relation to the general problem.* Blackwell *Scientific* Pub. Oxford, pp. 48-69.

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

Schmid W. (1976). The micronucleus test for cytogenetic analysis. In: *Chemical Mutagens: Principles and methods for their detection*. A. Hollaender Ed. Vol. 4 New York. Plenum Press, pp. 31-53.

Schoental R. (1975). Chromium carcinogenesis, formation of epoxyaldehides and tanning. Brit. J. Cancer *32*, 403-404.

Skeffington R. A., Shewrey P. R. and Peterson P. J. (1976). Chromium uptake and transport in barley seedlings *(Hordeum vulgare* L.). Planta Berl. *132*, 209-214.

Soane B. D. and Saunder D. H. (1959). Nickel and chromium toxicity of serpentine soils in Southern Rhodesia. Soil Sci. *88*, 322-330.

Sparrow A. H. (1951). Radiation sensitivity of cells during mitotic and meiotic cycles with emphasis on possible cytochemical changes. Ann. N. Y. Acad. Sci. *51*, 1508-1540.

——— and Singleton W. A. (1953). The use of radiocobalt as a source of gamma rays and some effects on chronic irradiation on growing plants. Am. Nat. *87*, 29-48.

——— and Sparrow R. C. (1976). Spontaneous somatic mutation frequencies for flower color in several *Tradescantia* species and hybrids. Environ. Exp. Bot. *16*, 23-46.

———, Moses M. J. and Dubow R. J. (1952). Relationships between ionizing radiation, chromosome breakage and certain other nuclear disturbances. Exp. Cell Res. Suppl. *2*, 305-322.

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

Starich G. H. and Blincoe C. (1983). Dietary chromium-forms and availabilities. Sci. Total Environ. *28*, 443-454.

Supuka J. (1974). Influence of the extracts of some iron-alloy powders on *Picea abies* (L.) Karst. seed germination. Biológia (Bratislava) *29*, 759-767.

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

Taylor F. G. and Parr P. D. (1978). Distribution of chromium in vegetation and small mammals adjacent to cooling towers. J. Tenn. Acad. Sci. *53*, 87-91.

Tsapakos M. J. and Wetterhahn K. E. (1983). The interaction of chromium with nucleic acids. Chem. Biol. Interactions *46*, 265-277.

———, Hampton T. H., Sinclair P. R., Sinclair J. F., Bemos W. J. and Wetterhahn K. E. (1983). The carcinogen chromate causes DNA damages in inhibition drug-mediated induction of porphyrin accumulation and glucuronidation in chick embryo hepatocytes. Carcinogenesis *4*, 959-966.

Tsuda H. and Kato K. (1977). Chromosomal aberrations and morphological transformation in hamster embryonic cells treated with potassium dichromate *in vitro*. Mutat. Res. *46*, 87-94.

Umeda M. and Nishiumura M. (1979). Inducibility of chromosomal aberrations by metal compounds in cultured mammalian cells. Mutat. Res. *67*, 221-229.

Van't Hof J. and Schairer L. A. (1982). *Tradescantia* assay system for gaseous mutagens. A report of the U. S. Environmental Protection Agency Gene-Tox. Program. Mutat. Res. *99*, 303-315.

Venitt S. and Levy L. S. (1974). Mutagenicity of chromates in bacteria and its relevance to chromate carcinogenesis. Nature *250*, 493-495.

Verfaillie G. R. M. (1974). Kinetics of chromium absorption by intact rice plants. IAEA, Vienna pp. 315-331.

Villalobos-Pietrini R. and Balderas M. (1982). Pollen abortion induced by gamma radiation and ethanol in *Gibasis pulchella*. An. Inst. Biol. Unive. Nal. Autón. Méx. *49*, Ser. Biol. Exp. (1): 89-107.

———, Hernández R., Guadarrama M. A. and Gómez-Arroyo S. (1986). Cytological detection of somatic mutations in *Tradescantia* induced by ethanol. Cytologia *51*, 623-630.

Wacker W. E. C. and Vallee B. L. (1959). Nucleic acids and metals. J. Biol. Chem. *234*, 3257-3261.

Wallace A., Soufi S. M., Cha J. W. and Rommey E. M. (1976). Some effects of chromium toxicity on bush bean plants grown in soil. Plant Soil *44*, 471-473.

Yamamoto A. M., Wade O. and Ono T. (1981). A low-molecular-weight chromium binding substance in mammals. Toxicol. Appl. Pharmacol. *59*, 515-523.