ELSEVIER

Mutation Research 321 (1994) 35–42

MR
Genetic Toxicology

# Genotoxicity of vanadium pentoxide in Chinese hamster V79 cells

B.-Z. Zhong, Z.-W. Gu **, W.E. Wallace, W.-Z. Whong, T. Ong *

*Microbiology Section, Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, 944 Chestnut Ridge Road, Morgantown, WV 26505–2888, USA*

(Received 28 June 1993)
(Revision received 25 September 1993)
(Accepted 4 October 1993)

**Abstract**

Workers in many mining and manufacturing industries are potentially exposed to vanadium. Inhalation of dust containing vanadium pentoxide ($V_2O_5$), a pentavalent compound of vanadium, has been reported to cause lung diseases. Information related to the genotoxicity and potential carcinogenicity of $V_2O_5$, however, is still limited. In this study, the effect of $V_2O_5$ on mitosis, sister-chromatid exchange (SCE), micronucleus formation (MN), and gene mutation in Chinese hamster V79 cells was determined. Cells were treated with varying concentrations of $V_2O_5$ for 24 h. The results showed that no significant increases in the frequencies of SCE or gene mutation occurred in $V_2O_5$-treated cultures. However, dose-related increases were noted for micronucleated cells in cultures exposed to this compound, and the number of binucleated cells in the presence of cytochalasin B was found to decrease with increasing $V_2O_5$ concentrations. Since the micronucleated cells induced by $V_2O_5$ contained kinetochore-positive micronuclei, their induction appears to be due to damage to the spindle apparatus. These results indicate that $V_2O_5$ is cytotoxic and aneuploidogenic to V79 cells.

*Key words:* Micronucleus assay; Antikinetochore antibody assay; SCE assay; HGPRT gene mutation; Chinese hamster V79 cells; Vanadium pentoxide

## 1. Introduction

Vanadium, a fairly abundant element in the earth's crust (0.02%), is an important industrial chemical. It is used in the steel industry for manufacture of alloys that are strong and resilient. Vanadium is also the principle catalyst used in the synthesis of sulfuric acid (Petrucci, 1989). This element is also used in the production of dyes and inks, paints and varnish dyers, insecticides and photographic materials (Browning, 1969). Potential exposure of human subjects to vanadium, often in the form of vanadium oxide, is not limited to mining and industries. High concentrations of this metal have been found in soil and plants near industrial operations (Roldán and Altamirano, 1990) and in the air of Bahrain while oil fields burned in Kuwait (Madany and

* Corresponding author.
** Visiting scientist from the Department of Occupational Health, Shanghai Medical University, Shanghai, People's Republic of China.


*SSDI* 0165-1218(93)E0118-8

Raveendran, 1992). The toxicity of vanadium compounds in vitro appears to depend on valence and is related to their solubility in assay media. The most toxic forms of vanadium to mammals are the pentavalent form ($V^{5+}$) compounds (Sharma et al., 1987). Numerous studies regarding the potential health hazards of vanadium-containing residual ash of high vanadium-content oil have been reported. The combustion of these materials provides an appreciable source of vanadium oxides in the environment. Epidemiological studies have indicated a correlation between exposure to airborne vanadium particles and the incidence of cancer in residents of metropolitan areas (Stock, 1960).

Many studies on the biological effects of vanadium have been reported (Nechay et al., 1986). This element has been shown to both inhibit and stimulate the activity of several enzymes including ATPases, phosphatases, kinases, DNA-polymerases and peroxidases (Sabbioni et al., 1983; Serra et al., 1990). These alterations in enzymatic activity by vanadium are most likely related to observed effects such as the destruction of cysteine and cystine (Carson et al., 1987), alterations in DNA synthesis in quiescent human fibroblasts and Swiss mouse 3T3 and 3T6 cells (Carpenter, 1981; Smith, 1983; Hansen and Stern, 1984), and lipid peroxidation in rodent kidney cells (Donaldson et al., 1985). Other biological effects include immunological changes such as decreases in alveolar macrophage number, viability, and phagocyte activity (Waters et al., 1974).

Data on the genotoxicity and carcinogenicity of several vanadium compounds have been reported in the literature. Conflicting results in regard to the mutagenic activity of vanadium have been shown in bacterial assay systems using *Escherichia coli* and *Salmonella typhimurium* (Graedel et al., 1986; Hansen and Stern, 1984; WHO, 1988). Ammonium metavanadate (V5) induced mitotic gene conversion and reverse mutation in the D7 strain of yeast (Bronzetti et al., 1990), but vanadyl sulfate (V4) did not. No mutagenic effect was observed in the presence or absence of the microsomal S9 fraction in V79 cells (Galli et al., 1991). However, potential aneuploidogenic effects have been shown in *Allium cepa*, where $V_2O_5$ poisons the spindle apparatus (Singh and Sharma, 1980). Aneuploidy effects were also induced by V5 and V4 in the D61M strain of *Saccharomyces cerevisiae* (Galli et al., 1991). Treatment of human lymphocytes with $V_2O_5$ was found not to increase the frequency of structural chromosomal aberrations (CAs) or SCEs, but did increase polyploidy cell frequency, average generation time, and the frequency of cells with satellite associations (Roldán and Altamirano, 1990). A comparative study of the activity of $V_2O_3$, $VOSO_4$, and $NH_4VO_3$ on the induction of SCE and CA showed that they are all genotoxic in cultured Chinese hamster ovary cells (Owusu-Yaw et al., 1990). In a transformation assay, V5 transformed BALB/3T3 cells; while V4 did not. This is consistent with the in vitro data showing that V5 is more cytotoxic than V4 (Sabbioni et al., 1991). Recently, the transforming activity of sodium orthovanadate ($V_5$) has been studied in BALB/3T3 cells by Sheu et al. (1992). Their results indicate that orthovanadate may have acted both as an initiator and a promotor for morphological transformation of cultured mammalian cells.

In regard to potential health hazards, $V_2O_5$ appears to be the most important compound among vanadium oxides. Acute $V_2O_5$ dust inhalation produced significant changes in pulmonary function in monkeys (Knecht et al., 1985). Inhalation of dust containing $V_2O_5$ has been reported to cause lung diseases (Carson et al., 1987; NIOSH, 1978). Workers in mining and manufacturing industries may be exposed to a high level of $V_2O_5$ (Clayton and Clayton, 1981). However, information related to the genotoxicity and potential carcinogenicity of $V_2O_5$ is still limited. In this study, the effects of $V_2O_5$ on mitosis, SCE, MN formation, and gene mutation at the HPRT locus in Chinese hamster V79 cells were determined.

## 2. Materials and methods

*Cell line and culture*

The Chinese hamster lung fibroblast cell line (V79) was kindly supplied by Dr. C.C. Chang

(Michigan State University, East Lansing, MI). Cultures were maintained in 75-$cm^2$ flasks with subculturing performed every 3–4 days to prevent the cultures from becoming confluent. Cells were grown exponentially in Minimum Essential Medium (MEM; Gibco, Grand Island, NY), supplemented with 10% fetal bovine serum (FBS, Gibco), 1 mM L-glutamine (Gibco), 100 units penicillin/ml, and 100 $\mu$g streptomycin/ml (Gibco). These were incubated at 37°C in a humidified atmosphere of 5% $CO_2$ in all experiments. Cells were subcultured every 3–4 days by treatment with trypsin–EDTA solution (Gibco) in phosphate-buffered saline (PBS).

*Chemical preparation and treatment*

Vanadium pentoxide (99.9% purity, Fisher Lot 713190), provided by Mr. William Moorman (NIOSH, Cincinnati, OH), was prepared prior to the assays in MEM at a concentration of 1 mg/ml and sonicated 30 min before use. Cytochalasin B (Cyt B), mitomycin C (MMC), vincristine sulphate (VS), and 2,4,7-trinitrofluorenone (TNF) were purchased from Sigma Chemical Co. (St. Louis, MO). *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was obtained from Aldrich Chemical Inc. (Milwaukee, WI). The positive controls, MMC and VS, were dissolved in MEM; MNNG and TNF were dissolved in dimethyl sulfoxide (DMSO). MEM or DMSO was used as a solvent control, and final concentrations did not exceed 1% (v/v). Cyt B, used for the cytokinesis-block, was dissolved in DMSO at a concentration of 2 mg/ml, stored at −20°C, and diluted with Hank's balanced salt solution (HBSS, Gibco) immediately prior to use.

*Cytotoxicity test*

The cell-cycle kinetic was used to determine the cytotoxicity of $V_2O_5$. Approximately $5.0 \times 10^4$ /ml of V79 cells in the exponential phase of growth were seeded on a slide in a square petri dish and allowed to adhere for 2 h. In each dish 12 ml of MEM was added and the cultures were incubated at 37°C for 24 h. $V_2O_5$ treatments were performed at concentrations of 1, 3, 6, 9 and 12 $\mu$g/ml for 24 h. Cyt B (4 $\mu$g/ml) was added 4 h after $V_2O_5$ treatment was initiated and incubated continuously for 20 h. Cells were harvested by trypsinization, hypotonicated with 0.075 M KCl, fixed in methanol, and stained with Diff-Quik (Dage Diagnostics, Aquada, Puerto Rico) stain. In each treatment group, the numbers of mononucleated and binucleated cells per 1000 cells (500 cells/culture) were determined for cell-cycle kinetic analysis.

*Immunofluorescent staining of kinetochores in micronuclei with antikinetochore antibodies*

The procedure used for chemical treatment and slide preparation was similar to that reported by Channarayappa et al. (1992). Trypsinized V79 cells were seeded directly onto pre-cleaned sterile glass slides ($0.5 \times 10^5$ cells in 1 ml of growth medium per slide) in a square petri dish (100 mm, Lab-Tak) and allowed to adhere for 2 h before 12 ml of MEM was added. Chemicals were added 24 h after initial incubation. $V_2O_5$ and MMC were left in the culture until 24 h harvest, VS treatment was carried out in serum-free MEM for 4 h, then cells were washed once and incubated with fresh MEM for an additional 20 h. Cyt B was added and remained in the cultures for the last 20 h. Upon termination of the experiments, cells on the slides were rinsed with PBS, subjected to hypotonic treatment by adding 15 ml 0.075 M KCl for 10 min at room temperature, and fixed in situ with pre-chilled (−20°C) absolute methanol for 15 min at −20°C. Slides were dried for a few minutes, washed twice with PBS (pH 7.4), and placed in PBS containing 0.1% Tween-20 for 5 min. Immunofluorescence staining of kinetochore (KC) in binucleated cells with antikinetochore antibodies was performed as described by Eastmond and Tucker (1989). Slides from each treatment were randomized and coded prior to scoring. Replicate cultures were examined for each concentration level by scoring 1000 binucleated cells per culture and a total of 2000 cells per treatment at a magnification of 1000×. The kinetochores appeared yellow/green against the orange/red nuclear material; the cytoplasm appeared faint green in fluorescent analysis. Criteria of Eastmond and Tucker (1989) was followed both for MN determination and measurement of nuclear division. For each treatment the nuclear

Table 1
Cell cycle delay in cytokinesis-blocked V79 cells following treatment with vanadium pentoxide

| Chemical treatment | Concentration ($\mu$g/ml) | Number of cells with [a] | | | |
|---|---|---|---|---|---|
| | | 1 Nucleus | % | 2 Nuclei | % |
| Solvent control | 0 | 109 | 10.9 | 891 | 89.1 |
| Vanadium pentoxide | 1 | 400 | 40.0 | 600 | 60.0 * |
| | 3 | 770 | 77.0 | 230 | 23.0 * |
| | 6 | 671 | 67.1 | 329 | 32.9 * |
| | 9 | 709 | 70.9 | 291 | 29.1 * |
| | 12 | 707 | 70.7 | 293 | 29.3 * |

[a] 18 h after addition of cytochalasin B (4 $\mu$g/ml). The numbers are based on 1000 cells scored.
* Significantly different from control at $p < 0.01$ (chi-square test).

division index (NDI) was determined by scoring the number of nuclei in 500 cells: NDI = $[(M_1) + (2XM_2) + (3XM_3) + (4XM_4)]/N$. Statistical analyses were performed using the Chi-square test.

*Gene mutation assay*

Testing for induction of 6-thioguanine resistant ($TG^r$) mutation was carried out according to the method of Abbondandolo et al. (1977). For these experiments, $1 \times 10^6$ cells were plated in 25-$cm^2$ tissue-culture flasks containing 5 ml MEM supplemented with 10% heat-inactivated FBS and incubated for 24 h. The cells were washed with PBS and treated with 5 ml MEM containing different concentrations of $V_2O_5$. After 24 h treatment, the cells were washed, trypsinized, and replated in 4, 100-mm dishes at $5 \times 10^5$ cells/dish for mutation studies and 6, 60-mm dishes at 100 cells/dish for measurement of relative survival. Replated cells were incubated to give an expression time of 7 days in each experiment. During which time, 2–3 subcultures were made. $2.5 \times 10^5$ cells were plated in each of 6 dishes, and 10 $\mu$g $TG^r$/ml was added 3 h after plating. Colony-forming efficiency (CFE) was measured following a 7-day incubation period by seeding 100 cells/dish, and mutation frequencies were estimated after 10 days. The dishes were fixed in methanol and stained with Giemsa. MEM and MNNG were tested simultaneously as solvent and positive controls, respectively.

*SCE assay*

The V79 cultures were set up uniformly with $6 \times 10^5$ cells in each 100-mm dish containing 10 ml MEM and incubated for 24 h. Cells were then treated with $V_2O_5$ at concentrations of 1, 2, 3 and 4 $\mu$g/ml for 24 h. After treatment, cells were trypsinized and reseeded into duplicate dishes

Table 2
Percent of kinetochore-positive and kinetochore-negative micronuclei in cytokinesis-blocked V79 cells following treatment with vanadium pentoxide

| Chemical treatment | Concentration ($\mu$g/ml) | Micronucleated cells [a] | | | | Percent of | | NDI [c] |
|---|---|---|---|---|---|---|---|---|
| | | Total [b] | % Cells | $KC^+$ Cells | $KC^-$ Cells | $KC^+$ | $KC^-$ | |
| Medium | 0 | 47 | 2.35 | 23 | 24 | 48.93 | 51.07 | 1.95 |
| Vanadium pentoxide | 1 | 83 ** | 4.15 | 58 ** | 25 | 69.88 | 30.12 | 1.97 |
| | 2 | 124 ** | 6.20 | 102 ** | 22 | 82.26 | 17.74 | 1.95 |
| | 3 | 151 ** | 7.55 | 134 ** | 17 | 88.74 | 11.26 | 1.96 |
| Mitomycin C | 0.04 | 72 * | 3.60 | 22 | 50 ** | 30.55 | 69.45 | 2.03 |
| Vincristine sulfate | 0.04 | 166 ** | 8.30 | 152 ** | 14 | 91.56 | 8.44 | 2.41 |

[a] Based on 2000 binucleated cells scored.
[b] A dose–response relationship was revealed by trend test $Z = 2.894$, $p < 0.005$.
[c] Nuclear division index = $[M1 + (2 \times M2) + (3 \times M3) + (4 \times M4)]/N$ where $M1$–$M4$ are the number of cells with 1–4 nuclei, and $N$ is the total number of cells scored.
* $p < 0.05$; ** $p < 0.01$ Compared to the control by Chi-square test.

per treatment in 10 ml fresh MEM. A solvent control, MEM, and a positive control, TNF, were run concurrently with each trial. 5'-Bromodeoxyuridine (BrdU, Sigma) was added to the medium at a final concentration of 12.5 $\mu$M. Cells were re-incubated for 28–30 h in the dark. 2 h before harvesting, colcemid was added at a final concentration of 1 $\mu$M. Cells were harvested by trypsinization, washed by centrifugation, resuspended in a 0.075 M KCl hypotonic solution for 20 min at 37°C, and fixed 3 times in 3:1 mixture of methanol–acetic acid. Two drops of cell suspension were dropped onto a cold and wet slide, and slides were waved through a flame. The slides were then allowed to air-dry overnight, and were stained using the fluorescence–Giemsa method (Perry and Wolff, 1974). From each treatment, 100 metaphases were counted for replicative index determination, and 30 cells having 20–23 distinguishable and well-spread chromosomes were scored for SCEs. The results were expressed as the mean number of SCEs per metaphase ± S.E. (standard error). Statistical analysis was performed using analysis of variance to compare the average frequencies of SCE induced by $V_2O_5$ with the solvent and positive controls.

## 3. Results

The cytotoxic effect of $V_2O_5$ as measured by the number of binucleated cells in the presence of Cyt B after 24 h treatment is shown in Table 1. The data indicate that with increases in concentration, the number of binucleated cells significantly decreases from 89% in the control to 23–32% at the range of concentrations tested (3–12 $\mu$g/ml).

The induction of MN in V79 cells following $V_2O_5$ treatment and the immunofluorescent staining of kinetochores is presented in Table 2. A clear concentration-related increase in MN frequencies was observed. In the concentration range from 1 to 3 $\mu$g/ml, the incidence of MN increased from 4.15 to 7.55%. The dose–response relationship was statistically significant by the trend test ($Z = 2.894$, $p < 0.005$). At the concentration of 3 $\mu$g/ml, a more than 3-fold increase in the frequency of MN cells over the control value was elicited. The results of immunofluorescent staining of kinetochores in MN with anticentromere antibody showed that almost one half (49%) of micronuclei in untreated cells contained kinetochores ($KC^+$); however, more than 69% of micronuclei induced by $V_2O_5$ (1 $\mu$g/ml) reacted positively with the antibody. The frequency of $KC^+$ MN also showed a concentration-related increase in the treated cells. No increase in the number of $KC^-$ micronucleated cells and no decrease in NDI was noted in the cultures treated with $V_2O_5$.

The frequencies of SCE in $V_2O_5$-treated V79 cells are shown in Table 3. No significant increase in SCE frequency was observed with the $V_2O_5$

Table 3
The frequency of sister chromatid exchanges in V79 cells after vanadium pentoxide treatment

| Chemical treatment | Concentration ($\mu$g/ml) | SCEs/cell [a] Mean ± SE | Replication index | Frequencies of endoreduplicative chromosomes (%) |
|---|---|---|---|---|
| Medium | – | 6.07 ± 0.29 | 2.18 | 0 |
| Vanadium pentoxide | 1.0 | 7.00 ± 0.38 | 1.75 | 6 |
| | 2.0 | 7.53 ± 0.37 * | 1.70 | 8 |
| | 3.0 | 7.18 ± 0.57 | 1.40 | 19 |
| | 4.0 [b] | – | – | – |
| Trinitrofluorenone | 4.0 | 17.27 ± 0.59 ** | 1.96 | 0 |

[a] Values shown represent the means of 2 cultures, 30 cells were scored from each of 2 cultures.
[b] Toxic.
* $p < 0.05$, ** $p < 0.01$, compared to the control by grouped $t$-test for multiple samples.

Table 4
The frequency of 6-thioguanine-resistant mutants in V79 cells after vanadium pentoxide treatment

| Chemical treatment | Concentration (μg/ml) | Relative survival [c] (%) | Mutants per $10^5$ survivors |
|---|---|---|---|
| DMSO | 4 [a] | 100 | 1.04 ± 0.43 |
| Vanadium pentoxide | 1 | 77.8 | 2.09 ± 1.27 |
| | 2 | 22.7 | 0.51 ± 0.21 |
| | 3 | 14.6 | 0.82 ± 0.44 |
| | 4 | 0.7 | 0 |
| MNNG [b] | 1 | 34.0 | 18.74 ± 3.01 |

[a] μl/ml.
[b] *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine.
[c] Relative survival = (the number of colonies in treatment flasks/the number of colonies in control flasks) × 100%.

concentrations tested. However, a decrease in the replicative index was found in the $V_2O_5$-treated cells. The concentration of 4 μg/ml was overly toxic to the cells. However, the frequencies of endoreduplication of chromosomes were 6%, 8% and 19% at 1 μg/ml, 2 μg/ml and 3 μg/ml of $V_2O_5$, respectively.

$V_2O_5$ was tested for the ability to induce gene mutation at the HPRT locus in V79 cells. Table 4 illustrates the frequencies of $TG^r$ mutants in cultures treated with different concentrations of $V_2O_5$ and MNNG. None of the 3 concentrations tested significantly increased the frequency of $TG^r$ mutants; no dose response or minimum 3-fold increase over background (Nestmann et al., 1991) was demonstrated. However, MNNG, a known carcinogen, showed a positive response. Data on relative survival indicate that the tested concentrations of $V_2O_5$ were toxic to V79 cells.

## 4. Discussion

The study reported here indicates that $V_2O_5$ is cytotoxic to the V79 cells as determined by cell cycle kinetics using the Cyt B-blocked binucleated cell assay, the BrdU-labeling technique, and the gene mutation assay (Tables 1, 3 and 4). In the Cyt B-blocked binucleated cell assay, nuclei continue to divide while the cells do not, so the number of cells undergoing mitotic cycles can be determined by quantifying the number of nuclei present within each cell (Krishna et al., 1989). An increase in the number of mononucleated cells, with a significant decrease in binucleated cells, at the concentrations 1–3 μg/ml, indicate that $V_2O_5$ inhibits mitosis even at low concentrations. The decreased replicative index in the SCE assay (Table 3) and the percent relative survival decreased with increased concentrations (Table 4) are also consistent with the cytotoxicity of $V_2O_5$ in a concentration-dependent response. These data are in agreement with the observation of Roldán and Altamirano (1990), who showed that low concentrations (2–6 μg/ml) of vanadium in the pentoxide form produced a significant decrease in mitotic index in human lymphocytes. This is also consistent with the work of Schiff and Graham (1984). The cytotoxic effect of $VOSO_4$, $V_2O_5$, and oil fired fly ash (vanadium-rich) on hamster tracheal epithelium was observed in vitro. Morphological alterations consisted of vacuolization of both nuclei and cytoplasm, and respiratory mucosa was markedly affected. It has been reported that the effects of some metals on cell division include spindle disturbance, metaphase arrest, stathmokinesis, diplochromatid formation, chromosome lagging, polyploidy and ultimately, a decrease in the mitotic index (Sharma et al., 1987). Some of these responses may be attributed to the affinity of metals for sulfur ligands and consequently for the spindle proteins (Cherian, 1985). Vanadate (sodium metavanadate) in vitro has been shown to inhibit microtubule–dynecin interactions in ciliated epithelial cells (Buckley and Stewart, 1983) and to reversibly inhibit anaphase movement of chromosomes as well as spindle enlongation (Cande and Wolniak, 1978).

Treatment with $V_2O_5$ produced a significant increase in the frequencies of MN cells. Since MN can be formed either by acentric chromosomal fragment or whole chromosome, it was of interest to distinguish the type of MN induced by $V_2O_5$. The results of the antikinetochore antibody study indicate that the MN (88.7% at 3 μg/ml) induced by $V_2O_5$ contained at least one kinetochore-positive MN. Thus, it appears that MN formation in V79 cells exposed to $V_2O_5$ is due to spindle dysfunction, leading to chromosome lagging and the improper execution of mitosis. Ap-

parently, $V_2O_5$ is an aneuploidogenic agent. These findings are consistent with the observations of Singh (1979): that low doses of $V_2O_5$ poison the spindle in *Allium cepa*.

$V_2O_5$ failed to induce SCE or gene mutation in V79 cells. These results, in part, are in agreement with those reported by Roldán and Altamirano (1990) who showed that treatment of human lymphocytes with $V_2O_5$ did not increase the frequency of structural CA or SCE. However, $V_2O_5$ caused an increase in polyploid cell frequency and the frequency of cells with satellite associations. This phenomenon has been considered to be due to the predisposition of cells to an increased tendency of non-dysjunction in satellite chromosomes and to the induction of trisomies as well (Houghton, 1979). CA and SCE induced by pentavalent vanadium ($NH_4VO_3$) at concentrations 4–16 and 0.5–4.0 $\mu$g/ml, respectively, have been reported by Owusu-Yaw et al. (1990). Analysis of individual aberrations showed both structural and numerical (endoreduplications) aberrations. It is interesting to note that a high frequency of endoreduplication of chromosomes was observed in our present study when SCE were scored. The frequencies of endoreduplicative chromosomes were 6%, 8%, and 19% at 1 $\mu$g/ml, 2 $\mu$g/ml and 3 $\mu$g/ml of $V_2O_5$, respectively. Somatic aneuploidy occurs as a result of exposing cells to vanadium, and there is accumulating cytogenetic evidence that somatic aneuploidy is a significant chromosomal change in the development of cancer (Thompson and Perry, 1988).

## 5. Acknowledgments

We are grateful to Mr. William Moorman for generously providing the $V_2O_5$ samples. We wish to thank Drs. Stamm and Hubbs for their valuable suggestions and Mrs. Michael for typing the manuscript.

## 6. References

Abbondandolo, A., S. Bonnatis, G. Corti, R. Fioro, N. Loprianno and A. Mazzaccaro (1977) Induction of 6-thioguanine-resistance mutants in V79 Chinese hamster cells by mouse-liver microsome-activated dimethylnitrosamine, Mutation Res., 46, 365–373.

Bronzetti, G., E. Morichetti, C.D. Croce, R.D. Carratore, L. Giromini and A. Galli (1990) Vanadium: genetical and biochemical investigations, Mutagenesis, 5, 293–295.

Browning, E. (1969) Vanadium, in: Toxicity of industrial metals, Second edition, Butterworth, New York, 340 pp.

Buckley, I., and M. Stewart (1983) Ciliary but not saltatory movements are inhibited by vanadate, Cell Motility, 3, 167–184.

Cande, W.Z., and S.M. Wolniak (1978) Chromosome movement in lysed mitotic cells in inhibited by vanadate, J. Cell Biol., 79, 573–580.

Carpenter, G. (1981) Vanadate epidermal growth factor and the stimulation of DNA synthesis, Biochem. Biophys. Res. Commun., 102, 1115–1121.

Carson, B.L., H.V. Ellis and J.L. McCann (1987) Toxicology and biological monitoring of metals in humans, including feasibility and need, Lewis, pp. 276–282.

Channarayappa, J. Nath and T. Ong (1992) Immunofluorescent staining of kinetochores in micronucleus for detection of aneuploidy inducing agents, J. Tiss. Cult. Meth., 14, 125–132.

Cherian, G. (1985) Metallothionein and metal toxicity, CRC Press, Boca Raton, FL.

Clayton, G.D., and F.E. Clayton (1981) Vanadium, in: Patty's Industrial Hygiene and Toxicology, 3rd edn., Vol. IIA, Wiley, New York, pp. 2013–2033.

Donaldson, J., R. Hemming and F. Labella (1985) The effects of acute and chronic vanadium exposure on kidney lipid peroxidation were studied in rats and mice, Can. J. Physiol. Pharmacol., 63, 196–199.

Eastmond, D.A., and J.D. Tucker (1989) Kinetochore localization in micronucleated cytokinesis-blocked Chinese hamster ovary cells: A new and rapid assay for identifying aneuploidy-inducing agents, Mutation Res., 224, 517–525.

Galli, A., R. Vellosi, R. Fiorio, C. Della Croce, R. Del Carrator, E. Morichetti, L. Giromini, D. Resellini and G. Bronzetti (1991) Genotoxicity of vanadium compounds in yeast and cultured mammalian cells, Teratogen. Carcinogen. Mutagen., 11, 175–183.

Graedel, T.E., D.T. Hawkins and D.L. Claxton (1986) Atmospheric Chemical Compounds, Sources, Occurrence, and Bioassay, Academic Press, New York.

Hansen, K., and R. Stern (1984) A survey of metal-induced mutagenicity in vitro and in vivo, Toxicol. Environ. Chem., 9, 87–91.

Houghton, J.A. (1979) Relationship between satellite association and the occurrence of non-disjunction in man, Mutation Res., 61, 103–114.

Knecht, E.A., W.J. Moorman, J.C. Clark, D.W. Lynch and T.R. Lewis (1985) Pulmonary effects of acute vanadium pentoxide inhalation in monkeys, Am. Rev. Resp. Dis., 132, 1181–1185.

Krishna, G., M.L. Kropko and J.C. Thesis (1989) Use of the

cytokinesis-block method for the analysis of micronuclei in V79 Chinese hamster lung cells, results with mitomycin C and cyclophosphamide, Mutation Res., 222, 63–69.

Madany, I.M., and E. Raveendran (1992) Polycyclic aromatic hydrocarbons, nickel and vanadium in air particulate matter in Bahrain during the burning of oil fields in Kuwait, Sci. Tot. Environ., 116, 281–289.

Nechay, B.R., B. Nanninga, P.S.C. Nechay, R.L. Post, J.J. Grantaham, J.G. Macara, L.R. Kubenal, T.D. Phillips and F.H. Nielsen (1986) Role of vanadium in biology, Fed. Proc. Fed. Am. Soc. Exp. Biol., 45, 122–132.

Nestmann, E.R., R.L. Brillinger, J.P.W. Gilman, C.J. Rudd and S.H. Swierenga (1991) Recommended protocols based on a survey of current practice in genotoxicity testing laboratories, II. Mutation in Chinese hamster ovary, V79 Chinese hamster lung and L 5178 Y mouse lymphoma cells, Mutation Res., 246, 255–284.

NIOSH (1978) Occupational Health Guideline for vanadium pentoxide fumes, US Department of Health and Human Services, Sept., pp. 1–3.

Owusu-yaw, J., M.D. Cohen, S.Y. Fernando and C.I. Wei (1990) An assessment of the genotoxicity of vanadium, Toxicol. Lett., 50, 327–336.

Perry, P., and S. Wolff (1974) New Giemsa method for the differential staining of sister chromatids, Nature (London), 261, 156–158.

Petrucci, R.H. (1989) Vanadium, in: General Chemistry–Principles and Modern Applications, 5th edn., Plenum, New York, 877 pp.

Roldán, R.E., and L.M.A. Altamirano (1990) Chromosomal aberrations, sister-chromatid exchanges, cell-cycle kinetics and satellite association in human lymphocytes cultures exposed to vanadium pentoxide, Mutation Res., 245, 61–65.

Sabbioni, E., L. Clerici and A. Brazzelli (1983) Different effects of vanadium ions on some DNA-metabolizing enzymes, J. Toxicol. Environ. Health, 12, 737–748.

Sabbioni, E., G. Pozzi, A. Pintar, L. Casella and S. Garattini (1991) Cellular retention, cytotoxicity and morphological transformation by vanadium(IV) and vanadium(V) in BALB/3T3 cell lines, Carcinogenesis, 12, 47–52.

Schiff, L.J., and J.A. Graham (1984) Cytotoxic effect of vanadium and oil-fired fly ash on hamster tracheal epithelium, Environ. Res., 34, 390–402.

Serra, M.A., E. Sabbioni, A. Marchesini, A. Pintar and M. Valoti (1990) Vanadate as an inhibitor of plant and mammalian peroxidase, Biol. Trace Element Res., 23, 151–164.

Sharma, R.P., S.J.S. Flora, D.B. Drown and S.G. Oberg (1987) Persistence of vanadium compounds in lungs after intratracheal instillation in rats, Toxicol. Ind. Health, 3, 321–329.

Sheu, C.W., I. Rodrignez and J.K. Lee (1992) Proliferation and morphological transformation of BALB/3T3 cells by a prolonged treatment with sodium orthovanadate, Fd. Chem. Toxicol., 301, 307–311.

Singh, O.P. (1979) Effects of certain chemical pollutants on plant chromosomes, Ph.D. Thesis, University of Calcutta, India.

Singh, O.P., and A. Sharma (1980) Effects of certain metallic pollutants in plants genetic systems: a review, Nucleus, 23, 15–29.

Smith, J.B. (1983) Vanadium ions stimulate DNA synthesis in Swiss mouse 3T3 and 3T6 cells, Proc. Natl. Acad. Sci. (U.S.A.), 80, 6162–6166.

Stock, P. (1960) On the relations between atmospheric pollution in urban and rural localities and mortality from cancer, bronchitis, pneumonia, with particular reference to 3,4-benzopyrene, beryllium, molybdenum, vanadium and arsenic, Br. J. Cancer, 14, 397–418.

Thompson, E.J., and P.E. Perry (1988) The identification of micronucleated chromosomes: A possible assay for aneuploidy, Mutagenesis, 3, 415–418.

Waters, M.D., D.E. Gardner and D.L. Coffin (1974) Cytotoxic effects of vanadium on rabbit alveolar macrophages in vitro, Toxicol. Appl. Pharmacol., 28, 253–263.

WHO (1988) Task group on environmental health criteria for vanadium, Environmental Health Criteria 81, Vanadium, UNEP, ILO and WHO, WHO, Geneva.