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Zinc Chromate Induces Chromosome Instability and DNA Double Strand Breaks in Human Lung Cells

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Abstract

Hexavalent chromium Cr(VI) is a respiratory toxicant and carcinogen, with solubility playing an important role in its carcinogenic potential. Zinc chromate, a water insoluble or ‘particulate’ Cr(VI) compound, has been shown to be carcinogenic in epidemiology studies and to induce tumors in experimental animals, but its genotoxicity is poorly understood. Our study shows that zinc chromate induced concentration-dependent increases in cytotoxicity, chromosome damage and DNA double strand breaks in human lung cells. In response to zinc chromate-induced breaks, MRE11 expression was increased and ATM and ATR were phosphorylated, indicating that the DNA double strand break repair system was initiated in the cells. In addition, our data show that zinc chromate-induced double strand breaks were only observed in the G2/M phase population, with no significant amount of double strand breaks observed in G1 and S phase cells. These data will aid in understanding the mechanisms of zinc chromate toxicity and carcinogenesis.

Introduction

Exposure to hexavalent chromium (Cr(VI)) has been known for more than a century to be associated with induction of cancer in humans, causing an 18–80 fold increased risk of lung

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

cancer (IARC, 1990; Léonard and Lauwerys, 1980; Levy and Vanitt, 1986). However, different types of Cr(VI) compounds have different carcinogenic potencies. One of the key factors in the carcinogenicity of Cr(VI) compounds is water solubility (Langård, 1993). In animal experiments, it is the slightly soluble to highly insoluble particulate forms of Cr(VI) administered in their nonsolubilized particulate forms induced tumors (Patierno et al., 1988; Elias et al., 1991). These particulate forms are more potent probably due to their persistence within the lungs (Ishikawa et al., 1994a, b). Recent cell culture studies have shown that particulate lead chromate undergoes dissolution at the cell surface where the chromate ion is released, providing cells with a chronic exposure to Cr(VI) (Singh et al., 1999). Water soluble chromate compounds are clearly genotoxic, but are less potent carcinogens (IARC, 1990; Léonard and Lauwerys, 1980; Levy and Vanitt, 1986; Langård, 1993; Patierno et al., 1988), presumably because they do not persist in exposed tissue and are rapidly reduced by extracellular body fluids to the poorly absorbed Cr(III) form and therefore rarely reach high enough local concentrations in the immediate cellular microenvironment to cause cancer (Mancuso, 1997; Bencko, 1985).

Zinc chromate is an insoluble Cr(VI) compound and widely used for corrosion prevention and in pigments. Zinc chromate causes cancer in experimental animals following intrapleural and intrabronchial implantation (Langård, 1990; Levy et al., 1986). Epidemiologic studies also show a clear association between exposure to zinc chromate and lung cancer (Kano et al., 1993; Sheffet et al., 1982; Dalager et al., 1980; Davies 1984). However, the carcinogenic mechanisms of zinc chromate are poorly understood and the full spectrum of zinc chromate-induced genotoxic damage has not been established.

Most of the early studies on zinc chromate focused on correlating zinc chromate exposure and lung cancer incidence (Levy et al., 1986; Kano et al., 1993; Sheffet et al., 1982; Dalager et al., 1980; Davies, 1984; Langård and Vigander, 1983). Zinc chromate was found to have higher cancer risk than other particulate chromium compounds and to be the “prime causative agent” among lung cancer cases in chromium exposed workers (Langård, 1990). Cell culture studies also showed that zinc chromate induced morphological transformation of Syrian hamster embryo cells (Elias et al., 1991). Surprisingly, follow-up studies have not been done to investigate its carcinogenic mechanisms. Despite the fact that insoluble Cr(VI) compounds are more potent carcinogens, only two particulate Cr(VI) compounds have been studied in their target cells, human bronchial cells. These reports show that lead chromate induces DNA damage and chromosome aberrations, centrosome amplification, spindle assembly checkpoint bypass and neoplastic transformation, and that barium chromate induces chromosome damage in human bronchial cells (Wise et al., 2003; Xie et al., 2005; Holmes et al., 2006; Wise et al., 2006a; Wise et al., 2006b; Xie et al., 2007). To fully understand these particles as a class of carcinogens, more studies on Cr(VI) particles are necessary and have important implications for the risk assessment and regulation of these compounds. Accordingly, this study evaluated key aspects of zinc chromate genotoxicity in human lung cells.

Materials and Methods

Chemicals and Reagents

Zinc chromate, colcemid, and potassium chloride were purchased from Sigma/Aldrich. Giemsa stain was purchased from Biomedical Specialties Inc. (Santa Monica, CA). Gurr's buffer, trypsin/EDTA, sodium pyruvate, penicillin/streptomycin, and L-glutamine were purchased from Invitrogen Corporation (Grand Island, NY). Crystal violet and methanol were purchased from J.T. Baker (Phillipsburg, NJ). Dulbecco's minimal essential medium and Ham's F-12 (DMEM/F-12) 50:50 mixture was purchased from Mediatech Inc. (Herndon, VA). Cosmic calf serum (CCS) was purchased from Hyclone, (Logan, UT). Tissue culture dishes, flasks, and plasticware were purchased from Corning Inc. (Acton, MA).

Cells and Cell Culture

WTHBF-6 cells, a clonal cell line derived from primary human bronchial fibroblasts with reconstituted telomerase activity and a clastogenic and cytotoxic response to metals which is the same as their parent cells (Wise et al., 2004), were routinely cultured in DMEM/F-12 supplemented with 15% CCS, 2 mM L-Glutamine, 100 U/ml penicillin/100 ug/ml streptomycin, and 0.1 mM sodium pyruvate. Cells were maintained as adherent subconfluent monolayers by feeding at least twice weekly and subculturing at least once a week using 0.25% trypsin/1mM EDTA solution. Cells were tested routinely for mycoplasma contamination. All experiments were conducted on logarithmically growing cells.

Preparation of Chemicals

Zinc chromate (ACS reagent minimum 98% purity, Alfa Aesar, Ward Hill, MA) was used as a particulate Cr(VI) salt and suspensions of zinc chromate particles in cold sterile water were prepared. Dilutions were maintained as a suspension using a vortex mixer and treatments were dispensed into cultures directly from these suspensions.

Cytotoxicity and Clastogenicity Assays

Cytotoxicity was determined by a clonogenic assay measuring the reduction in plating efficiency in treatment groups relative to controls as previously described (Wise et al., 2002). Briefly, 200,000 cells were seeded in 5 ml of medium in a 60 mm tissue culture dish and then treated for 24 h with suspensions of zinc chromate. After treatment, cells were reseeded at colony forming density (1000 cells per 100 mm dish). The colonies were allowed to grow for 14 days; fixed with 100% methanol; stained with crystal violet; and the colonies counted. There were four dishes per treatment group and each experiment was repeated at least three times. Clastogenicity was determined by measuring the amount of chromosomal damage in treatment groups and controls exactly as previously described (Wise et al., 2002). Briefly, one hour before the end of the treatment time 0.1 ug/ml colcemid was added to arrest the cells in metaphase. Cells were then washed and collected by trypsinization. Cell pellets were resuspended in 0.075 M potassium chloride (KCl) hypotonic solution for 17 min to swell the cells and the nuclei followed by fixing with methanol: acetic acid (3:1). Finally, the cells were dropped on a clean wet slide and

uniformly stained using a 5% Giemsa stain in Gurr's buffer. One hundred metaphases per data point were analyzed in each experiment. Each experiment was repeated at least three times.

Immunofluorescence microscopy

Gamma-H2A.X (g-H2A.X) foci were used as a measurement of DNA double strand breaks and measured as previously described (20). Briefly, 7000 cells were seeded per chamber in an 8-well chamber slides. After treatment with Cr(VI) for 24 h, the cells were fixed in 4% paraformaldehyde, permeabilized with 0.2% Triton X-100 and blocked. Cells were then incubated with anti-g-H2A.X antibody (Cell Signaling, Beverly, MA) at 4 °C overnight and a FITC AlexaFluor 484-conjugated goat anti-rabbit Ig G second antibody (Molecular Probes, Eugene, OR) for 1 h. Images were obtained using Olympus BH2-RFCA fluorescence microscope fitted with a xenon lamp and a 100× objective.

Flow Cytometry for Cell Cycle Arrest and g-H2A.X Staining

For determining position in the cell cycle: Cell cycle position was determined based on our published methods (Xie et al., 2005). Briefly, cells were collected after zinc chromate treatment and fixed in ice cold 70% ethanol. After fixation, cells were digested in RNase A and stained with propidium iodide (PI) for 30 min. DNA content stained with PI in each cell cycle were detected by a BD FACS Calibur flow cytometer and analyzed with the Modifit LT modeling software package (version 3.0, Verity Software House, Topsham ME).

For determining DNA double strand breaks during the cell cycle: g-H2A.X expression was measured according to methods described by Huang and Darzynkiewicz (Huang and Darzynkiewicz, 2006). Cells were collected after zinc chromate treatment and fixed in 4% paraformaldehyde followed by ice cold 70% ethanol. Cells then incubated with anti-g-H2A.X antibody in BSA-Triton-X-PBS buffer overnight at 4 °C. Cells were washed with BSA-Triton-XPBS and then incubated with a FITC AlexaFluor 484-conjugated goat anti-rabbit IgG secondary antibody for 1 h. Cells then were washed again and stained with PI for 30 min. The intensity of FITC and PI fluorescence of the cells were measured by a BD FACS Calibur flow cytometer and analyzed with the WinList flow cytometry software package (version 6.0, Verity Software House, Topsham ME).

Mitotic Index

To determine if cells were accumulating in mitosis, we measured mitotic index. 500,000 cells were seeded per 100 mm dish and treated with varying concentrations of zinc chromate for 24 h. After treatment, cells were collected and incubated in 0.075 M KCl hypotonic solution for 10 min. Cells were then fixed, dropped on slides and stained as described in clastogenicity assays methods. The mitotic index was determined as the number of mitotic cells per 3000 total relative to control cells using light microscopy. Each experiment was repeated at least three times.

Western Blot Analysis

Protein expression was determined with western blot as previously described (Xie et al., 2005). Cells were lysed with 1× SDS sample buffer. Equal samples were resolved by

3%-8% tris-acetate SDS-PAGE or 10% Bis-Tris Gels (Invitrogen, Carlsbad, CA) and transferred to nitrocellulose membranes. The membranes were probed with rabbit polyclonal antibody against phosphorylated ATM (Rockland, Gilbertsville, PA) or phospho-ATR (Cell signaling, Danvers, MA) and Mre11 (GeneTex, San Antonio, TX). Equal protein loading was confirmed by immunoblotting with an antibody to constitutively expressed β -actin (Abcam, Cambridge, MA). The membranes were then probed with horseradish peroxidase conjugated secondary antibody for 1 h and the blots visualized by enhanced chemiluminescent (ECL) plus western blotting detection reagents (GE Healthcare, Buckinghamshire UK). Images were obtained with Storm Image System (Amersham Biosciences, Piscataway, NJ).

Apoptosis Assay

Apoptosis analyses were done based on a commercial kit protocol (Abcam, Cambridge, MA). Cells were seeded at 200,000 per 60 mm dish and treated with varying concentrations of zinc chromate for 24 h and then washed with PBS. 100,000 cells were collected with trypsin and suspended in Annexin V binding buffer. Cells then were incubated with Annexin V-FITC and PI. After incubation, cells were analyzed on a flow cytometer and the data analyzed with the WinList flow cytometry software package (version 6.0, Verity Software House, Topsham ME). 3 μ M camptothecin was used as a positive control.

Statistical Analysis

Where mentioned, values are shown as means $\pm$ SEM (standard error of the mean). Since the percentages calculated in repeated experiments at each treatment level are considered to be independent binomial measurements which can be approximated by a normal distribution, the standard independent two sample t test is valid to test the significance of differences between groups. It is expected that the variances of measurements at different treatment levels are different. We choose to use Satterthwart's approximated t test which assumes unequal variances between the two groups ($p < 0.05$ was considered significant).

Results

Zinc Chromate Is Cytotoxic and Genotoxic to Human Lung Cells

Zinc chromate induced concentration-dependent cytotoxicity in WTHBF-6 cells. Specifically, zinc chromate concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 μ g/cm² induced 76, 53, 29, 15 and 6 percent relative survival, respectively (Figure 1). Zinc chromate also induced a concentration-dependent increase in chromosome damage. Concentrations of 0, 0.1, 0.2, 0.3, 0.4 and 0.5 μ g/cm² zinc chromate damaged 4, 18, 28, 34, 45 and 50 percent of metaphases and induced 4, 21, 36, 41, 60 and 71 total chromosomal damage per 100 metaphases, respectively (Figure 2). No increase in aneuploid metaphases was observed (data not shown). The spectrum of chromosome damage included chromatid lesions, isochromatid lesions, dicentrics, double minutes, chromatid exchanges and acentric fragments with chromatid lesions the most common form of aberration.

Zinc Chromate Causes G2 Arrest

The G2/M checkpoint allows the cell to repair DNA damage before entering mitosis. Zinc chromate induced a G2/M arrest as evidenced by an accumulation of cells in G2 and a decrease of cells in mitosis in a concentration-dependent manner (Figure 3A). Specifically, a 24 h exposure to 0.1, 0.2, 0.3, 0.4 and 0.5 $\mu\text{g}/\text{cm}^2$ zinc chromate increased the cells in G2/M from 13.9 percent to 17.7, 20.4, 28.0, 30.6 and 35.2 percent respectively (Figure 3B). These concentrations also decreased the relative number of cells in mitosis (measured by a mitotic index) to 73, 63, 39, 32 and 20 percent of control, respectively (Figure 3C). Considered together, the increase in G2/M combined with a reduction in mitotic cells indicates that zinc chromate induced a G2 arrest.

Zinc Chromate Induces DNA Double Strand Breaks in Human Lung Cells

One of the most dangerous types of DNA damage is DNA double strand breaks. If not repaired or improperly repaired, double strand breaks will cause chromosome deletions or translocations, eventually leading to cell death or oncogenic transformation (Iliakis et al., 2004; Vamvakas et al., 1997). Induction of double strand breaks triggers phosphorylation of histone H2A.X on Ser 139 and it forms repair foci at the sites of damaged DNA. The presence of γ -H2A.X repair foci correlates with the frequency of double strand breaks (Rogakou et al., 1998; Rogakou et al., 1999). To test if zinc chromate induces DNA double strand breaks, we measured γ -H2A.X foci formation using immunofluorescence analysis. Concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 $\mu\text{g}/\text{cm}^2$ zinc chromate induced an average of 1.8, 3.4, 6.2, 10.4 and 9.0 foci per cell, respectively (Figure 4). Apoptosis was also measured after zinc chromate treatment by flow cytometry. No significant increase in apoptosis was found immediately after zinc chromate exposure when the DNA double strand breaks were measured (Figure 5), indicating that the foci we observed were from double strand breaks and not from apoptotic cells.

Double Strand Break Repair Proteins Are Activated in Human Lung Cells Exposed to Zinc Chromate

The DNA double strand break repair machinery involves many candidate sensor proteins and repair proteins which remain partly identified. The double strand break sensor Mre11–Rad50–Nbs1 (MRN) complex functions at an early stage of DNA double strand break repair pathway (Paull and Gellert, 1998). Ataxia-telangiectasia (A-T) mutated (ATM) is a major double strand break signal transducer targeting proteins that are critical for checkpoint signaling (Lee and Paull 2005) and is critical for activating the G1, S, and G2/M checkpoints. We found that Mre11 levels were increased in a concentration-dependent manner with some reduction at the highest dose (Figure 6, Table 1). ATM phosphorylation was also increased in a concentration-dependent manner (Figure 6, Table 1). ATR phosphorylation was also induced, though the greatest response was at the lower doses (Figure 6, Table 1.). These observations are consistent with a response to the production of DNA double strand breaks and cell cycle arrest.

Zinc Chromate-Induced Double Strand Breaks Occur in G2/M Phase

The origin of particulate Cr(VI)-induced breaks is uncertain. If breaks were direct, it would be expected that they would occur in all phases of the cell cycle. By contrast, if the breaks were indirect such as from a collapsed replication fork, then the breaks would likely be more prevalent in S or G2 phase. Based on differences in cellular DNA content, we subdivided cells into G1, S, G2/M phases. By gating-analysis of the gamma-H2A.X versus DNA content distributions the percent of gamma-H2A.X expressed cells was estimated for each phase of cell cycle. As the zinc chromate concentration increased more double strand breaks formed and that damage formation occurred in G2/M phase (Figure 7).

Discussion

Epidemiology (Davies, 1984; Langård and Vigander, 1983) and cell culture studies (Elias et al., 1989, 1991) all show that zinc chromate is a strong carcinogen. It targets human bronchial cells and causes lung cancer. However, the genotoxicity has not been investigated. This is the first study to report that zinc chromate induces chromosome damage and DNA double strand breaks and induces a DNA repair response. Zinc chromate is similar to other particulate chromate compounds, as it induces a concentration-dependent increase in chromosome damage. Zinc chromate is a more potent genotoxic agent than lead chromate, but similar to barium chromate. For example, at a concentration of 0.5 ug/cm², lead chromate damaged 25 percent of metaphases in these human bronchial cells (Wise et al., 2004b), while zinc chromate damaged 46 percent of metaphases at the same concentration (Figure 2). Barium chromate, however, was very similar to zinc chromate as 0.5 ug/cm² damaged 47 percent of metaphases in these cells (Wise et al., 2004b). The likely explanation for this difference in potency is a difference in cellular Cr ion uptake. Previously, we found that barium chromate and lead chromate had different genotoxic potency based on administered dose, but the same potency when compared by intracellular Cr level (Wise et al., 2004b). Alternatively, it could be a differential effect of their respective cations. For example, our previous data showed that lead ions are not involved in the cytotoxicity or clastogenicity of lead chromate (Wise et al., 2004c). Although zinc (Zn²⁺) is not categorized as a carcinogen, it can inhibit the activity of human N-methylpurine-DNA glycosylase in base excision repair (Wang et al., 2006). This inhibition may make zinc a contributing factor in zinc chromate genotoxicity. Regardless of the underlying mechanism, our observations support the epidemiological finding that the zinc chromate has a higher cancer risk than lead chromate (Levy, 1990).

We found that zinc chromate induced G2 arrest. This finding is consistent with some studies with soluble chromate that reported growth arrest at G2/M phase in human lung epithelial carcinoma cells (Zhang et al., 2001) and yeast (O'Brien et al., 2002). However, they differ from a report of soluble Cr(VI)-induced S-phase arrest in human skin fibroblasts (Ha et al., 2003), and a second report that soluble Cr(VI) induces cell accumulation in S-phase in which SMC1 was phosphorylated to facilitate S-phase arrest (Wakeman et al., 2004). The likely explanation for these differences are administered dose as the studies which found S-phase arrest used relatively high concentrations of Cr(VI) (over 10 uM) and did not consider possible G2/M arrest in their study.

In this study we found that the zinc chromate-induced DNA double strand breaks are predominantly generated at G2/M phase. This finding is consistent with Reynolds et al. report (2007) in which they found that soluble Cr(VI)-induced breaks were G2-phase specific as a result of proficient mismatch repair of the Cr-DNA adducts during DNA replication in human lung cells (Reynolds et al., 2007). Another study (Ha et al., 2004) reported that the Cr(VI)-induced double strand breaks were S-phase specific in human skin cells after 3 h treatment. The explanation for this difference is uncertain but it may be due to cell type and treatment time. The studies reporting G2-induced DNA double strand breaks used human lung cells treated for 24 h, while the study reporting S-phase induced DNA double strand breaks used skin fibroblasts treated for 3 h. Previously, we noted that skin cells appeared to be dramatically more sensitive to the cytotoxic effects of Cr(VI) than lung cells (Wise et al., 2002). It is also notable that Cr(VI) causes skin ulcers but not skin cancer, but does cause lung cancer (IARC, 1990). One hypothesis, albeit untested, is that lung cells can rapidly repair the initial S-phase induced breaks so that they do not show up after a 24 h exposure, while with skin cells they can not repair those breaks and instead undergo more rapid apoptosis. Such a hypothesis would be consistent with the greater cytotoxic sensitivity of skin, but more work is needed to see if it is accurate. Thus, these data are consistent with the conclusion that Cr(VI)-induced DNA double strand breaks are caused by indirect action, likely involving repair of ternary Cr-DNA adducts manifested as DNA crosslinks (Brooks et al., 2008; Reynolds et al., 2007; Ha et al., 2004). Several studies have shown that Cr(VI)-induced DNA double strand breaks require proficient excision repair and mismatch repair (Couv-Privat et al., 2007; Dronkert et al., 2001; Peterson-Roth et al., 2005), consistent with this hypothesis. One study showed that excision repair is required for Cr(VI) mutagenesis and Cr(VI)-induced chromosome damage (Brooks et al., 2008), suggesting that excision repair could mediate incision of cross-links leading to the formation of double strand breaks. Another study found that Cr(VI)-treated cells with proficient mismatch repair produced much higher levels of DNA double strand breaks resulting from Cr-DNA cross-links (Peterson-Roth et al., 2005). Given that nucleotide excision repair and mismatch repair are involved in repair of Cr DNA adducts and Cr-DNA cross-links (Dronkert et al., 2001; Peterson-Roth et al., 2005), it is likely that the formation of double strand breaks in G2/M phase are a consequence of DNA repair during replication.

ATR is believed to be required for checkpoint responses to agents that block DNA replication forks (Pichierri and Rosselli, 2004). ATR is an upstream initiator of the checkpoint response from various types of DNA lesions and is recruited by ATR-interacting protein (ATRIP) to stalled DNA replication forks (Falck et al., 2005; Cortez et al., 2001; Zou and Elledge, 2003). After its recruitment to sites of damage, ATR then phosphorylates a number of substrates which in turn target other proteins to induce cell-cycle arrest and facilitate DNA repair (Falck et al., 2005). We observed ATR phosphorylation in human lung cells after zinc chromate exposure at all concentrations considered, which is consistent with the observations by us here and others that Cr(VI) induces DNA double strand breaks via blocked replication forks (Reynolds et al., 2007; Ha et al., 2004). The Mre11-Rad50-Nbs1 (MRN) complex acts as a double strand break sensor and recruits ATM to broken DNA molecules (Lee and Paull, 2005). ATM kinase signals the presence of DNA double strand breaks by phosphorylating downstream proteins that initiate cell-cycle arrest, apoptosis, and

DNA repair. We observed that zinc chromate also induced concentration-dependent increases in Mre11 expression and ATM phosphorylation. These observations are consistent with our previous study using particulate lead chromate and soluble sodium chromate (Xie et al., 2005) and with studies showing that soluble Cr(VI) activates ATM in response to Cr(VI) exposure (Ha et al., 2003; Wakeman et al., 2004).

It is interesting to note that the ATR activation was greatest at the lower concentration, while ATM activation continued to increase with concentration. The explanation for this effect is uncertain but suggests that there is some switching in the signaling pathways with ATM predominating at the higher concentrations studied here. Future research is aimed at exploring the relationship of these changes in ATR and ATM activation and Cr(VI)-induced DNA repair and cell cycle arrest.

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Figure 1. Zinc Chromate Induces Cytotoxicity in Human Lung Cells

This figure shows that a 24 h exposure to zinc chromate induced concentration-dependent cytotoxicity in WTHBF-6 cells. Data represent an average of 3 independent experiments. Error bars = standard error of the mean. # Significantly different ($p < 0.005$) compared to control.

Figure 2. Zinc Chromate Induces Chromosome Instability

This figure shows that a 24 h exposure to zinc chromate induced concentration-dependent increases in the frequency of chromosome damage and in the total amount of chromosome damage in WTHBF-6 cells. Data represent an average of 3 independent experiments. Error bars = standard error of the mean. * Significantly different (p<0.001) compared to control. # Significantly different (p<0.005) compared to control.

A

B

C

Figure 3. Zinc Chromate Induces G2 Arrest in Human Lung Cells

This figure shows that a 24 h exposure to zinc chromate induced G2 arrest in WTHBF-6 cells. (A). Cells were analyzed for cell cycle position by flow cytometry. These representative flow figures show DNA content in each cell cycle phase. (B). Zinc chromate induced an increase in DNA content in G2/M phase. (C) Zinc chromate induced concentration-dependent decreases in the number of mitotic cells. * Significant ($p < 0.001$) differences compare to vehicle control. Significantly different ($p < 0.005$) compared to

control. ** Significantly different ($p < 0.05$) compared to control. Data represent a minimum of three independent experiments. Error bars = standard error of the mean.

Figure 4. Zinc Chromate Induces DNA Double Strand Breaks in Human Lung Cells

This figure shows a 24 h exposure to zinc chromate induced concentration-dependent increase in the average number of DNA double strand breaks (measured as gamma-H2A.X foci) in WTHBF-6 cells. Data represent a minimum of three independent experiments. Error bars = standard error of the mean. # Significant ($p < 0.005$) differences compare to vehicle control. ** Significant ($p < 0.05$) differences compare to vehicle control.

Figure 5. Zinc Chromate Does Not Induce Apoptosis

This figure shows a 24 h exposure to zinc chromate did not induce apoptosis in WTHBF-6 cells. Cells were treated with zinc chromate for 24 h and then immediately incubated with Annexin VFITC and PI which was detected by flow cytometry. 3 uM camptothecin was used as positive control. Data represent three independent experiments. Error bars = standard error of the mean.

Figure 6. Double Strand Break Repair Proteins Are Activated in Human Lung Cells After Zinc Chromate Exposure

This figure shows that a 24 h exposure to zinc chromate induced expression of Mre11 and activation of ATM and ATR in WTHBF-6 cells. Equal protein loading was confirmed by immunoblotting with anti β-Actin antibody. X-ray exposure was used as a positive control.

Figure 7. Zinc Chromate-Induced Double Strand Breaks Are G2/M Phase Specific

This figure shows that zinc chromate-induced DNA double strand breaks occur in G2/M phase in WTHBF-6 cells. The expression of gamma-H2A.X was measured concurrently with cellular DNA content by flow cytometry, and the data are shown as bivariate g-H2A.X vs DNA content distribution. Data represent a minimum of three independent experiments. Error bars = standard error of the mean. ** Significant ($p < 0.05$) differences compare to vehicle control.

Table 1

Particulate Cr(VI) Induces p-ATR, p-ATM and Mre11 Expression

Zinc Chromate Treatment (ug/cm ²)	p-ATR Expression (Percent of Control)	p-ATM Expression (Percent of Control)	Mre11 Expression (Percent of Control)
0	100	100	100
0.1	291.8	262.7	156.4
0.2	267.1	298.1	205.1
0.3	137.1	464.5	203.7
0.4	148.3	503.0	392.8
0.5	181.2	705.2	218.0
X-ray	598.5	2579.7	134.7