Chronic Exposure to Lead Chromate Causes Centrosome Abnormalities and Aneuploidy in Human Lung Cells


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Abstract
Hexavalent chromium [Cr(VI)] compounds are established human lung carcinogens. The carcinogenicity of Cr(VI) is related to its solubility, with the most potent carcinogens being the insoluble particulate Cr(VI) compounds. However, it remains unknown why particulate Cr(VI) is more carcinogenic than soluble Cr(VI). One possible explanation is that particulates may provide more chronic exposures to chromate over time. We found that aneuploid cells increased in a concentration- and time-dependent manner after chronic exposure to lead chromate. Specifically, a 24-hour lead chromate exposure induced no aneugenic effect, whereas a 120-hour exposure to 0.5 and 1 μg/cm² lead chromate induced 55% and 60% aneuploid metaphases, respectively. We also found that many of these aneuploid cells were able to continue to grow and form colonies. Centrosome defects are known to induce aneuploidy; therefore, we investigated the effects of chronic lead chromate exposure on centrosomes. We found that centrosome amplification in interphase and mitotic cells increased in a concentration- and time-dependent manner with 0.5 and 1 μg/cm² lead chromate for 120 hours, inducing aberrant centrosomes in 18% and 21% of interphase cells and 32% and 69% of mitotic cells, respectively; however, lead oxide did not induce centrosome amplification in interphase or mitotic cells. There was also an increase in aberrant mitosis after chronic exposure to lead chromate with the emergence of disorganized anaphase and mitotic catastrophe. These data suggest that one mechanism for lead chromate-induced carcinogenesis is through centrosome dysfunction, leading to the induction of aneuploidy. (Cancer Res 2006; 66(8): 4041-8)

Introduction
Hexavalent chromium compounds [Cr(VI)] are well-known human lung carcinogens (1–4). However, their carcinogenic mechanisms remain poorly understood. Epidemiologic, whole animal, and cell culture studies indicate that water solubility plays a key role in the carcinogenicity of Cr(VI) with water-insoluble or “particulate” Cr(VI) compounds as the most potent form (1–4). Human pathology studies show that Cr(VI) deposits and persists at bronchial bifurcations where Cr-associated cancers occur (5, 6), which is consistent with a particulate exposure.

It remains unclear why particulate Cr(VI) compounds have a greater carcinogenic potential than soluble Cr(VI) compounds. Studies using lead chromate as a model particulate Cr(VI) compound show that the particles partially dissolve outside the cell releasing Pb cations and chromate anions (7–9). The internalized Cr ions induce chromosome aberrations, DNA adducts, and DNA double-strand breaks (10–15), whereas the internalized Pb ions are generally nongenotoxic (8, 16). Two studies investigating potential epigenetic effects of Pb in lead chromate–induced carcinogenesis also show that Pb does not interfere with Cr-induced cell death (17) or cause mitotic stimulation of Cr-damaged cells (18). In addition, internalized particles seem to have no toxic effect except for possibly a small contribution to cytotoxicity (17). Thus, the differences between particulate and soluble exposures remain unclear.

One possible explanation is that particulates may provide more chronic exposures to chromate over time. The particulates may have an effect at bronchial bifurcation sites, leading to more chronic exposures, whereas soluble chromate is more rapidly cleared. However, no studies have considered chronic exposures to particulate chromate. All of the studies thus far focused on 24 hours or shorter time points (8, 10–16). Thus, in this study, we investigated longer exposure times to better mimic the exposures to particulate chromate in humans.

Lung cancers exhibit a high incidence of chromosomal instability (19). Specifically, 70% to 80% of malignant lung tumors exhibit a complex karyotype with severe aneuploidy, often a triploid to tetraploid complement of chromosomes (19). One possible mechanism of chromosomal instability is centrosome amplification, which is also a common event in lung cancer (19). Centrosome amplification is characterized by either an increase in centrosome number (>2) or in size. These centrosome abnormalities result in multipolar spindle formation and/or an increased microtubule nucleating capacity, which in turn causes chromosome missegregation and aneuploidy (as reviewed in ref. 20).

Metals do induce chromosomal instability and abnormal centrosomes. For example, one study found that vanadium inhibited the separation of the centrosome after duplication causing a monopolar spindle apparatus (21). Three studies on dimethylarsinic all revealed that arsenic induces multiple centrosomes and multipolar spindle formation, which leads to the induction of aneuploidy (22–24). One study on mercury revealed that methylmercury but not inorganic mercury induced aberrant centrosomes and multipolar spindles (25). Cell culture studies show that particulate chromate induces structural chromosomal instability in the form of chromosomal aberrations (8, 10–14). However, the effects of particulate chromate on numerical chromosomal instability and centrosome amplification have not been...
investigated. Accordingly, this study investigates the hypothesis that chronic exposures to lead chromate induce chromosomal instability and centromere dysfunction in human bronchial cells.

**Materials and Methods**

**Chemicals and reagents.** Lead chromate, lead oxide, colloidal, potassium chloride, magnesium sulfate, EGTA, PIPES, anti-γ-tubulin (clone GTU-88), anti-a-tubulin-FITC conjugate antibody, and Triton X-100 were purchased from Sigma-Aldrich (St. Louis, MO). Giemsa stain was purchased from Biomedical Specialties, Inc. (Santa Monica, CA). Alexa Fluor 555 goat anti-mouse IgG, TOPRO-3, and Prolong were purchased from Molecular Probes (Eugene, OR). Trypsin/EDTA, sodium pyruvate, penicillin/streptomycin, α-glutamine, and Gurr’s buffer were purchased from Invitrogen Corp. (Grand Island, NY). Methanol, acetone, and acetic acid were purchased from J.T. Baker (Phillipsburg, NJ). DMEM 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 normal human bronchial fibroblasts that ectopically express human telomerase, were used in all experiments. These cells have a similar clastogenic and cytotoxic responses to metals compared to their parent cells (13). Cells were maintained as subconfluent monolayers in DMEM/F-12 supplemented with 15% CCS, 2 mM/s/L α-glutamine, 100 units/mL penicillin/100 μg/mL streptomycin, and 0.1 mM/L sodium pyruvate (where incubated in a 5% CO₂ humidified environment at 37°C). They were fed thrice a week and subcultured at least once a week using 0.25% trypsin/1 mM/L EDTA solution. All experiments were done on logarithmically growing cells.

**Preparation of chemicals.** Lead chromate and lead oxide were administered as a suspension in acetone, as previously described (11). We treated with a concentration range of 0.1 to 1 μg/cm² lead chromate as it is consistent with the published literature on lead chromate, and we and others have shown this range to model both environmental and occupational exposures (10, 11).

**Aneuploid analysis.** Aneuploidy was determined by counting the number of chromosomes in solid stained metaphases. Preparation of chromosomes was done as previously described (11). Each experiment was repeated at least thrice. A minimum of 100 metaphases were analyzed per concentration.

**Clonogenic aneuploidy.** Cells were seeded into 60-mm dishes, and the monolayer of cells was treated with 0 or 0.5 μg/cm² lead chromate for 96 or 120 hours. The cells were then harvested and plated onto coverslips at a very low density so they would form colonies derived from a single surviving cell as opposed to forming a monolayer. Once the colonies were at least 20 to 30 cells, the cells were incubated for 23 minutes in 0.8% sodium citrate and fixed with 3:1 methanol/acetic acid. Cells were dried, aged, and stained with Giemsa. The number of chromosomes from metaphases in each colony was counted. All available colonies were scored up to 50 colonies.

**Mitotic stage analysis.** A monolayer of cells was seeded onto chamber slides and treated with 0 or 0.5 μg/cm² lead chromate for 96 or 120 hours. Cells were fixed in situ with 20:1 methanol/acetic acid, aged overnight, and stained with 5% Giemsa for 5 minutes. Mitotic figures were analyzed using light microscopy. Mitotic figures were scored, as defined in Yih et al. (26), considering stage (prophase, metaphase, anaphase, and telophase) or abnormal appearance, such as metaphase with lagging chromosomes (defined as containing some chromosomes that are not lined along the metaphase plate like the rest of chromosomes), ball metaphase (where chromosomes are all located in the center of the cell), c-metaphase (chromosomes are located throughout the cell and are highly condensed and still attached to its sister chromatid), anaphase with lagging chromosomes (one or more chromosomes are lagging behind the other chromosomes as they move to the two separate poles), disorganized anaphase (chromosomes have separated from its sister chromatid and are scattered throughout the cell in an unorganized fashion), and mitotic catastrophe (chromosomes are also very disorganized and located throughout the cell but have a streaky appearance to them). One hundred metaphases per concentration were scored.

**Centromere and microtubule analysis.** A monolayer of cells was seeded onto chamber slides and treated with 0, 0.1, 0.5, or 1 μg/cm² lead chromate for 24, 96, or 120 hours. Cells were washed twice in a microtubule stabilizing buffer (3 mM/L EGTA, 50 mM/L PIPES, 1 mM/L magnesium sulfate, 25 mM/L potassium chloride), fixed with 20°C methanol for 10 minutes, and rehydrated with 0.05% Triton X-100 for 3 minutes. Cells were then incubated in blocking buffer for 30 minutes followed by an hour incubation with a primary anti-γ-tubulin antibody (Sigma, St. Louis, MO; T-6357). Cells were washed four times with PBS and then incubated with Alexa Fluor 555 goat anti-mouse IgG secondary antibody for an hour in the dark. Cells were washed four times in PBS and then incubated with anti-a-tubulin/FITC conjugated antibody for 1 hour in the dark followed by four washes with PBS. A post-fix was done using 4% paraformaldehyde followed by two PBS washes for 3 minutes each. DNA was stained with TOPRO-3 for 30 minutes and then washed once with water. Coverslips were mounted with Prolong, and cells were analyzed using fluorescence microscopy. One hundred mitotic cells and 1,000 interphase cells were analyzed per concentration.

**Statistical analysis.** Statistical analyses were done on the number and percentage of cells having a particular abnormality. These values were averaged across the replicates (typically three) for a particular condition defined by dose and time. For each mean value, SEs were calculated based on the unbiased estimate of variance. Differences among means were evaluated using Student’s t test, with P < 0.05 taken as the criterion for statistical significance. All analyses were conducted using the SAS software package (27). Because all comparisons among means were considered to be of substantive interest a priori, no adjustment for multiple comparisons was incorporated into the analysis (28).

**Results**

**Chronic exposure to lead chromate causes aneuploidy.** To determine if chronic exposure to lead chromate induces chromosomal instability, manifested as aneuploidy, we treated WTHBF-6 cells with varying concentrations of lead chromate for 24, 48, 72, 96, or 120 hours. Chronic exposure to lead chromate induced a concentration- and time-dependent increase in aneuploidy (Fig. 1A). A 24-hour exposure to 0.1, 0.5, and 1 μg/cm² lead chromate induced no aneugenic effect, whereas after 120 hours, these concentrations induced 24%, 56%, and 60% aneuploid metaphases, respectively. Grouping the aneuploid cells based on chromosome number into hypodiploidy (<46 chromosomes), hyperdiploidy (between 47 and 91 chromosomes), and tetraploidy (92 chromosomes) revealed two different patterns depending on concentration.

One pattern occurred after chronic exposure to a very low concentration of lead chromate and manifested as an increase in tetraploid cells at 96 and 120 hours. Specifically, 0.1 μg/cm² lead chromate induced tetraploidy in 5.4% and 8.4% of metaphases at 96 and 120 hours, respectively. For this pattern, there was no increase in either hypodiploid or hyperdiploid cells. A second pattern emerged at low to moderate concentrations of lead chromate. Specifically, chronic exposure to these concentrations induced both tetraploidy, which steadily increased with time, as well as hypodiploidy. For example, 1 μg/cm² lead chromate induced tetraploidy in 5% of metaphases at 72 hours, which increased to 15% by 120 hours (Fig. 1D). It also induced an increase in the number of hypodiploid cells with 21% at 96 hours and 39% at 120 hours (Fig. 1B). Once again with this pattern, there was only a slight increase in hyperdiploid cells (Fig. 1C).
Lead Chromate Induces Chromosome Instability

Thus, chronic exposure to lead chromate induces tetraploidy at low concentrations and both tetraploidy and hypodiploidy at higher concentrations. These data suggest that lead chromate induces aneuploidy via two different mechanisms: one that drives the induction of tetraploidy and one that induces hypodiploidy cells.

To determine if these aneuploid cells were viable, we assessed their ability to form colonies. Figure 2A depicts a normal diploid colony and a representative metaphase, whereas Fig. 2B depicts an aneuploid colony and a representative metaphase with 92 chromosomes. We found that after 96 or 120 hours of exposure to 0.5 μg/cm² lead chromate, 7% and 14% of the colonies that formed had an aneuploid complement of chromosomes compared with 0.9% and 0% in the controls, respectively (Fig. 2C). Therefore, lead chromate induces a time- and concentration-dependent increase in aneuploidy, and a portion of these aneuploid cells is able to grow and form colonies.

Role of centrosome amplification in lead chromate–induced aneuploidy. Centrosome amplification is known to induce chromosomal instability; therefore, we investigated the possible role it plays in lead chromate–induced aneuploidy. We investigated lead chromate’s effect on both interphase and mitotic cells, focusing on 96 and 120 hours where the aneugenic effect was the greatest (Fig. 1A). Normal interphase cells have 1 or 2 centrosomes (Fig. 3A). Aberrant interphase cells have >2 centrosomes as illustrated by the lead chromate–treated cell in Fig. 3B. Lead chromate induced increases in aberrant centrosome number in interphase cells in a concentration- and time-dependent manner (Fig. 3C). After a 24-hour exposure to lead chromate, there was no centrosome amplification, but 96 and 120 hours of exposures caused significant increases in the number of cells with >2 centrosomes (Fig. 3C). Specifically, at 120 hours, 0.1, 0.5, and 1 μg/cm² lead chromate induced centrosome amplification in 7%, 18%, and 21% of interphase cells, respectively. The majority of these aberrant interphase cells had 3 or 4 centrosomes (Fig. 3C).

Normal mitotic cells have 2 centrosomes located on opposite poles that produce bipolar spindles and organize the chromosomes.
that formed from a cell that was treated with 0.5 \( \mu g/cm^2 \) lead chromate at 96 and 120 hours. We categorized normal mitoses into prophase, metaphase, anaphase, and telophase and placed abnormal mitoses into six categories, including metaphase with lagging chromosomes, c-metaphase, ball metaphase, anaphase with lagging chromosomes, disorganized anaphase, and mitotic catastrophe (Fig. 5A). We found that 34% of mitotic cells were abnormal after a 96-hour exposure to 0.5 \( \mu g/cm^2 \) lead chromate and 46% were abnormal after 120 hours of exposure (Fig. 5B). The majority of aberrant mitoses after 96 hours were in disorganized anaphase (6%), c-metaphase (7%), or mitotic catastrophe (15%). After 120 hours of exposure, the percentage of cells in mitotic catastrophe remained the same, but the percentage of cells in disorganized anaphase increased to 24%. Therefore, lead chromate induced a time-dependent increase in abnormal mitosis with the majority of aberrant mitoses in mitotic catastrophe and disorganized anaphase.

Many of the cells with extreme centrosome amplification had very disorganized chromosomes similar to those seen in disorganized anaphase or mitotic catastrophe in the mitotic progression analysis. Figure 6 shows a picture of a mitotic stage analysis cell in disorganized anaphase stained with Giemsa (Fig. 6A) alongside a centrosome analysis cell stained with TOPRO-3 (Fig. 6B). The chromosomes of these two cells look highly similar, suggesting that both cells are in disorganized anaphase. When we merged the centrosomes and microtubules with the cell stained with TOPRO-3, we found that the disorganized anaphase cell had 15 centrosomes and highly disorganized microtubule formation (Fig. 6C). Therefore, it seems that the mitotic figures we categorized as aberrant are most likely due to centrosome amplification.

Discussion

Hexavalent chromium is a well-known human lung carcinogen. Solubility plays a key role in Cr(VI) carcinogenicity, with the particulate Cr(VI) compounds being the most potent. Chromosomal instability is a common feature of lung tumors; however, the mechanism by which particulate Cr(VI) induces chromosomal instability remains unknown (19).

This is the first study to show that particulate chromate induces aneuploidy. One previous study found that a 30-hour exposure to soluble potassium dichromate induced hypodiploid cells but not hyperdiploid or tetraploid cells (29). In our study, hypodiploid cells did not start to increase until after 48 hours of exposure. We also observed tetraploidy after 72 hours of exposure (Fig. 1). Thus, it seems that it takes longer for particulate chromate to induce hypodiploid cells than soluble chromate. Longer exposures to particulate chromate also induce tetraploid cells, which did not occur after a soluble chromate exposure. The explanation for this discrepancy is uncertain, but it may be due to solubility or cation differences between particulate and soluble Cr(VI) compounds.

There is an interesting difference between exposure to very low concentrations of lead chromate and low to moderate concentrations. All three concentrations induced an increase in tetraploid
cells, but only the low to moderate concentrations of lead chromate also induced an increase in hypodiploid cells. This suggests that there are different mechanisms for lead chromate–induced hypodiploidy and tetraploidy. One important difference between these concentrations is that the very low concentration of lead chromate does not induce a significant increase in centrosomes amplification, whereas the low to moderate concentrations of lead chromate induce a high percentage of mitotic cells with centrosome amplification. Therefore, we hypothesize that centrosome amplification is inducing hypodiploidy, but tetraploidy is induced by a different mechanism possibly by effects on the spindle assembly checkpoint. It is important to note that the hypodiploidy occurs before tetraploidy at the two higher concentrations. Previous studies have suggested that tetraploidy is the first outcome of centrosome amplification (29). Our data indicate that this outcome is not the case for lead chromate–induced centrosome amplification.

Another more recent report by Shi and King shows that chromosome nondisjunction and the induction of binucleated tetraploid cells can induce aneuploid cells and aberrant centrosome numbers (30). However, we do not believe that lead chromate–induced aneuploidy and centrosome amplification occurs via this mechanism for several reasons. First, we found no increase in binucleated cells observed after chronic exposures to lead chromate (data not shown). Second, chromosome nondisjunction and the induction of tetraploidy can account for cells with 4 centrosomes but may not explain the cells with 3 centrosomes or >4 centrosomes that we also observe after longer exposures to lead chromate. It might be argued that a subsequent division of binucleated tetraploid cells can produce greater centrosome numbers; however, if this occurred, it would require either a tetraploid cell to fail to divide leading to a cell with 8 centrosomes and 184 chromosomes or an aneuploid cell with <8 centrosomes but between 46 and 92 chromosomes. In our aneuploid analysis, we did not see any cells with 184 chromosomes and relatively no increase in hyperdiploid cells. Lastly, centrosome dysfunction did not correlate with tetraploidy, because we observed more cells with aberrant centrosome number than cells with a tetraploid complement of chromosomes. Therefore, we do not believe that chromosome nondisjunction is a mechanism by which lead chromate induces centrosome amplification.

Aneuploidy is considered a well-known hallmark of cancer; however, whether it is an early-stage or late-stage event in tumorigenesis is still debated. Our data are consistent with pathology studies showing that the majority of lung tumors exhibit aneuploidy (19). It also suggests that aneuploidy may be an early

Figure 3. Chronic exposure to lead chromate induces centrosome amplification in interphase cells. A to B, depict a normal and an aberrant interphase cell.

**Table 1.**

<table>
<thead>
<tr>
<th>Chemical Concentration (μg/cm²)</th>
<th>Time (h)</th>
<th>Normal Centrosome Number</th>
<th>Aneuploid Centrosome Number</th>
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<td></td>
<td></td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>12 ± 0.7</td>
<td>59 ± 0.7</td>
</tr>
<tr>
<td>0.1 μg/cm²/LO</td>
<td></td>
<td>12 ± 0.7</td>
<td>59 ± 0.7</td>
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<tr>
<td>0.5 μg/cm²/LO</td>
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<td>10 μg/cm²/LO</td>
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<td>12 ± 0.7</td>
<td>59 ± 0.7</td>
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</table>

**Figure 3.** Chronic exposure to lead chromate induces centrosome amplification in interphase cells. A to B, depict a normal and an aberrant interphase cell.
event in tumorigenesis because we are seeing aneuploidy after only 2 to 3 days of lead chromate treatment. Other studies have also shown that relatively short exposures to other metals induce aneuploidy. Ochi et al. showed that both organic and inorganic arsenic can induce aneuploidy after only 48 hours of exposures in Syrian hamster embryo cells. Another study in normal human lung fibroblasts showed that a 30-hour exposure to cadmium chloride, potassium dichromate, or dimethylarsinic acid had an aneugenic effect (31). Therefore, these data suggest that aneuploidy is an early step in the development of cancer.

One mechanism by which aneuploidy occurs is via centrosome amplification. Aberrant centrosome numbers can cause multipolar spindle formation and unequal pulling of the chromosomes resulting in chromosome missegregation and aneuploidy. This study is the first to investigate and report that chronic exposure to particulate chromate induces centrosome amplification. No studies have considered soluble Cr(VI). There was no centrosome amplification after a 24-hour exposure to lead chromate, which is consistent with the aneuploid data showing no aneugenic effect after 24 hours. However, after 96 and 120 hours, lead chromate induced a concentration- and time-dependent increase in centrosome amplification in both mitotic and interphase cells, which is also consistent with the aneuploid data showing a concentration- and time-dependent increase in aneuploid cells as well (Figs. 1A, 3C, and 4D). These data are consistent with reports showing that organic arsenic and methylmercury are able to induce centrosome amplification (22–25) and aneuploidy (24).

The mitotic stage analysis data are consistent with the centrosome amplification data. The majority of aberrant mitoses induced after chronic lead chromate exposure were in mitotic catastrophe or disorganized anaphase (Fig. 5B), which was most likely a direct result of centrosome amplification (Fig. 6). Mitotic

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**Figure 4.** Chronic exposure to lead chromate induces centrosome amplification in mitotic cells. A, to C, normal and aberrant mitotic cells. Blue (TOPRO-3), DNA; green (FITC), microtubules; red (Alexa 555), centrosomes. A, a normal mitotic cell with two centrosomes, well-organized bipolar spindles, and chromosomes lined up along the metaphase plate. B, this cell is an aberrant mitotic figure with four centrosomes, multipolar spindle fiber, and disorganized DNA. C, an aberrant mitotic figure with 15 centrosomes and completely disorganized spindle fibers and DNA. The cells in (B) and (C) were treated with 0.5 μg/cm² lead chromate for 96 hours. D, centrosome amplification in mitotic cells increases in a time- and concentration-dependent manner after treatment with lead chromate (LC) but does not increase after exposure to lead oxide (LO). 

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<tr>
<th>Chemical Concentration (μg/cm²)</th>
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<th>Aberrant Centrosome Number</th>
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catastrophe is a type of cell death that occurs during mitosis. Cells with damaged DNA and/or aberrant spindle fibers combined with abrogation of the G2-M and/or the spindle assembly checkpoints often result in mitotic catastrophe (32). One study suggests that a possible outcome of overduplication of centrosomes is mitotic catastrophe (33). Cells that are able to escape mitotic catastrophe result in asymmetrical cell division or mitotic slippage, leading to the generation of aneuploid cells, which could possibly be an important mechanism in tumorigenesis (32). Our data are consistent with these findings.

The physicochemical mechanism of lead chromate–induced centrosome amplification is unknown. It is established that lead chromate partially dissolves outside the cell, releasing Pb and Cr ions that then enter the cell (9). Once inside the cell, the Cr ions induce the cytotoxic, growth-inhibiting, and genotoxic effects observed after exposure to lead chromate and the lead ions have no

![Figure 5. Chronic exposure to lead chromate induces aberrant mitosis. A, representative examples of normal and aberrant mitotic figures: 1, prophase; 2, metaphase; 3, anaphase; 4, telophase. 5 to 11, abnormal mitotic figures: 5, aberrant metaphase with lagging chromosomes (circled); 6, c-metaphase; 7, ball metaphase; 8, anaphase with lagging chromosome (circled), 9 to 10, disorganized anaphase. 11, mitotic catastrophe. B, effects of a 96- or 120-hour exposure to 0.5 μg/cm² lead chromate on the number of aberrant mitoses. The percentage of aberrant mitoses increases in a time-dependent manner with 96 or 120 hours of 0.5 μg/cm² lead chromate exposure inducing 34% and 46% abnormal mitoses.](image)

<table>
<thead>
<tr>
<th>Lead Chromate Concentration (μg/cm²)</th>
<th>Time (h)</th>
<th>Prophase</th>
<th>Metaphase</th>
<th>Anaphase</th>
<th>Telophase</th>
<th>Subtotal</th>
<th>Metaphase with Lagging Chromosomes</th>
<th>C-Metaphase</th>
<th>Ball Metaphase</th>
<th>Anaphase with Lagging Chromosomes</th>
<th>Disorganized Anaphase</th>
<th>Mitotic Catastrophe</th>
<th>Subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>96</td>
<td>15</td>
<td>8</td>
<td>22</td>
<td>29</td>
<td>29</td>
<td>0.3</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.5</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>21</td>
<td>28</td>
<td>1ann</td>
<td>0.3</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1ann</td>
<td>1ann</td>
</tr>
<tr>
<td>Control 0.5</td>
<td>120</td>
<td>35</td>
<td>44</td>
<td>17</td>
<td>24</td>
<td>90</td>
<td>0.3</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15ann</td>
<td>34ann</td>
</tr>
<tr>
<td>0.5</td>
<td>9</td>
<td>26</td>
<td>7</td>
<td>14</td>
<td>45</td>
<td>56</td>
<td>0.4</td>
<td>0.4</td>
<td>0.3</td>
<td>23.7</td>
<td>13</td>
<td>46ann</td>
<td>46ann</td>
</tr>
</tbody>
</table>

* Data reflect an average of three independent experiments; 100 mitotic cells per experiment were analyzed

† Vehicle control: acetone

‡ Statistically significant compared to control (p<0.003)
apparent effect (14, 15, 17, 18). Thus far, the potential effects of Cr ions on centrosome amplification have not been studied, but a 30-hour exposure to soluble chromate induced aneuploidy, which suggests that Cr ions could have the capacity to cause centrosome amplification, although that study did not consider it (31). On the other hand, it is possible that lead ions could contribute to lead-chromate–induced centrosome amplification. Studies show that lead ions do enter the cell after lead chromate exposure (17), and a recent study by Bonacker et al. showed that soluble lead ions disrupt microtubule function and formation (34). However, lead’s effect on centrosome function is unknown. A third possibility is that lead chromate–induced centrosome amplification could be due to physical interactions with the lead chromate particles themselves. Studies show that asbestos particles disrupt spindle formation and induce aneuploidy (35), suggesting that particles may also induce centrosome amplification. Thus, determining the relative contribution of chromium ions, lead ions, or the particles in lead chromate–induced centrosome amplification is an important future direction.

The overall hypothesis for particulate chromate-induced carcinogenesis indicates that particles dissolve outside the cell and enter the cell as their respective ions (9). Once inside the cell, the chromate ions are reduced to Cr(III) through a series of redox reactions releasing Cr(V), Cr(IV), and free radicals as intermediates (36). Cr(III), one of the intermediates or some combination of them induce chromosomal aberrations, adducts, cross-links, and strand breaks (7, 8, 11, 12, 37). It has been proposed that this genetic damage may result in mutations and ultimately carcinogenesis (17). However, it seems that not only does lead chromate damage DNA, but it also has a significant effect on centrosome and chromosome number. Therefore, we propose that lead chromate–induced carcinogenesis involves chronic exposure to both the chromate anion and the divalent cation inducing structural changes in the DNA and centrosome amplification leading to numerical changes in the chromosome complement. Further work is aimed at determining how lead chromate induces centrosome amplification and determining the potential mechanistic role of the spindle assembly checkpoint.

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