Comparative Effects of Particulate Hexavalent Chromium on Mitotic Stages in Human and Whale Lung Fibroblasts

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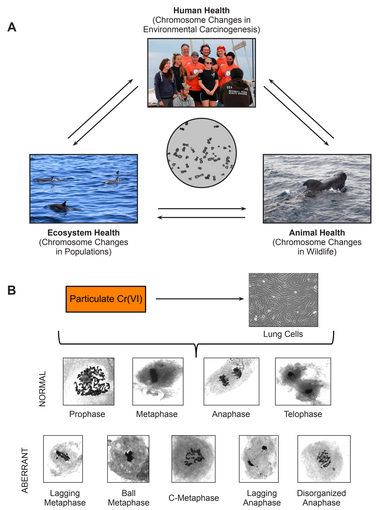
Research Question

Hexavalent chromium [Cr(VI)] is a known carcinogen and genotoxicant. The carcinogenic mechanism is unknown, but the prevailing theory is that it causes cancer by inducing chromosome instability. One possible mechanism for chromium-induced numerical chromosome instability is by disrupting centrosomes. Centrosome dysfunction leads to aberrant mitosis. Here we study the effects of zinc chromate on mitosis. Using a One Environmental Health approach, we analyzed whale lung cells and human lung cells.

Therefore, this project asks the question: Is there a difference in mitotic stage disruption in human and whale lung fibroblasts when exposed to hexavalent chromium?

A One Environmental Health Approach



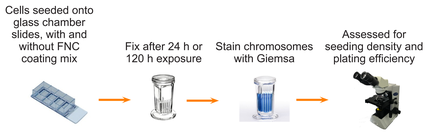
A) We pursue a One Environmental Health approach, focusing on genomic instability. **B)** In this study, we studied the effects of particulate Cr(VI) on whale and human lung cells. In Aim 1, We optimized cell densities to the needs of the study. In Aim 2, the effect of the particulate Cr(VI) on mitotic index determined. In Aim 3, mitotic stage analysis was conducted to classify the stages as normal or aberrant to see if whale cells are more or less resistant to mitotic damage induced by particulate Cr(VI), compared to human lung cells.

Aim 1: Optimizing Protocol for Slide Seeding

Why we did it:

In this Aim we determined the seeding densities need to study normal human lung cells (NHLF) and immortalized human lung cells (Cl6) in order reduce the number of experiments with too high/too low seeding density. This will maximize time to perform experiments, analyze the data, and reduce lab equipment use.

How we did it:



Human lung fibroblasts (NHLF and Cl6) were seeded in two-well slides for 24 h tests and one-well chamber slides for 120 h tests. Cells were tested both with and without FNC coating mix on the slides. After 24 h or 120 h, the cells were fixed and stained with Giemsa. Slides were evaluated for confluency, even distribution of cells, and plating efficiency.

What we found:

Cell Line	NHLF	NHLF	NHLF	NHLF	NHLF	Cl6
Test Number	1	1	1	1	2	1
Time Point	24 h	24 h	120 h	120 h	120 h	120 h
FNC-Coated?	Yes	No	Yes	No	Yes	Yes
Seeding Density	18K 24K	18K 24K	8K	8K	12K	10K
Confluency	18K: 80% 24K: 87%	18K: 60% 24K:65%	32%	40%	15%	15%
Results	18K: Nice and even 24K: Good, not patchy	18K: Scorable 24K: Dense in some places	Little patchy, empty space, seed more than 8K	Patchy, blank spaces	Essentially no cells	Essentially no cells

What does it mean?

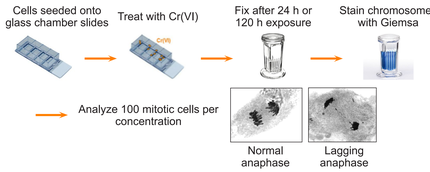
The optimal seeding density for FNC-coated NHLF cells were 18K-24K for a 24 h exposure. For 120 h exposure, both Cl6 and NHLF were difficult to grow, possibly due to experimental error. The 120 h slides should have FNC-coated slides and more than 8K for both NHLF and Cl6 cells.

Aim 2: Assessing Mitotic Index in Human and Whale Cells

Why we did it:

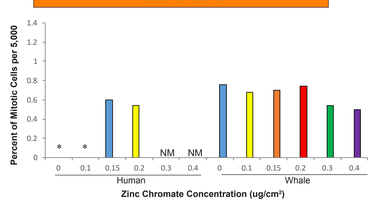
Zinc chromate is known to arrest human cells in interphase. Thus, we investigated whether this outcome occurs in whale cells.

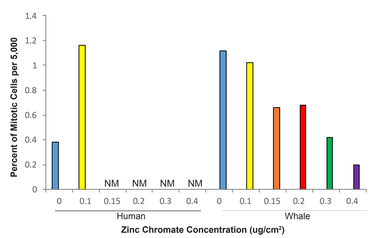
How we did it:



Primary whale lung fibroblasts (BHW200Lu) and human lung fibroblasts (Cl6) were seeded in either two-well or one-well chamber slides. The cells were treated with varying concentrations of zinc chromate. After 24 h or 120 h, the cells were fixed and stained with Giemsa. The mitotic index was counted based on 5,000 cells for both time points and all treatment concentrations.

What we found:





*These slides have yet to be scored for mitotic index. NM = No mitosis. Treatments yielding fewer than 10 mitotic cells were classified as NM.

What does it mean?

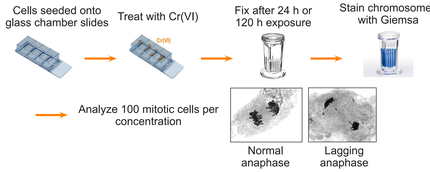
Human cells appear to be more sensitive to Cr(VI)-induced cell cycle arrest. Both cell types show decreased mitotic index after 120 h exposure compared to 24 h. This outcome means zinc chromate arrested cells in interphase at higher concentrations and over a long period of time. This experiment will need to be repeated to gather more evidence and confirm these results.

Aim 3: Assessing Aberrant Mitosis in Human and Whale Cells

Why we did it:

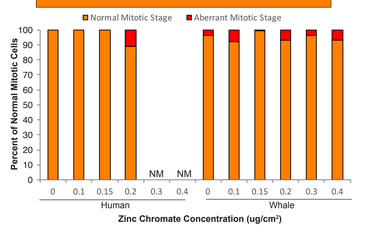
Zinc chromate is a known carcinogen and genotoxicant and may induce cancer by causing chromosome instability. Chromosome instability can result from aberrant mitosis, which may lead to cancer. Zinc chromate induces aberrant mitosis such as lagging metaphase/anaphase, C-metaphase, ball metaphase, disorganized anaphase, and mitotic catastrophe in human cells, but its effects in whale cells are unknown

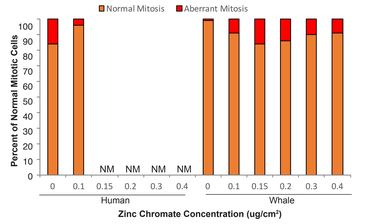
How we did it:



Primary whale lung fibroblasts (BHW200Lu) and human lung fibroblasts (Cl6) were seeded in either two-well or one-well chamber slides. The cells were treated with varying concentrations of zinc chromate. After 24 h or 120 h, the cells were fixed and stained with Giemsa. Normal and aberrant mitotic stages were scored. A total of 100 mitotic cells were analyzed per concentration.

What we found:





NM = No mitosis. Treatments yielding fewer than 10 mitotic cells were classified as NM.

What does it mean?

Human cells appear to be more sensitive to Cr(VI)-induced cell cycle arrest. It appears that instances of aberrant mitosis increase after 120 h exposure compared to 24 h. This means, zinc chromate may disrupt mitotic mechanisms, which leads to aberrant mitosis. Aberrant mitoses are evidence of centrosome abnormalities. This experiment will need to be repeated in order to confirm these results.

Take Home Message

Whale cells appear to be less affected by the ability of zinc chromate to prevent cells from entering mitosis. This outcome suggests there is a different response to particulate Cr(VI) in humans and whales at a cellular level.

Further Reading

Holmes, A.L., Wise, S.S., Sandwick, S.J., Lingle, W.L, Negron, V.C., Thompson, W.D., and Wise, J.P. Chronic Exposure to Lead Chromate Causes Centrosome Abnormalities and Aneuploidy in Human Lung Cells. Cancer Res, 66(8): 4041-4048, 2006

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Acknowledgements

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