Published in final edited form as:

Comp Biochem Physiol C Toxicol Pharmacol. 2010 June ; 152(1): 91–98. doi:10.1016/j.cbpc.2010.03.001.

The Cytotoxicity and Genotoxicity of Hexavalent Chromium in Steller Sea Lion Lung Fibroblasts Compared to Human Lung Fibroblasts

John Pierce Wise Sr^{a,b,c,d,e,*}, Sandra S. Wise^{a,b,c,d}, Amie L. Holmes^{a,b,c}, Carlyne LaCerte^{a,b,c,d}, Fariba Shaffiey^{a,b}, and AbouEl-Makarim Aboueissa^f

^aWise Laboratory of Environmental and Genetic Toxicology, P.O. Box 9300, 96 Falmouth St. Portland, ME 04104-9300 USA

^bMaine Center for Toxicology and Environmental Health, University of Southern Maine, P.O. Box 9300, 96 Falmouth St. Portland, ME 04104-9300 USA

^cDepartment of Applied Medical Sciences, 96 Falmouth St., Portland, ME 04104 USA

^dOcean Alliance, 191 Weston Rd., Lincoln, MA 01773 USA

^eMystic Aquarium, 55 Coogan Blvd., Mystic, CT 06355 USA

^fDepartment of Mathematics & Statistics, University of Southern Maine, ME, 04104 USA

Abstract

In this study we directly compared soluble and particulate chromate cytotoxicity and genotoxicity in human (*Homo sapiens*) and sea lion (*Eumetopias jubatus*) lung fibroblasts. Our results show that hexavalent chromium induces increased cell death and chromosome damage in both human and sea lion cells with increasing intracellular chromium ion levels. The data further indicate that both sodium chromate and lead chromate are less cytotoxic and genotoxic to sea lion cells than human cells, based on administered dose. Differences in chromium ion uptake explained some but not all of the reduced amounts of sodium chromate-induced cell death. By contrast, uptake differences could explain the differences in sodium chromate-induced chromosome damage and particulate chromate-induced toxicity. Altogether they indicate that while hexavalent chromium induces similar toxic effects in sea lion and human cells, there are different mechanisms underlying the toxic outcomes.

Keywords

chromate; chromium; cytotoxicity; genotoxicity; sentinel; Steller sea lion

© 2010 Elsevier Inc. All rights reserved

*Correspondence: Dr. John Pierce Wise, Sr. Phone: +1 (207) 228-8050 FAX: +1 (207) 228-8518 John.Wise@usm.maine.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest There are no conflicts of interest.

Introduction

Hexavalent chromium [(Cr(VI))] is an occupational and environmental concern (ATSDR, 2008; IARC, 1990). Cr-contaminated waste is released to the environment, affecting air, soil and water including the oceans (ATSDR, 2008). Cr(VI) is widely acknowledged to be a human carcinogen with genotoxicity including chromosomal aberrations and DNA strand breaks recognized as the major mechanism for its carcinogenicity (Wise S et al., 2008). Cr(VI) is also a reproductive concern. Human and rodent studies show that Cr(VI) accumulates in the testes and cause a reduction in testicular weights, seminiferous tubule degeneration, decreased sperm counts and altered reproductive behaviors (Al-Hamood et al., 1998; Witmer et al., 1991), and Cr(VI) genotoxicity may contribute to or exacerbate these concerns as well.

The oceans are a common sink for pollutants, putting aquatic species at risk (Chung, 1986; Hixon et al., 1991). In the ocean, Cr(VI) is the predominant state for Cr (Geisler and Schmidt, 1991; Pettine and Millero, 1990), and we recently reported that Cr levels can accumulate to high levels in marine mammal species (Wise J et al., 2008; 2009). We also recently found that Cr(VI) is genotoxic to Steller sea lion cells, inducing chromosomal aberrations (Wise S et al., 2009). The Steller sea lion is an endangered species whose population has dramatically declined in recent years for uncertain reasons (Calkins et al., 1999; Loughlin and York, 2000). These declines are not considered natural population fluctuations, but rather due to changes in environmental conditions (Pascual and Adkison, 1994). Possible factors include nutritional stress, reduced fecundity and environmental contaminants, among others (Barron et al., 2003; Merrick, 1999).

Comparing our genotoxicity results to published literature, we noted that Cr(VI) appeared to be less genotoxic to Steller sea lion lung cells than human lung cells. These comparisons suggested that sea lions may have evolved cellular mechanisms to better protect themselves from genotoxic threats (Wise S et al., 2009), though no direct comparisons between the species have been made. Accordingly, in this study we directly compared the cytotoxicity and clastogenicity of Cr(VI) in Steller sea lion lung cells and human lung cells to see if there is a difference in response.

Materials and Methods

Chemicals and Reagents

Sodium chromate (Na_2CrO_4), lead chromate (PbCrO_4), demecolchicine and potassium chloride (KCl) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Giemsa stain was purchased from Biomedical Specialties Inc. (Santa Monica, CA, USA). Cytoseal 60 slide mounting medium was purchased from VWR (Bridgeport, NJ, USA). Gurr's buffer, trypsin/EDTA, sodium pyruvate, penicillin/streptomycin, and L-Glutamine were purchased from Invitrogen Corporation (Grand Island, NY, USA). Crystal violet, methanol and acetone were purchased from J.T. Baker (Phillipsburg, NJ, USA). Dulbecco's minimal essential medium and Ham's F-12 (DMEM/F-12) 50:50 mixture was purchased from Mediatech Inc. (Herndon, VA, USA). Cosmic calf serum (CCS) was purchased from Hyclone, (Logan, UT, USA). Tissue culture dishes, flasks, and plasticware were purchased from Corning Inc. (Acton, MA, USA).

Cells and Cell Culture

Steller sea lion lung cells were primary cell lines, isolated from tissue explants obtained from stranded animal biopsies, as described previously (Wise S et al., 2009). WTHBF-6 cells are hTERT-expressing human lung fibroblasts, a clonal cell line derived from primary human bronchial fibroblasts (PHBF), exhibiting a diploid karyotype, normal growth parameters and an extended lifespan (Wise S et al., 2004a). Both cell lines were cultured as adherent

monolayers of cells, being sub-cultured at least once a week. Cells were maintained in Dulbecco's minimal essential medium and Ham's F-12 (DMEM/F-12) 50:50 mixture, supplemented with 15% cosmic calf serum, 2 mM L-glutamine, 100 U/mL penicillin/100 µg/mL streptomycin, and 0.1 mM sodium pyruvate. Sea lion and human cells were maintained in a 37 °C humidified incubator with 5% CO₂. Cells were regularly tested for mycoplasma contamination. All experiments were conducted on logarithmically growing cells with a doubling time of 24 h for both cell lines.

Preparation of Chromium Compounds

Sodium chromate (Na₂CrO₄, CAS #7775-11-3, ACS reagent minimum 98% purity), was used as a representative soluble Cr(VI) compound and administered as a solution in water as previously described (Wise S et al., 2004a). Briefly, Na₂CrO₄ was weighed and dissolved in deionized water. Desired treatment concentrations were made from a filtered sample of the stock solution.

Lead chromate (PbCrO₄, CAS# 7758-97-6, ACS reagent minimum 98% purity), was used as a representative particulate Cr(VI) compound and administered as suspensions of particles as previously described (Wise S et al., 2004a) to ensure that cells were exposed to intact particles. Briefly, PbCrO₄ was weighed, suspended in acetone to sterilize the particles and desired treatment concentrations were made from this stock solution.

Cytotoxicity Assays

Cytotoxicity was measured by the colony-forming assay, as described previously (Wise S et al., 2004a; 2009). Briefly, logarithmically growing cells were seeded into each well of a 6-well plate and treated for 24 h with 0.05, 0.1, 0.5, 1, 5, or 10 µg/cm² lead chromate or 0.1, 0.5, 1, 2.5, 5, 10, or 25 µM sodium chromate. At the end of the exposure time, cells were washed with phosphate-buffered saline (PBS) and then removed from the dish with 0.25 % trypsin/EDTA. Cells were counted and seeded into 100 mm tissue culture dish at a density of 1000 cells per dish; four dishes were seeded for each concentration and allowed to grow until colonies formed. Colonies were then stained with crystal violet. Three to eight independent experiments were performed and four dishes were analyzed for each concentration for each experiment.

Chromosome Preparation

Cells were prepared as described previously (Wise S. et al., 2004a; 2009). Briefly, logarithmically growing cells were seeded into 100 mm tissue culture dishes and treated for 24 h with 0.1, 0.5, 1, or 5 µg/cm² lead chromate or 1, 2.5, 5, or 10 µM sodium chromate. At the end of the treatment cells were harvested and placed in a hypotonic solution of 0.075 M KCl for 17 min followed by fixation in 3:1 methanol:acetic acid. The fixative was changed twice. The cells were dropped onto clean wet slides and uniformly stained with Giemsa stain. Three to five independent experiments were performed. One hundred metaphases per each treatment dose were analyzed for chromosome aberrations, according to standard criteria previously described (Wise S et al., 2004a; 2009).

Determination of Intracellular Chromium Ion Levels

Cr(VI) enters cells via an anion transport system and once inside the cells, while its valence state may change, the amount of chromium does not (Wise S et al., 2008). In addition, while there are chromate efflux pumps in bacteria, none have yet been identified in mammalian cells. This difference is probably due to both the rapidity with which Cr(VI) is reduced to Cr(III) in these cells and the affinity for the intracellular Cr(VI) species for intracellular ligands (Wise S et al., 2008). Thus, our measure of total intracellular Cr serves as a measure of the amount of chromium uptake.

Cell preparation

Cells were prepared as previously described (Holmes A et al., 2005), with minor changes. Briefly, logarithmically growing cells were seeded into 100 mm tissue culture dishes and then treated for 24 h with 0.1, 0.5, 1, 5, or 10 $\mu\text{g}/\text{cm}^2$ lead chromate or 0.5, 1, 2.5, 5, 10, or 25 μM sodium chromate. At the end of the treatment, cells were washed with PBS and the number and volume of cells were determined. Cells were then washed three times in PBS and then resuspended in 0.075 M KCl hypotonic solution for 5 min followed by 15 min in 2% SDS. Finally the solution was sheered through an 18 gauge needle and filtered through a 0.2 μm filter. Samples were stored at -20°C until metal analysis. Three to nine independent experiments were performed.

Ion levels measurements

Intracellular Cr ion levels were determined using the Inductively Coupled Plasma-Optical Emission Spectrometers (ICP-OES), equipped with a gem cone low flow nebulizer, according to previously published methods (Holmes A et al., 2005). Solutions were introduced to the nebulizer using a peristaltic pump operating at 2 mL/min. Samples of intracellular fluids were diluted 5 $\times$ in 0.16 M aqueous HNO_3 prior to analysis. Chromium was determined using emission wavelength at 267.716 with a minimum detection limit of 2 ppb. Yttrium (Y) was used as an internal standard for chromium determinations. The intracellular concentrations were converted from $\mu\text{g}/\text{L}$ to μM by dividing by the volume of the sample, the atomic weight of the chemical, the number of cells in the sample and the average cell volume.

Statistics

The T-test was used to determine significant differences between the Steller sea lion cells and human cells at the same concentration. The analysis of covariance (ANCOVA) was used to determine whether there was a significant difference between Steller sea lion and human lung cells lines and between lead chromate and sodium chromate lines for each graph based on intracellular Cr concentration.

Results

Steller sea lion cells take up less Cr than human cells

Administered dose does not necessarily reflect the intracellular ion concentration. To account for potential differences in Cr uptake between the two cell lines, the intracellular levels of Cr were determined after a 24 h exposure. Both cell lines took up increasing amounts of Cr ions in a concentration-dependent manner (Figure 1). Specifically, 2.5, 5 and 10 μM sodium chromate produced intracellular Cr levels of 440, 713, and 1520 μM , respectively in Steller sea lion lung cells, and 1195, 2740 and 4313 μM , respectively, in human lung cells (Figure 1A). The pattern was similar for lead chromate with human cells again taking up more chromium. Specifically, 0.1, 0.5, 1 and 5 $\mu\text{g}/\text{cm}^2$ lead chromate produced intracellular Cr levels of 44, 198, 229 and 583 μM , respectively, in Steller sea lion lung cells and 131, 677, 1095 and 2062 μM , respectively, in human lung cells (Figure 1B). These data indicate the Steller sea lion cells had less chromium uptake after exposure to either particulate or soluble chromate.

Cr(VI) is less cytotoxic to Steller sea lion cells than human cells

Lead chromate and sodium chromate were cytotoxic to both cell lines in a concentration-dependent manner (Figure 2). In sea lion lung cells, concentrations of 1, 2.5, 5, 10 and 25 μM reduced relative survival to 104, 99, 92, 58 and 11%, respectively, and 68, 41, 5, 0 and 0%, respectively, in human lung cells (Figure 2A). For lead chromate, in sea lion lung cells, concentrations of 0.1, 0.5, 1, 5 and 10 $\mu\text{g}/\text{cm}^2$ lead chromate reduced relative survival to 95,

88, 91, 70 and 52 %, respectively, and 88, 67, 42, 9 and 1%, respectively, in human lung cells (Figure 2B).

These data indicate that both sodium chromate and lead chromate are less cytotoxic to sea lion cells than to human cells, based on administered dose. Of course it is possible that the mechanism for these differences is differential Cr uptake into the cells, since Figure 1 shows less uptake in sea lion cells. Correcting for uptake differences, the data show that for sodium chromate (Figure 2C), the difference in Cr(VI) potency between the cell lines lessens when the data are compared by intracellular level. These data indicate that differential uptake contributes to the differences in cytotoxicity, but it cannot explain all of it; thus, other mechanisms must be involved. In contrast, differences in uptake appear to account for the differences in lead chromate-induced cytotoxicity (Figure 2D).

It is interesting to note that lead chromate and sodium chromate seem to have different cytotoxic mechanisms. Previous studies in humans indicated that the Cr(VI) ions are responsible for lead chromate's cytotoxicity and that Pb ions play no role (Holmes et al., 2005). Accordingly, we compared the cytotoxicity of lead chromate and sodium chromate, in sea lion cells, based on administered dose. The data show that there is less cytotoxicity in sodium chromate-treated cells than the lead chromate-treated cells at similar intracellular Cr levels (Figure 2E).

Cr(VI) is less genotoxic to Steller sea lion cells than human cells

Sodium chromate was genotoxic to both cell lines in a concentration-dependent manner (Figure 3). First considering the frequency of damage in human cells, 1 and 2.5 μM sodium chromate damaged chromosomes in 19 and 39% of metaphases, respectively, while 5 μM induced cell cycle arrest with no metaphases seen (Figure 3A). In Steller sea lion lung cells, sodium chromate was less genotoxic, as concentrations of 1.0, 2.5, 5 and 10 μM damaged chromosomes in 11, 21, 36 and 39 % of metaphases, respectively (Figure 3A). When considering the effect by the amount of total chromosome damage in 100 metaphases, the same trend was found. Concentrations of 1.0, 2.5, 5 and 10 μM sodium chromate damaged 12, 27, 49, and 57 chromosomes per 100 metaphases in Steller sea lion lung cells, respectively; and in human lung cells, 1 and 2.5 μM damaged 22 and 47 chromosomes per 100 metaphases, respectively (Figure 3B). The spectrum of sodium chromate-induced damage consisted mainly of chromatid and isochromatid lesions and was not different between the cell lines (Table 1 and 2).

Lead chromate also induced chromosomal damage in a concentration-dependent manner (Figure 4). Considering the frequency of damage in human lung cells, concentrations of 0.1, 0.5, and 1 $\mu\text{g}/\text{cm}^2$ damaged chromosomes in 8, 18 and 32% of metaphases, respectively. Cell cycle arrest was induced at 5 $\mu\text{g}/\text{cm}^2$ and no metaphases were seen (Figure 4A). In Steller sea lion cells, lead chromate was less genotoxic, as concentrations of 0.1, 0.5, 1 and 5 $\mu\text{g}/\text{cm}^2$ lead chromate damaged chromosomes in 9, 13, 18 and 23 % of metaphases, respectively (Figure 4A). The same trend was found when considering total chromosome damage in 100 metaphases (Figure 4B). Concentrations of 0.1, 0.5, 1 and 5 $\mu\text{g}/\text{cm}^2$ damaged 9, 15, 20 and 30 chromosomes, respectively, in Steller sea lion lungs cells; and in human lung cells, 0.1, 0.5, and 1 $\mu\text{g}/\text{cm}^2$ damaged 8, 20 and 41 chromosomes, respectively (Figure 4B). The spectrum of lead chromate-induced damage also consisted mainly of chromatid and isochromatid lesions and was not different between the cell lines (Table 1 and 2).

These data indicate that both sodium chromate and lead chromate are less genotoxic to sea lion cells than human cells, based on administered dose. Of course it is possible that the mechanism for these differences is differential Cr uptake into the cells, since Figure 1 shows less uptake in sea lion cells. Correcting for the greater chromium uptake in the human cells shows that, for sodium chromate (Figures 3C, 3D), the difference in Cr(VI) genotoxicity is explained by differential uptake. However, for cytotoxicity, differential uptake contributed to the

differences, but it could not explain all of it indicating that other mechanisms must be involved. By contrast, for lead chromate-induced cytotoxicity and genotoxicity, uptake does appear to account for the difference between the cell lines (Figures 4C, 4D).

It is interesting to note that lead chromate and sodium chromate seem to operate by different mechanisms. Previous studies in human cells indicated that the Cr(VI) ions are responsible for lead chromate's cytotoxicity and genotoxicity and that Pb ions play no role (Holmes et al., 2005; Wise S et al., 2004b). Accordingly, we compared the genotoxicity of lead chromate and sodium chromate in sea lion cells based on intracellular Cr concentration (Figure 5). The data suggest that the two compounds are similar.

Discussion

Few data have been presented directly comparing the response of human and marine mammal model systems to test whether marine mammals and humans respond similarly to toxicants. This study is the first to directly compare the cytotoxic and genotoxic effects of a pollutant of concern (Cr(VI)) for the marine environment in both human and Steller sea lion cells. Our data indicate that sea lion lung cells respond similarly to Cr(VI) as human lung cells, but that there are some different intracellular mechanisms occurring.

In particular, for soluble Cr(VI), there appear to be differences in the mechanisms underlying its cytotoxicity. We found, after correcting for differences in Cr uptake, that the sea lion cells are less sensitive than human cells to soluble Cr(VI)-induced cytotoxicity. The reason for this decreased sensitivity is uncertain. The current paradigm for Cr(VI)-induced toxicity was recently reviewed (Wise S et al., 2008). It indicates that Cr(VI) ions cross the cell membrane through an anion transport system and are reduced inside the cell to Cr(III) via a series of intermediates. Then Cr(III) and/or one or more of these intermediates adducts DNA to cause a stalled replication fork, which the cell attempts to repair, and during that repair, DNA double strand breaks are created. These breaks then go on to form the chromosomal aberrations. One possibility, albeit untested, is that human cells may be more efficient in recognizing this damage and signaling to the apoptosis machinery more effectively than the sea lion cells. Alternatively, the sea lion cells may have a different intracellular metabolism that reduces the amount of the adducting Cr species produced. A different outcome was observed for particulate chromate. Differential Cr uptake appeared to account for all of the differences in lead chromate-induced cytotoxicity and genotoxicity. This outcome is inconsistent with the results we observed for soluble chromate and suggest that the Pb ion may be playing a role in lead chromate's effects. Comparisons of soluble chromate and lead chromate in sea lion cells alone, at similar intracellular Cr levels, are consistent with these observations and show more cell death after lead chromate exposure, but similar amounts of genotoxicity for both compounds. Previous studies in human cells indicated that Pb ions do not play a role in lead chromate-induced cytotoxicity and genotoxicity (Holmes et al., 2005; Wise S et al., 2004b). Thus, Steller sea lion cells appear to be different in this regard, and an interesting hypothesis that emerges from our study is that Pb ions may be contributing to lead chromate-induced apoptosis in sea lion cells. This hypothesis would explain our observations with soluble chromate (less cytotoxicity, but similar genotoxicity) and lead chromate (similar cytotoxicity and genotoxicity due to Pb inducing apoptosis), but more work is needed to confirm this hypothesis. Pb ions have been shown to induce apoptosis in a number of human and rodent cell types (Pulido and Parrish, 2003), though it is unknown if Pb induces apoptosis in Steller sea lion lung cells.

It is interesting to note that the Steller sea lion lung cells take up less Cr(VI) than the human lung cells. The mechanism for this difference is uncertain. Chromate uptake is mediated by the anion transport proteins responsible for phosphate and sulfate transport into the cells and is not dependent on intracellular reducing agents for that uptake (Wise S et al., 2008). Lead chromate

particles can enter the cell by phagocytosis, but when they do, they not dissolve inside the cell or contribute to chromium uptake or chromosome damage (Xie et al., 2004; Holmes et al., 2005; Wise S et al., 2004b). Thus, the mostly likely, albeit untested, explanation is that the sea lion cells simply have lower levels of these transport proteins. Alternatively, the sea lion cells may have evolved a different and less efficient transport system for chromate; perhaps one more specific to sulfate and phosphate. Further research is needed to distinguish between these possibilities.

The Western Steller sea lion population, which used to be abundant in Alaskan waters, has suffered a 75% population reduction from 1979 to 1997 (Calkins et al., 1999) and is still decreasing (Loughlin and York, 2000). The decline is considered to be the result of as of yet undetermined change(s) in environmental conditions (Pascual and Adkison, 1994) including nutritional stress (Merrick, 1995), reduced fecundity (Pitcher et al., 1998) and environmental contaminants (Barron et al., 2003). These new data suggest that, with respect to metal contaminants, the sea lion response is likely to reflect the human response. Thus, it may be possible to extend human risk assessments to the sea lion population.

In summary, our data demonstrate that Steller sea lion lung fibroblasts are less sensitive than human cells to the cytotoxic but not the genotoxic effects of sodium chromate and exhibit a similar sensitivity to human cells after lead chromate treatment. In addition, the data generate an important hypothesis concerning relative DNA repair in the two cell lines and the potential ability of Pb to inhibit that repair. Further work is aimed at investigating this hypothesis.

Acknowledgments

The authors would like to acknowledge the late David St. Aubin for his efforts and enthusiasm in helping to start this project. We would like to thank Carrie Goertz, Larry Dunn and Frances Gulland for technical assistance and Christy Gianios, Jr. for administrative and information technology support. This work was supported by Grant number NA16FX1412 from the United States Department of Commerce, National Oceanic and Atmospheric Administration (J.P.W.); National Institute of Environmental Health Sciences Grant numbers ES10838 (J.P.W.) and ES016893 (J.P.W.), and the Maine Center for Toxicology and Environmental Health.

References

- Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for Chromium. U.S. Department of Health and Human Services, Public Health Service; Atlanta, GA: 2008.
- Al-Hamood MH, Elbetieha A, Bataineh H. Sexual maturation and fertility of male and female mice exposed prenatally and postnatally to trivalent and hexavalent chromium compounds. *Reprod. Fertil. Dev* 1998;10:179–183. [PubMed: 9801270]
- Barron MG, Heintz R, Krahn MM. Contaminant exposure and effects in pinnipeds: implications for Steller sea lion declines in Alaska. *Sci. Total Environ* 2003;311:111–133. [PubMed: 12826388]
- Bataineh H, Al-Hamood MH, Elbetieha A, Bani Hani I. Effect of long-term ingestion of chromium compounds on aggression, sex behavior and fertility in adult male rat. *Drug. Chem. Toxicol* 1997;20:133–149. [PubMed: 9292274]
- Calkins DG, McAllister DC, Pitcher KW. Steller sea lion status and trend in southeast Alaska: 1979–1997. *Mar. Mamm. Sci* 1999;15:462–477.
- Chowdhury AR, Mitra C. Spermatogenic and steroidogenic impairment after chromium treatment in rats. *Indian J. Exp. Biol* 1995;33:480–484. [PubMed: 7590955]
- Chung YS. Air pollution detection by satellites: The transport and deposition of air pollutants over oceans. *Atmos. Environ* 1986;20:617–630.
- Geisler CD, Schmidt D. An overview of chromium in the marine environment. *Dt. hydrogr. Z* 1991;44:185–196.
- Hixon, MA.; Boersma, PD.; Hunter, ML., Jr.; Micheli, F.; Norse, EA.; Possingham, HP.; Snelgrove, PVR. Oceans at Risk: Research Priorities in Marine Conservation Biology. In: Soulé, ME.; Orians,

- GH., editors. Conservation Biology: Research Priorities for the Next Decade. Island Press; Washington, D.C.: 2001. p. 125-154.
- Holmes AL, Wise SS, Xie H, Gordon N, Thompson WD, Wise JP Sr. Lead ions do not cause human lung cells to escape chromate-induced cytotoxicity. *Toxicol. Appl. Pharmacol* 2005;203:167–176. [PubMed: 15710177]
- IARC. Monographs on the Evaluation of Carcinogenic Risks to Humans: Chromium, Nickel and Welding. Vol. Vol. 49. International Agency for Research on Cancer; Lyons, France: 1990.
- Loughlin TR, York AE. An accounting of the sources of Steller sea lion, *Eumetopias jubatus*, mortality. *Mar. Fish. Rev* 2000;62:40–45.
- Mancuso TF. Chromium as an industrial carcinogen: Part II. Chromium in human tissues. *Am. J. Ind. Med* 1997;2:140–7. [PubMed: 9028429]
- Merrick, RL. PhD thesis. University of Washington; Seattle: 1995. The relationship of the foraging ecology of Steller sea lions (*Eumetopias jubatus*) to their population decline in Alaska.
- Pascual MA, Adkison MD. The decline of the Steller Sea Lion in The Northeast Pacific: Demography, Harvest or Environment? *Ecol. Appl* 1994;4:393–403.
- Pettine M, Millero FJ. Chromium speciation in seawater: The probable role of hydrogen peroxide. *Limnol. Oceanogr* 1990;35:730–736.
- Pitcher KW, Calkins DG, Pendleton GW. Reproductive performances of female Steller sea lions: an energetics-based reproductive strategy? *Can. J. Zool* 1998;76:2075–2083.
- Pulido MD, Parrish AR. Metal-induced apoptosis: mechanisms. *Mutat. Res* 2003;533:227–241. [PubMed: 14643423]
- Witmer CM, Harris R, Shupack SI. Oral bioavailability of chromium from a specific site. *Environ. Health Perspect* 1991;92:105–110. [PubMed: 1935839]
- Witmer CM, Park H-S, Shupack SI. Mutagenicity and disposition of chromium. *Sci. Total Environ* 1989;86:131–148. [PubMed: 2690337]
- Wise JP Sr. Wise SS, Little JE. The Cytotoxicity and Genotoxicity of Particulate and Soluble Hexavalent Chromium in Human Lung Cells. *Mutat. Res* 2002;517:221–229. [PubMed: 12034323]
- Wise JP Sr. Wise SS, Kraus S, Shaffiey F, Grau M, Li Chen T, Perkins C, Thompson WD, Zheng T, Zhang Y, Romano T, O'Hara T. Hexavalent chromium is cytotoxic and genotoxic to the North Atlantic right whale (*Eubalaena glacialis*) lung and testes fibroblasts. *Mutat. Res* 2008;650:30–38. [PubMed: 18006369]
- Wise JP Sr. Payne R, Wise SS, LaCerte C, Wise J, Gianios C Jr. Thompson WD, Perkins C, Zheng T, Zhu C, Benedict L, Kerr I. A global assessment of chromium pollution using sperm whales (*Physeter macrocephalus*) as an indicator species. *Chemosphere* 2009;75:1461–1467. [PubMed: 19324391]
- Wise SS, Elmore LW, Holt SE, Little JE, Bryant BH, Wise JP Sr. Telomerase-mediated lifespan extension of human bronchial cells does not affect hexavalent chromium-induced cytotoxicity or genotoxicity. *Mol. Cell. Biochem* 2004a;255:103–111. [PubMed: 14971651]
- Wise SS, Holmes AL, Ketterer ME, Hartsock WJ, Fomchenko E, Katsifis SP, Thompson WD, Wise JP Sr. Chromium is the proximate clastogenic species for lead chromate-induced clastogenicity in human bronchial cells. *Mutat. Res* 2004b;560:79–89. [PubMed: 15099827]
- Wise SS, Holmes AL, Wise JP Sr. Particulate and soluble hexavalent chromium are cytotoxic and genotoxic to human lung epithelial cells. *Mutat. Res* 2006;610:2–7. [PubMed: 16872863]
- Wise SS, Holmes AL, Wise JP Sr. Hexavalent chromium-induced DNA damage and repair mechanisms. *Rev. Environ. Health* 2008;23:39–57. [PubMed: 18557597]
- Wise SS, Shaffiey F, LaCerte C, Goertz CEC, Dunn JL, Gulland FMD, Aboueissa A, Zheng T, Wise JP Sr. Particulate and soluble hexavalent chromium are cytotoxic and genotoxic to Steller sea lion lung cells. *Aquat. Toxicol* 2009;91:329–335. [PubMed: 19157580]
- Xie H, Holmes AL, Wise SS, Gordon N, Wise JP Sr. Lead chromate-induced chromosome damage requires extracellular dissolution to liberate chromium ions but does not require particle internalization or intracellular dissolution. *Chem. Res. Toxicol* 2004;17:1362–1367. [PubMed: 15487897]

Figure 1. Human Lung Cells Take Up Greater Amounts of Cr(VI) than Steller Sea Lion Lung Cells
 This figure shows that exposure to Cr(VI) induces Cr ion uptake in both human and Steller sea lion lung cells in a concentration-dependent manner. Data represent mean $\pm$ S.E. ($n = 3$ or 9). (A) Cr uptake after soluble chromate treatment. $25 \mu\text{M}$ sodium chromate was not measured for WHTBF-6 cells, because this concentration yielded 0% relative survival and no metaphases. (B) Cr uptake after particulate chromate treatment. $10 \mu\text{g}/\text{cm}^2$ lead chromate was not measured for WHTBF-6 cells, because this concentration yielded 0% relative survival and no metaphases. ^aSignificant differences between human and Steller sea lion cells using t-test (p -value < 0.05). ^bSignificant differences between human and Steller sea lion lines using ANCOVA (p -value < 0.05).

A)

B)

c)

D)

E)

Figure 2. Cr(VI) Is More Cytotoxic to Human Lung Cells than Steller Sea Lion Lung Cells

This figure shows that exposure to Cr(VI) induces cytotoxicity in human and Steller sea lion lung cells in a concentration-dependent manner based on administered dose ($p < 0.01$). Data represent mean $\pm$ S.E. ($n = 3$ or 8). (A) Soluble Cr(VI) cytotoxicity based on administered dose. (B) Particulate Cr(VI) cytotoxicity based on administered dose. (C) Cytotoxicity of soluble chromate based on intracellular Cr ion level. (D) Cytotoxicity of particulate chromate based on intracellular Cr ion level. (E) Cytotoxicity of both particulate and soluble chromate based on intracellular Cr ion level in Steller sea lion lung cells. ^aSignificant differences between human and Steller sea lion cells using t-test (p -value < 0.05). ^bSignificant differences between human and Steller sea lion lines or lead chromate and sodium chromate lines using ANCOVA (p -value < 0.05).

Figure 3. Soluble Cr(VI) Is More Genotoxic to Human Lung Cells than Steller Sea Lion Lung Cells
This figure shows that exposure to soluble Cr(VI) induces genotoxicity in both human and Steller sea lion cells in a concentration-dependent manner. Genotoxicity is presented as a

percent of metaphases with damage and total chromosome damage for each cell line. NM – no metaphases observed. Data represent mean $\pm$ S.E. (n = 3 or 5). **(A)** Frequency of metaphases with chromosome damage based on administered dose. Human cells showed more metaphases with damage than Steller sea lion cells. At higher concentrations, no metaphases were observed in human lung cells. **(B)** Soluble Cr(VI)-induced total chromosome damage in 100 metaphases based on administered dose. Human cells showed more chromosome damage than Steller sea lion cells. At higher concentrations no metaphases were observed in human lung cells. **(C)** Frequency of metaphases with chromosome damage based on intracellular Cr ion level. **(D)** Total chromosome damage in 100 metaphases based on intracellular Cr ion level. ^aSignificant differences between human and Steller sea lion cells using t-test (p-value < 0.05). ^bSignificant differences between human and Steller sea lion lines using ANCOVA (p-value < 0.05).

A)

B)

Figure 4. Particulate Cr(VI) Is More Genotoxic to Human Lung Cells than Steller Sea Lion Lung Cells

This figure shows that exposure to particulate Cr(VI) induces genotoxicity in both human and Steller sea lion cells in a concentration-dependent manner. Genotoxicity is presented as a percent of metaphases with damage and total chromosome damage for each cell line. NM – no metaphases observed. Data represent mean $\pm$ S.E. (n = 3–9). **(A)** Particulate Cr(VI)-induced metaphases with chromosome damage based on administered dose. Human cells showed more metaphases with damage than Steller sea lion cells. At 5 $\mu\text{g}/\text{cm}^2$, no metaphases were observed in human lung cells. **(B)** Particulate Cr(VI)-induced total chromosome damage in 100 metaphases based on administered dose. Human cells showed more chromosome damage than Steller sea lion cells and at 5 $\mu\text{g}/\text{cm}^2$, no metaphases were observed in human lung cells. **(C)** Frequency of metaphases with chromosome damage based on intracellular Cr ion level. **(D)** Total chromosome damage in 100 metaphases based on intracellular Cr ion level. ^aSignificant differences between human and Steller sea lion cells based on administered concentration using t-test (p-value < 0.05). ^bSignificant differences between human and Steller sea lion lines using ANCOVA (p-value < 0.05).

Figure 5. Cr(VI)-Induced Genotoxicity in Steller Sea Lion Cells Based on Intracellular Cr Ion Levels

This figure shows that exposure to Cr(VI) induces genotoxicity with increasing intracellular Cr ion levels in Steller sea lion cells in a concentration-dependent manner. Soluble and particulate Cr(VI) induce a similar amount of damage in Steller sea lion lung cells. Data represent mean $\pm$ S.E. (n = 3–9). (A) Genotoxicity presented as a percent of metaphases with chromosome damage. (B) Genotoxicity presented as total chromosome damage in 100 metaphases.

Table 1

Spectrum of Chromosome Damage in Steller Sea Lion Lung Cells

Sodium Chromate Concentration (uM) ^a	Chromatid Lesions				Isochromatid Lesions			
	Chromatid Gaps	Chromatid Breaks	Total Chromatid Lesions (Gaps + Breaks)	Isochromatid Gaps	Isochromatid Breaks	Total Isochromatid Lesions (Gaps + Breaks)	Chromatid Exchange	Acentric Fragment
0	3 ± 1.5	2 ± 0.6	5 ± 1.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0.3
1	7 ± 1.3	4 ± 1.5	12 ± 1.9 ^d	0 ± 0.3	0 ± 0	0 ± 0.3	0 ± 0	0 ± 0
2.5	14 ± 5	11 ± 2.2	24 ± 6.6	1 ± 0.6	2 ± 0.7	2 ± 0.9	0 ± 0	0 ± 0
5	28 ± 6.4	16 ± 5	45 ± 11.2	0 ± 0.3	4 ± 0.3 ^c	4 ± 0.6 ^c	0 ± 0	0 ± 0
10	27 ± 7.4	21 ± 5	48 ± 12.4	1 ± 0.6	3 ± 1.5	4 ± 1.8	0 ± 0	0 ± 0
Lead Chromate Concentration (ug/cm ²) ^b	Chromatid Gaps	Chromatid Breaks	Total Chromatid Lesions (Gaps + Breaks)	Isochromatid Gaps	Isochromatid Breaks	Total Isochromatid Lesions (Gaps + Breaks)	Chromatid Exchange	Acentric Fragment
	Chromatid Gaps	Chromatid Breaks	Total Chromatid Lesions (Gaps + Breaks)	Isochromatid Gaps	Isochromatid Breaks	Total Isochromatid Lesions (Gaps + Breaks)	Chromatid Exchange	Acentric Fragment
0	4 ± 1.3	1 ± 0.6	5 ± 1.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.1	5 ± 0.6	3 ± 0.6	8 ± 1	0 ± 0	1 ± 0.6	1 ± 0.6	0 ± 0	0 ± 0
0.5	9 ± 1.7	6 ± 1.5	15 ± 3.2	0 ± 0	0 ± 0.3	0 ± 0.3	0 ± 0	0 ± 0
1	10 ± 3.5	8 ± 2.4	18 ± 5.3	1 ± 0.7 ^d	0 ± 0	1 ± 0.7 ^d	0 ± 0	0 ± 0.3
5	17 ± 3.3	12 ± 2 ^c	29 ± 5.3 ^c	0 ± 0.3	1 ± 0.3	1 ± 0.6	0 ± 0	0 ± 0

^a Administered as a solution in water

^b Administered as a suspension in acetone

^c Statistically different from control

^d Statistically different from human lung.

Table 2

Spectrum of Chromosome Damage in Human Lung Cells

Sodium Chromate Concentration (uM) ^a	Chromatid Lesions				Isochromatid Lesions			
	Chromatid Gaps	Chromatid Breaks	Total Chromatid Lesions (Gaps + Breaks)	Isochromatid Gaps	Isochromatid Breaks	Total Isochromatid Lesions (Gaps + Breaks)	Chromatid Exchange	Acentric Fragment
0	2 ± 0	0 ± 0.3	2 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1	10 ± 0.7 ^c	9 ± 1.8 ^c	19 ± 1.2 ^{c,d}	1 ± 0.9	1 ± 0.7	2 ± 0.6	0 ± 0.3	1 ± 0.7
2.5	23 ± 3.1 ^c	18 ± 4.7	41 ± 4.7 ^c	2 ± 0.5	2 ± 1.5	5 ± 1.9	1 ± 0.6	0 ± 0.3
5	NM	NM	NM	NM	NM	NM	NM	NM
10	NM	NM	NM	NM	NM	NM	NM	NM
Lead Chromate Concentration (ug/cm ²) ^b	Chromatid Gaps	Chromatid Breaks	Total Chromatid Lesions (Gaps + Breaks)	Isochromatid Gaps	Isochromatid Breaks	Total Isochromatid Lesions (Gaps + Breaks)	Chromatid Exchange	Acentric Fragment
0	2 ± 0.3	1 ± 0	3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
0.1	5 ± 0.9	3 ± 1.5	8 ± 0.7 ^c	0 ± 0.3	0 ± 0	0 ± 0.3	0 ± 0.3	0 ± 0
0.5	10 ± 1.3 ^c	6 ± 1.3	17 ± 1.3 ^c	1 ± 0.6	1 ± 0.6	2 ± 1.2	0 ± 0.3	1 ± 1
1	20 ± 3.3 ^c	13 ± 1.9 ^c	33 ± 5.1 ^c	4 ± 0.1 ^{c,d}	1 ± 0.3	5 ± 0.6 ^{c,d}	1 ± 1.3	2 ± 2
5	NM	NM	NM	NM	NM	NM	NM	NM

^a Administered as a solution

^b Administered as a suspension

^c Statistically different from control

^d Statistically different from Steller sea lion lung.