



Particulate and soluble hexavalent chromium are cytotoxic and genotoxic to Steller sea lion lung cells

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ABSTRACT

Hexavalent chromium is an environmental contaminant. Within the environment, marine waters are a common site for hexavalent chromium deposition. We have recently reported significantly high levels of chromium in skin tissue from North Atlantic right whales. These findings demonstrate that marine species are being exposed to chromium. It is possible that such exposures may be playing a role in population declines evident among certain marine mammals, such as the Steller sea lion. We developed a Steller sea lion lung cell line from Steller sea lion lung tissue. Hexavalent chromium was cytotoxic to these primary lung fibroblasts as 1, 2.5, 5, 10 and 25 μM sodium chromate induced 104, 99, 92, 58 and 11% relative survival, respectively. It was also genotoxic as 0, 1, 2.5, 5 and 10 μM sodium chromate damaged chromosomes in 6, 11, 21, 36, and 39% of metaphases and damaged 6, 12, 27, 49 and 57 total aberrations in 100 metaphases, respectively. We also considered the toxicity of particulate hexavalent chromium, as it is the more potent carcinogen in humans. We found that 0.1, 0.5, 1, 5 and 10 $\mu\text{g}/\text{cm}^2$ particulate chromate induced 95, 88, 91, 70, and 52% relative cell survival, respectively. These concentrations were genotoxic and damaged chromosomes in 9, 13, 18, and 23% of metaphases and induced 9, 15, 20 and 30 total aberrations per 100 metaphases, respectively. These data indicate that if sufficiently exposed, chromium may adversely affect the struggling Steller sea lion population. It would be prudent to investigate the effects chromium has in other Steller sea lion organs in order to derive a better understanding of how chromium in the marine environment may be affecting the declining Steller sea lion population.

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1. Introduction

Hexavalent chromium (Cr(VI)) continues to be a major occupational and environmental health concern because of its genotoxic effects leading to carcinogenic and other toxic outcomes. Cr(VI) is known to be present in the marine environment as sea water favors Cr(VI) over the trivalent form (Chiffolleau, 1994). The U.S. estimated annual release of total Cr is approximately 16,400 tons to air, soil and water (ATSDR, 2000). These numbers are considered under-

estimates since not all sources are required to report. In addition, the amount of Cr(VI) is unclear as most reporting requirements are for total Cr. For air releases reported, 2700–2900 tons of total Cr is released on an annual basis, of which at least 35% is thought to be in the hexavalent form (ATSDR, 2000).

Cr is released into the marine environment by both natural and anthropogenic sources with anthropogenic sources considered the greater factor (USDHHS, 1993). Anthropogenic sources include fuel combustion, emissions from metal industries and wastewaters from a variety of industries such as electroplating, leather tanning and textile manufacturing (USDHHS, 1993). While these general sources of chromium to the marine environment are known, little is known about the environmental fate, transport and speciation of Cr from these various sources in marine environments. Cr(VI) is the predominant form in sea

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water with residence times ranging from 4.6 to 18 years (Pettine and Millero, 1990; USDHHS, 1993). Reported levels of total dissolved Cr(VI) in natural waters range from 1.2 in unpolluted areas, to 365 nM in areas influenced by wastewater effluents (Aboul Dahab, 1989; Kamala-Kannan et al., 2008; Georgescu et al., 1988).

Marine air measurements of Cr are infrequent; however a few measurements have been done. Total Cr levels in Baltimore harbor and Hawaii were 0.226 and 0.067 $\mu\text{g m}^{-3}$, respectively (IARC, 1990; Bowen, 1979). Total Cr levels in areas bordering marine environments have been reported ranging from a low in Sydney, Australia (0.0002–0.0013 $\mu\text{g m}^{-3}$) to a high of 6.842 $\mu\text{g m}^{-3}$ in Shoubra, Egypt (Li et al., 2002; Borai et al., 2002). The lack of data is largely due to technical shortcomings with respect to an inability to determine the initial Cr speciation that enters the environment, and the complexity of the analyses needed in different environmental media. In situ analytical techniques are being developed, however, much work still needs to be done before reliable results are available (Jena and Raj, 2008; Khlystov and Ma, 2006; Elci et al., 2008).

Because of the genotoxicity of Cr(VI), its impacts on public health and its presence in the marine environment, we have become interested in the hypothesis that Cr(VI) may impact the health of marine mammals and their ability to recover from large population losses. However, while Cr(VI) is a known genotoxicant for humans and terrestrial mammals, its effects in marine mammals are poorly understood. Recently, we reported that chromium may be a significant risk factor for the critically endangered North Atlantic right whale (Wise et al., 2008). Cr(VI) was cytotoxic and genotoxic to North Atlantic right whale lung and testes cells in a concentration-dependent manner indicating that it has the potential to pose a toxic threat to these great whales. These data suggest that Cr(VI) may pose a problem for marine mammals in general, however, its genotoxicity in other marine species has not been studied.

The Steller sea lion is another marine mammal species that is endangered. The Western population of Steller sea lion (*Eumetopias jubatus*), 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 at a rate of 5.2% per year (Loughlin and York, 2000). These declines are not considered to be the consequence of natural population fluctuation, but rather the result of undetermined change(s) in environmental conditions (Pascual and Adkison, 1994). Some of the population decline is attributed to nutritional stress (Merrick, 1995) and reduced fecundity (Pitcher et al., 1998). However, there are likely to be other contributing factors such as environmental contaminants (Barron et al., 2003).

We recently reported data that Western Steller sea lions are exposed to chromium (Wise et al., 2006). The tissue levels were not particularly high because the study focused on sea lion pups, but the data do indicate that exposure to Cr occurs in the population and levels in the adults are expected to be much higher as metals are known to accumulate with age in this species (Hamanaka et al., 1982). However, the ability of Cr(VI) to induce genotoxicity in this species or the class of marine mammals it represents (Pinnipedia) is unknown. In humans, particulate Cr(VI) is considered more potent than soluble Cr(VI), it is considered the carcinogenic form of chromium. Particulate Cr(VI) is genotoxic to human cells inducing chromosomal aberrations and DNA double strand breaks (Wise et al., 2002; Wise et al., 2004; Xie, 2005). The potential toxicity of particulate Cr(VI) has not been reported for any marine mammal. Accordingly, in this paper, we investigated the cytotoxicity and genotoxicity of both particulate and soluble Cr(VI) in cultured Steller sea lion lung cells.

2. Materials and methods

2.1. Chemicals and reagents

Sodium chromate, lead chromate, demecolchicine and potassium chloride (KCl) 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)

2.2. Cells and cell culture

Stellar sea lion lung fibroblasts were isolated from tissue explants obtained during necropsy of a male Steller sea lion pup according to our published methods (Wise et al., 2006). Briefly, tissue samples were isolated from the lung by a trained marine mammal pathologist and were placed in L-15 medium supplemented with 100 U/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin, and 10 mg/ml gentamicin and then shipped with cold packs to the Wise Laboratory. Tissue explants were rinsed several times in phosphate-buffered saline (PBS) with penicillin–streptomycin and gentamicin. Explants were then sliced into small pieces (≤ 1 mm) with a scalpel, rinsed repeatedly and placed into T-25 flasks with complete culture media consisting of DMEM/F-12 supplemented with 15% cosmic calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin and 0.1 mM sodium pyruvate, and placed in a 37 °C humidified incubator with 5% CO₂, cells were observed growing out of the explants.

Cells were routinely cultured in a 50:50 mixture of DMEM/F12 supplemented with 15% CCS, 2 mM L-glutamine, 100 U/ml penicillin/100 $\mu\text{g/ml}$ streptomycin and 0.1 mM sodium pyruvate. Cells were maintained as adherent subconfluent monolayers and fed at least twice a week and subcultured at least once a week using 0.25% trypsin in 1 mM EDTA solution. Cells were tested routinely for mycoplasma contamination. All experiments were conducted on logarithmically growing cells with a doubling time of 24 h.

Fibroblasts serve as a surrogate model for other cells in the body. We focused on lung fibroblasts because of the availability of tissue and the practical realities of attempting cell culture of a wild species. For example, the time necessary to transport the tissue is often too long (more than 24 h) to allow for epithelial cell growth; as these cells are not as hardy as fibroblasts. However, we feel this approach is appropriate because metals accumulate in fibroblasts and not epithelial cells and epithelial cells are typically more sensitive to metal effects, thus we would expect to see an even stronger effect in epithelial cells (Kondo et al., 2003). Moreover, fibroblasts are also known to be a target of metals such as Cr(VI) in disease (DeFlora et al., 1990).

2.3. Preparation of Cr(VI) compounds

Sodium chromate (CAS #7775-11-3, ACS reagent minimum 98% purity), a soluble hexavalent chromium compound (OSHA, 2006) was administered as a solution in water as previously described (Wise et al., 2002). Lead chromate (CAS #7758-97-6, ACS reagent minimum 98% purity), an insoluble hexavalent chromium compound, was administered as a suspension in acetone as previously described (Wise et al., 2004b).

2.4. Cytotoxicity assay

Cytotoxicity was determined with a clonogenic assay, which measures a reduction in plating efficiency in treatment groups relative to the controls based on our published methods with minor modifications (Wise et al., 2002). Briefly, cells (100,000) were seeded in 2.3 ml of medium per well in a 6-well tissue culture dish, allowed to grow for 48 h and then treated for 24 h with sodium chromate or lead chromate. After 24 h treatment, medium was collected (to include any loosely adherent mitotic cells); the cells were rinsed with PBS; detached from the dish with 0.25% trypsin/1 mM EDTA; and trypsinized cells were transferred to the collected medium to stop the trypsin. Cells were then resuspended in fresh medium; counted with a Coulter Multisizer III; and reseeded at colony forming density (1000 cells per 100 mm dish). Colonies were allowed to grow for 14 days; fixed and stained with crystal violet; and the colonies counted. There were four dishes per treatment group and each experiment was repeated at least three times. All treatment groups were compared to control and expressed as a percentage of the control.

2.5. Clastogenicity assay

Clastogenicity was determined by measuring the amount of chromosomal damage in treatment groups and controls and chromosomes were prepared based on our published methods with minor modifications (Wise et al., 2002). Cells were seeded at 800,000 per 100 mm dish; allowed to grow for 48 h; and then treated for 24 h with sodium chromate or lead chromate. Five hours before the end of the treatment time 0.1 g/ml colcemid was added to block the cells in metaphase. At the end of treatment, the medium was collected (to include any loosely adherent mitotic cells); the cells were rinsed with PBS, trypsinized with 0.25% trypsin/1 mM EDTA and added to the collected medium to stop the trypsin. Cells were then resuspended in 0.075 M KCl hypotonic solution for 17 min to swell the cells and the nuclei. At the end of the hypotonic time cells were fixed in 3:1 methanol:acetic acid fixative and the fixative was changed twice. Finally, cells were dropped onto clean wet slides and uniformly stained using a 5% Giemsa stain in Gurr's buffer.

Clastogenesis was measured through the production of chromosomal aberrations, which were scored using previously published criteria (Wise et al., 2002). One hundred diploid metaphases per data point were analyzed in each experiment. Slides were coded and each experiment was repeated at least three times.

2.6. Statistical analysis

Both one-way ANOVA and Kruskal–Wallis test were used to test if the intracellular concentration levels, cytotoxicity, and percent and total chromosome damage differ. Both methods showed that there is sufficient statistical evidence that at least two intracellular Cr ion concentration levels, cytotoxicity, and percent and total chromosome damage levels differ at a significance level of $\alpha = 0.05$. Then the *t*-test and the Mann–Whitney test were used to find which levels significantly differ at a significance level of $\alpha = 0.05$.

3. Results

Cr(VI) induced concentration-dependent increases in cytotoxicity in Steller sea lion lung cells. For particulate Cr(VI), concentrations of 0.1, 0.5, 5 and 10 $\mu\text{g}/\text{cm}^2$ lead chromate reduced relative survival by 95, 88, 70 and 52%, respectively (Fig. 1). For soluble Cr(VI), concentrations of 1.0, 2.5, 5, 10 and 25 μM sodium chromate induced 104, 99, 92, 58 and 11% relative survival, respectively (Fig. 2).

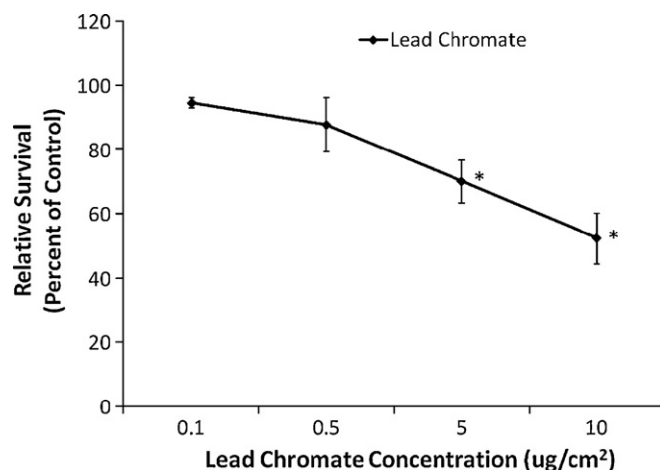


Fig. 1. Effect of lead chromate on the relative survival of Steller sea lion lung fibroblasts. This figure shows lead chromate induced concentration-dependent increases in cytotoxicity measured as a reduction in relative survival. All cells were derived from the same animal. Cells were exposed to lead chromate for 24 h. Data represent the mean of at least 3 independent experiments. Error bars = standard error of the mean. Concentrations of 5 and 10 $\mu\text{g}/\text{cm}^2$ were statistically significant ($P < 0.05$) from the control. *Indicates statistical significance.

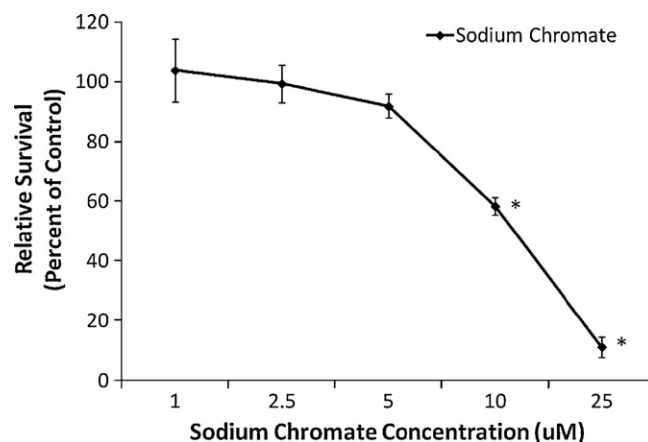


Fig. 2. Effect of sodium chromate on the relative survival of Steller sea lion lung fibroblasts. This figure shows sodium chromate induced concentration-dependent increases in cytotoxicity measured as a reduction in relative survival. All cells were derived from the same animal. Cells were exposed to sodium chromate for 24 h. Data represent the mean of at least 3 independent experiments. Error bars = standard error of the mean. Concentrations of 10 and 25 μM were statistically significant ($P < 0.001$) from the control. *Indicates statistical significance.

Cr(VI) also induced concentration-dependent increases in genotoxicity in Steller sea lion lung cells. For particulate Cr(VI), concentrations of 0, 0.1, 0.5, 1, and 5 $\mu\text{g}/\text{cm}^2$ lead chromate damaged 5, 9, 13, 18 and 23% of metaphases, respectively (Fig. 3). For soluble Cr(VI), concentrations of 1.0, 2.5, 5, 10 and 25 μM sodium chromate damaged 6, 11, 21, 36 and 39% of metaphases, respectively (Fig. 4). The same pattern was seen for total chromosome damage; for lead chromate the same concentrations induced 5, 9, 15, 20 and 30 aberrations per 100 metaphases; for sodium chromate the same concentrations induced 6, 12, 27, 49, and 57 aberrations per 100 metaphases (Figs. 3 and 4). There are more total aberrations than percent metaphases with damage at treatments of 2.5, 5 and 10 μM sodium chromate and 5 $\mu\text{g}/\text{cm}^2$ lead chromate indicating that at these concentrations cells began to show multiple aberrations per cell. Table 1 shows a representative spectrum of damage from one experiment for sodium chromate and lead chromate. Chromatid and isochromatid lesions were the major lesion with acentric

Table 1
Spectrum of chromosome damage in Steller sea lion lung fibroblasts.

Sodium chromate concentration (μM)	Chromatid lesion ^a	Isochromatid lesion	Chromatid exchange	Ring	Double minute	Acentric fragment	Dicentric
0	3	0	0	0	0	0	0
1	14	1	0	0	0	0	0
2.5	36	1	0	0	0	0	0
5	67	5	1	0	0	0	0
10	84	8	1	0	0	0	0
Lead chromate concentration ($\mu\text{g}/\text{cm}^2$)	Chromatid lesion	Isochromatid lesion	Chromatid exchange	Ring	Double minute	Acentric fragment	Dicentric
0	6	0	0	0	0	0	0
0.1	9	1	0	0	0	0	0
0.5	15	0	0	0	0	0	0
1	16	2	0	0	1	0	0
5	34	1	0	0	0	0	0

^a Total number of the specific type of lesion found in 100 cells.

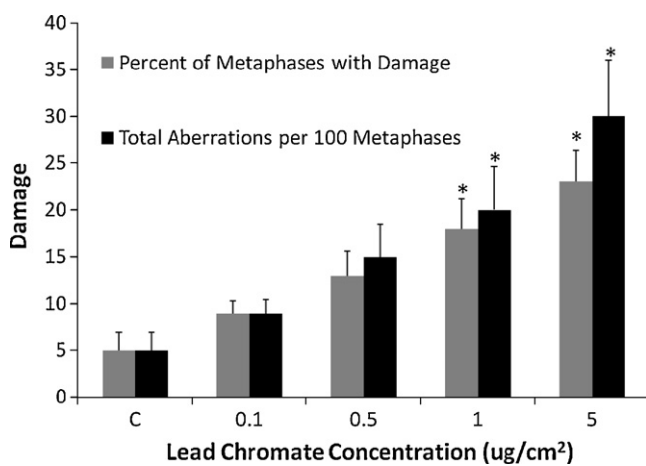


Fig. 3. Effect of lead chromate on percent metaphase with damage and total chromosome damage in Steller sea lion lung fibroblasts. This figure shows lead chromate induced concentration-dependent increases in clastogenicity. All cells were derived from the same animal. Cells were exposed to lead chromate for 24 h. Data represent the mean of at least 3 independent experiments. Error bars = standard error of the mean. Concentrations of 1 and 5 $\mu\text{g}/\text{cm}^2$ were statistically different from control ($P < 0.05$) for both the percent of metaphases with damage and total damage (i.e. total aberrations per 100 metaphases). *Indicates statistical significance.

fragments, double minutes, and chromatid exchanges less frequent.

4. Discussion

These data are the first to report that Cr(VI) is cytotoxic and clastogenic to Steller sea lion cells. The effects occurred in a concentration-dependent manner producing a spectrum of chromosome damage consistent with both carcinogenesis and teratogenesis. The results extend our understanding of the genotoxic effects of Cr(VI) from cetaceans (whales and dolphins) to the pinnipeds (seals and sea lion), both of which are exposed to Cr(VI) in their environment. The data are consistent with observations that Cr(VI) is cytotoxic and genotoxic to primary right whale and human lung cells (Wise et al., 2008, 2002).

It is important, but challenging to put these cell culture doses into an environmental exposure context. We were unable to locate

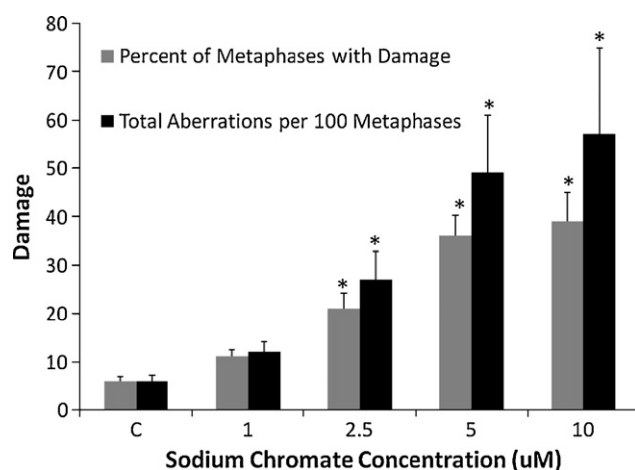


Fig. 4. Effect of sodium chromate on percent metaphase with damage and total chromosome damage in Steller sea lion lung fibroblasts. This figure shows sodium chromate induced concentration-dependent increases in clastogenicity. All cells were derived from the same animal. Cells were exposed to sodium chromate for 24 h. Data represent the mean of at least 3 independent experiments. Error bars = standard error of the mean. Concentrations of 2.5, 5 and 10 μM were statistically different from control ($P < 0.05$) for both the percent of metaphases with damage and total damage (i.e. total aberrations per 100 metaphases). *Indicates statistical significance.

any reports of marine air Cr levels in the Steller sea lion home range or places near it. The two marine air reports we did find reported total Cr levels in Hawaii ($0.067 \mu\text{g}/\text{m}^3$) and in Baltimore harbor ($0.226 \mu\text{g}/\text{m}^3$) (IARC, 1990; Bowen, 1979). If we consider an average sized male Steller sea lion (566 kg), then an average sea lion lung volume would be 0.031m^3 (based on a reported California sea lion lung volume of 55 ml/kg (L'enfant et al., 1970 as cited in Fahlman et al., 2008)) and an average resting respiration rate would be 438 breaths/h (based on a reported rate of 7.3 breaths/min for New Zealand sea lions (Gales and Mattlin, 1998)). Using both the Hawaii and Baltimore harbor air levels for Cr, the sea lions could potentially breathe in 21.8–73.6 μg Cr in a 24 h period (e.g. $0.067 \mu\text{g}/\text{m}^3 \times 0.031 \text{m}^3$ lung volume/breath \times 438 breaths/24 h = 21.8 μg Cr) if all of the Cr was hexavalent. If only 35% was hexavalent as suggested (ATSDR, 2000), this range would be 7.63–25.76 μg Cr(VI). In our studies

Table 2
Comparison of Cr(VI)-induced cytotoxicity in Steller sea lion cells with published values for humans and whales.

Dose	Steller sea lion lung cells (this report)	Cytotoxicity in human lung cells (Wise et al., 2002)	Cytotoxicity in right whale lung cells (Wise et al., 2008)
10 μM sodium chromate	58%	0	32
5 $\mu\text{g}/\text{cm}^2$ lead chromate	88	15	Not done

Table 3
Comparison of Cr(VI)-induced genotoxicity in Steller sea lion cells with published values for humans and whales.

Dose	Genotoxicity in Steller sea lion lung cells (this report)		Genotoxicity in human lung cells (Wise et al., 2002)		Genotoxicity in right-whale lung cells (Wise et al., 2008)	
	Percent of metaphases with chromosome damage	Total aberrations per 100 metaphases	Percent of metaphases with chromosome damage	Total aberrations per 100 metaphases	Percent of metaphases with chromosome damage	Total aberrations per 100 metaphases
2.5 μM sodium chromate	21	27	33	43	20	23
0.5 $\mu\text{g}/\text{cm}^2$ lead chromate	13	16	27	33	Not done	Not done

we dosed cells for 24 h with 0.89–4.4 μg of Cr from lead chromate or 0.68–16.9 μg from sodium chromate. Thus, the lower doses we studied are about an order of magnitude lower than these calculated sea lion exposures with our highest doses overlapping the lower end of this possible exposure scenario. Of course it is tempting to speculate that the exposures could still be different because a sea lion lung is much bigger than a culture dish. This observation is true, and we can only partially model a lung exposure in a culture dish. However, Cr particles, when inhaled do not universally diffuse throughout the lung. Instead, human studies indicate that Cr particles deposit and persist at bronchial bifurcation sites causing much higher localized exposures in a much smaller surface area (Ishikawa et al., 1994a,b). Thus, while our doses cannot precisely mimic an environmental exposure; these scenarios suggest that they are a reasonable starting point.

However, while outcomes are similar amongst species there are some differences in results. For example, sodium chromate is much less cytotoxic to Steller sea lion lung cells compared to human lung cells as a 24 h exposure to 10 μM sodium chromate induced 58% relative survival in the sea lion lung fibroblasts compared to a report of 0% relative survival at this dose and time for human lung fibroblasts (Table 2, Wise et al., 2002). Sodium chromate is also more cytotoxic to Steller sea lion cells than Northern right whales cells as a 24 h exposure of 10 μM in right whale lung fibroblasts induced 32% relative survival (Table 2, Wise et al., 2008). In addition, there are differences in the effects seen with lead chromate treatment. Lead chromate is also much less cytotoxic to Steller sea lion lung cells compared to human lung cells. A 24 h exposure to 5 $\mu\text{g}/\text{cm}^2$ of lead chromate has been reported to induce a relative survival of 15% (Table 2, Wise et al., 2002) while the same concentration in Steller sea lion lung cells induces relative survival of 88%. The explanation for these differences is uncertain. The difference between these species may be due to differences in uptake of Cr ions into the cell and in genotoxicity.

Sodium chromate is also less genotoxic to Steller sea lion lung cells than human lung cells, but toxicity in sea lion and whale cells are similar. For example, sea lion lung fibroblasts exposed to 2.5 μM sodium chromate for 24 h resulted in 21% of metaphases with damage and 27 total aberrant chromosomes per 100 metaphases compared to 20 and 23, respectively for right whale lung fibroblasts and 33 and 43, respectively, for human lung fibroblasts reported at this exposure dose and time (Table 3, Wise et al., 2008, 2002). The difference with regard to lead chromate showed a similar pattern. Lead chromate concentrations of 0.5 $\mu\text{g}/\text{cm}^2$ induced 27% of chromosomes with damage and 33 total aberrations per 100 metaphases in human lung cells but only 13% and 16 total aberrations in Steller sea lion lung cells (Table 3). The explanations for these differences are also uncertain, but may be due to differences in cellular uptake of Cr ions or DNA repair.

Steller sea lion population declines have been associated with a failure in juvenile recruitment into the breeding population (Chumbley et al., 1997; York, 1994). Blood chemistry and hematology analysis of Steller sea lion pups (<1 month old) from areas of population decline revealed that the pups were not nutritionally compromised therefore indicating that the inability of Steller sea lion pups to survive was not due to nutritional scarcity (Rea et al., 1998). Rather, some other factor was hindering survival. It is feasible that exposure to contaminants could be adversely affecting developmental processes in the maturing pups thereby posing a significant impediment to pup survival and population recovery for the entire species. Our data indicate that chromosome damage increased after Cr(VI) exposure in Steller sea lion cells. Both the frequency of metaphases with damage and the total amount of chromosome damage increased. The spectrum of chromosomal aberrations is consistent with the type of karyotypic deletions found with neoplastic transformation and teratogenesis [Yunis,

1983; Cavanee et al., 1983]. Thus, these data are consistent with a hypothesis that Cr(VI) is genotoxic to Steller sea lions.

Steller sea lion pups can have elevated Cr levels in their lungs (Holmes et al., 2008) and Cr(VI) can damage testes, alter sperm counts and reproductive behaviors (Witmer et al., 1989, 1991; Chowdhury and Mitra, 1995; Mancuso, 1997; Bataineh et al., 1997; Al-Hamood et al., 1998). If a genotoxic exposure were to occur during reproduction or embryogenesis, it could have significant effects on pup development. Our data support a hypothesis that chromium exposure may play a role in the population decline of the Steller sea lions and highlight the need for additional research on Cr(VI) in this population.

Our genotoxicity data are consistent with the current proposed mechanism of Cr(VI) toxicity. Specifically, Cr(VI) crosses the cell membrane by an anion transport system and is reduced inside the cell to Cr(III) via a series of intermediates. The ultimate genotoxic agents are some combination of Cr(III) and one or more of these intermediates (Singh et al., 1999; Xie et al., 2004; Wise et al., 2004a; Holmes et al., 2006; Xie et al., 2005). The use of a cell line allows for controlled toxicological investigations of the cellular mechanism of Cr(VI) toxicity in sea lions and also allows investigations into other contaminants.

In summary, our data demonstrate that Cr(VI) is cytotoxic and genotoxic to Steller sea lion lung fibroblasts. Further work is needed to determine how sea lions are exposed, potential differences in repair and apoptosis between sea lions, whales and humans and to further assess the potential contribution of Cr(VI) to the decline of the Western Steller sea lion population.

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