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Comparative cytotoxicity and genotoxicity of soluble and particulate hexavalent chromium in human and hawksbill sea turtle (*Eretmochelys imbricata*) skin cells

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Abstract

Chromium is both a global marine pollutant and a known human health hazard. In this study, we compare the cytotoxicity and genotoxicity of both soluble and particulate chromate in human and hawksbill sea turtle (*Eretmochelys imbricata*) skin fibroblasts. Our data show that both soluble and particulate Cr(VI) induce concentration-dependent increases in cytotoxicity, genotoxicity, and intracellular Cr ion concentrations in both human and hawksbill sea turtle fibroblasts. Based on administered concentration, particulate and soluble Cr(VI) were more cytotoxic and clastogenic to human cells than sea turtle cells. When the analysis was based on the intracellular concentration of Cr, the data showed the response of both species was similar. The one exception was the

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Conflict of Interest

The authors declare that there are no conflicts of interest.

cytotoxicity of intracellular Cr ions from soluble Cr(VI), which caused more cytotoxicity in sea turtle cells ($LC_{50}=271\text{ }\mu\text{M}$) than human cells ($LC_{50}=471\text{ }\mu\text{M}$), but its clastogenicity was similar between the two species. Thus, adjusting for differences in uptake indicated the explanation for the difference in potency was mostly due to uptake rather than differently affected mechanisms. Overall these data indicate sea turtles may be a useful sentinel for human health responses to marine pollution.

Keywords

chromium; chromate; sea turtle; hawksbill; genotoxicity; marine pollution; skin

1. Introduction

Ocean pollution is now a global problem. For example, we have recently documented metal pollution in all of the world's oceans (Holmes et al., 2008a; Martino et al., 2013; Savery et al., 2013a, 2013b, 2014a, 2014b; Wise et al., 2009, 2011a). Aquatic reptiles are key species in the ocean and inhabit both coastal and pelagic ecosystems. They can be long-lived and consequently can bioaccumulate environmental pollutants. Some, such as the American alligator (*Alligator mississippiensis*) have been classified as endangered and recovered, while others such as the hawksbill sea turtle (*Eretmochelys imbricata*) remain critically endangered and at risk of extinction (IUCN, 2015). Some human impacts, like hunting and loss of egg-laying habitat, have been well-identified as important factors on the health of aquatic reptiles (e.g. see Meylan and Donnelly, 1999). The impact of environmental pollution is poorly understood. In fact, reptile toxicology, particularly genetic toxicology, is one of the most underdeveloped areas of study despite the importance of these species.

Aquatic reptiles have been used very effectively as environmental sentinels for human exposure and human disease. The most developed model species is the American alligator, which brought attention to the problem of pesticide pollution in Florida lakes and launched the field of endocrine disruption with the discovery of reduced penis length in males as a consequence of pesticide exposure in Lake Woodruff (Delany et al., 1988; Guillette et al., 1994, 1995a, 1995b, 1999; Guillette 1998). Applications of other aquatic reptiles as sentinels and models for human disease have lagged behind particularly for genetic toxicology.

We have been developing sea turtles as an aquatic animal model for metal pollution and impacts for both ecosystem and human health focusing on hexavalent chromium. We chose chromium because our recent data in whales show elevated chromium levels around the globe with some locations having levels so high they fall in the range of those found in chromate workers who died of lung cancer (Wise et al., 2009). Given the high exposures and its known ability to damage DNA and cause reproductive effects (Agency for Toxic Substances and Disease Registry, "ATSDR", 2012), chromium must be considered as a major marine health concern (Neff, 2002). While we do use whales as model species, it is important to consider other species, particularly from different taxa such as aquatic reptiles.

Data show metal pollutants accumulate in several sea turtle species (Storelli et al., 1998, 2008; Anan et al., 2002; Franzellitti et al., 2004; Maffucci et al., 2005; Gardner et al., 2006; Frias-Expericuet et al., 2006; Andreani et al., 2008; Garcia-Fernandez et al., 2009; Jerez et al., 2010). However, chromium levels were only reported in two of these studies. Suzuki et al (2012) found low plasma Cr levels and did not consider other tissues. The other study reported lung, liver, kidney and muscle levels with lung showing the highest level of 2.29 ppm (mg/kg) (Storelli et al., 1998). While of limited insight, the data do indicate that like whales, sea turtles are being exposed to chromium. Studies of chromium's impacts on turtles are also limited to two reports. One study found chromium was the most cytotoxic of 4 metals considered (Tan et al., 2010). The other was our report that chromium was cytotoxic and genotoxic to cultured hawksbill turtle cells (Wise et al., 2014). No studies have compared the response of sea turtle and human model systems.

Accordingly, to further develop sea turtles as a model system for human health, we compared the cytotoxic impacts of chromium in cultured human and sea turtle cells. We focused on hexavalent chromium [Cr(VI)] because the chemistry of the marine environment favors the hexavalent form and the data in whales strongly implicate Cr(VI) as the source of exposure in the marine environment (Geisler and Schmidt, 1991; Pettine and Millero, 1990; Li Chen et al., 2009a; Wise et al., 2009, 2011a). We considered both particulate and soluble Cr(VI) because the data in whales and the limited data in sea turtles indicate inhalation is a major route of exposure (Li Chen et al., 2009a; Wise et al., 2011a) and human and rodent studies show particulate Cr(VI) is more potent than soluble Cr(VI) (IARC, 1990; Holmes et al., 2008b; Wise et al., 2008a, 2008b).

2. Methods

2.1 Chemicals and Reagents

RPMI was purchased from Mediatech (Manassas, VA). Penicillin/streptomycin, Gurr's buffer, sodium, L-glutamine and trypsin/EDTA were purchased from Invitrogen Corporation (Grand Island, NY). Dulbecco's minimal essential medium and Ham's F-12 (DMEM/F12) 50:50 mixture was purchased from Mediatech Inc. (Herndon, VA). Cosmic calf serum (CCS) was purchased from Hyclone (Logan, UT). Fetal Bovine Serum (FBS) was purchased from Gibco Life Technologies (Grand Island, NY). Crystal violet, acetic acid, and methanol were purchased from J.T. Baker (Phillipsburg, NJ). Tissue culture dishes, flasks, and plasticware were purchased from BD (Franklin Lakes, NJ). Lead chromate (PbCrO₄), sodium chromate (NaCrO₄), potassium chloride (KCl), and demecolchicine were purchased from Sigma/Aldrich (St. Louis, MO). Giemsa stain was purchased from Biomedical Specialties Inc. (Santa Monica, CA). Cytoseal 60 slide mounting medium was purchased from VWR (Bridgeport, NJ). MycoAlert detection kits were purchased from Lonza Rockland, Inc (Rockland, ME).

2.2 Cells and Cell Culture

We used hawksbill sea turtle fibroblast cells that were established from a skin biopsy of a healthy, juvenile hawksbill sea turtle (Fukuda et al., 2012) and primary human skin cells (BJ cells) previously described in Vaziri and Benchimol (1998). All cells were cultured as

monolayers of adherent cells and allowed to grow to near confluence before they were subcultured or used for experiments. All cultures were fed at least two times a week and subcultured at least once a week. Hawksbill sea turtle cells were grown in RPMI supplemented with 10% FBS and maintained in a humidified incubator with 5% CO₂ set at 26°C. BJ cells were grown in DMEM/F-12 50:50 mixture, supplemented with 15% CCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 ug/ml streptomycin, and 0.1 mM sodium pyruvate. The BJ cells were maintained in a humidified incubator with 5% CO₂ set at 37°C. Cells were tested routinely for mycoplasma contamination. All experiments were tested on logarithmically growing cells.

2.3 Preparation of Chemicals

Sodium chromate (Na₂CrO₄) was used as a representative soluble Cr(VI) compound (CAS# 7775-11-3, ACS reagent minimum 98% purity) and was administered as a solution in water as previously described (Wise et al., 2002). Lead chromate (PbCrO₄) was used as a representative particulate Cr(VI) compound (CAS# 7758-97-6, ACS reagent minimum 98% purity) and was administered as a suspension in water as previously described (Wise et al., 2002). Sodium chromate treatment concentrations are expressed as uM because sodium chromate dissolves fully in cell culture. Lead chromate treatment concentrations are expressed as weight per surface area (ug/cm²) because it is an insoluble compound that only partially dissolves in cell culture. (Holmes et al., 2005; Wise et al., 2005)

2.4 Cytotoxicity

Cytotoxicity was determined using a clonogenic assay based on our published methods (Wise et al., 2002). Briefly, logarithmically growing cells were seeded in each well of a 6-well tissue culture plate and allowed to rest for 48 hours before being treated with either sodium chromate or lead chromate for 24 hours. After treatment, cells were resuspended in fresh medium and reseeded in four 100 mm tissue culture dishes per concentration at a density of 1000 cells/dish. Once colonies formed (about two weeks) the dishes were rinsed twice with phosphate buffered saline (PBS) and fixed in methanol for 20 minutes. The dishes were then stained with crystal violet for 30 minutes and the colonies counted. All experiments were repeated at least three times and results were expressed as a percentage of the control.

2.5 Clastogenicity

Clastogenicity was measured using a chromosomal aberration assay based on our published methods (Wise et al., 2002). Briefly, logarithmically growing cells were seeded in 100 mm tissue culture dishes and allowed to rest for 48 hours before being treated with either sodium chromate or lead chromate for 24 hours. Cells were arrested in metaphase by adding 0.1 g/ml demecolcine to each 100 mm dish 1 hour prior to the end of the treatment period. At the end of the treatment period, cells were harvested and resuspended in a hypotonic solution of 0.075 M KCl and then fixed with a 3:1 methanol:acetic acid solution. The fixative was changed twice before the cells were dropped on to clean, wet microscope slides and stained with 5% Giemsa stain in Gurr's Buffer. Slides were analyzed for chromosome aberrations in 100 metaphase per treatment concentration, as described in our published methods (Wise et al., 2002).

2.6 Determination of Intracellular Chromium Ion levels

2.6.1 Cell Preparation—Intracellular chromium ion levels were measured using the ion uptake assay, as previously described (Holmes et al., 2005). Briefly, a monolayer of logarithmically growing cells were seeded into 100 mm tissue culture dishes, allowed to rest for 48 hours to re-enter the normal cell cycle pattern, and then treated with various concentrations of sodium chromate and lead chromate for 24 h. At the completion of treatment, 3 mL of treated culture media was saved for the determination of extracellular chromium ion levels. Cells were then harvested and the number and volume of cells determined. The cells were washed twice with PBS, resuspended in hypotonic solution, followed by the addition of 2% SDS. This solution was sheered through a needle and filtered. The filtered samples were stored at -20°C until analysis.

2.6.2 Ion Level Measurement—Intracellular chromium levels were determined using an Inductively Coupled Plasma-Optical Emissions Spectrometer (ICP-OES) using previously published methods (Holmes et al., 2005). Solutions were introduced to the nebulizer using a peristaltic pump operating at 2 mL/min. Prior to analysis, samples of intracellular fluid were diluted 5x in 0.16 M aqueous HNO_3 . Chromium was determined using emission wavelength at 267.716 with a minimum detection limit of 2 ppb. Yttrium was used as an internal standard. The intracellular concentrations were converted from $\mu\text{g/L}$ to μM by dividing the volume of the sample, the atomic weight of the chemical, the number of cells in the sample and the average cell volume. 0 h treatments were conducted, and the values subtracted from the 24 h measurements to account for the possibility of particulate Cr(VI) passing through the filter.

2.7 Statistical Analysis

We used the statistical test SCHEFFE for each dose compared to control and each dose compared to each other for each species. A probit model was used to calculate the LC_{50} and the TD_{20} because the scatter plot showed a logistic curve.

3. Results

3.1 Cytotoxicity

Soluble and particulate Cr(VI) induced a concentration-dependent decrease in cell survival in both human and hawksbill sea turtle cells. At lower concentrations, the cytotoxic response to soluble Cr(VI) was similar in both sea turtle and human cells. For example, concentrations of 0.5, and 1 sodium chromate induced 79, and 52 percent relative survival in human cells, and 69 and 46 percent relative survival in hawksbill sea turtle cells, respectively (Fig. 1A). At higher concentrations, hawksbill cells were more resistant as 2.5 and 5 μM sodium chromate induced 24, and 4 percent relative survival, respectively compared to 4, and 0 percent relative survival in human cells (Fig. 1A). Based on administered concentration, the calculated LC_{50} for sodium chromate in sea turtle cells was 0.9 μM (95% confidence interval: 0.9 to 1.0) and 0.9 μM (95% confidence interval: 0.8 to 1.0) in human cells, further reflecting the similar outcomes at lower concentrations.

For particulate Cr(VI), hawksbill sea turtle cells were more resistant to lead chromate-induced cytotoxicity than human cells. For example, concentrations of 0.1, 0.5, 1 and 5 $\mu\text{g}/\text{cm}^2$ lead chromate induced 64, 28, 10, and 0 % relative survival, respectively in human cells. In hawksbill sea turtle cells the same concentrations of particulate Cr(VI) induced 108, 79, 54 and 7 percent relative survival, respectively (Fig. 1B). This difference is further shown by the calculated LC_{50} for lead chromate which was 6.7-fold higher in sea turtle cells than in human cells (1.2 $\mu\text{g}/\text{cm}^2$ compared to 0.18 $\mu\text{g}/\text{cm}^2$ (95% confidence intervals for each respectively: 1.1 to 1.4 and 0.15 to 0.21)).

3.2. Clastogenicity

Soluble and particulate Cr(VI) induced a concentration-dependent increase in clastogenicity. Clastogenicity was quantified through two different measurements of chromosome aberrations. One measure was the percent of metaphases, which used the cell as the unit of comparison. The other measure, total chromosome damage, used the chromosome as the unit of comparison. Consistent with the cytotoxicity data, sea turtle cells were more resistant to the clastogenic effects of both soluble and particulate Cr(VI). For example, in human cells, sodium chromate concentrations of 0.5, 1, and 2.5 μM damaged chromosomes in 16, 27, and 39 percent of metaphases and induced 18, 30, and 64 total aberrations in 100 metaphases, respectively. In hawksbill sea turtle cells, the same sodium chromate concentrations damaged chromosomes in 9, 14, and 21% of metaphases and induced 10, 16, and 26 total aberrations in 100 metaphases, respectively. At 5 μM sodium chromate, no metaphases were observed in human cells; however, in sea turtle cells, 5 μM sodium chromate induced damage in 29 % of the metaphases and induced 39 total aberrations (Fig. 2).

We calculated a TC_{20} (concentration at which 20 % of cells were damaged or 20 aberrations were produced). Based on administered concentration, the calculated TC_{20} s for the percent of metaphases with damage and total aberrations in 100 metaphases for sodium chromate in sea turtle cells were 2.1 and 1.4 μM (95% confidence intervals for each respectively: 1.6 to 2.9 and 1.1 to 1.8), respectively, compared to 0.67 and 0.59 μM , respectively (95% confidence intervals for each respectively: 0.47 to 0.84 and 0.49 to 0.68), for these same endpoints in human cells. In other words, the TC_{20} s for sodium chromate were 2.4-3.1 times higher in sea turtle cells than in human cells showing the sea turtle cells are more resistant.

Sea turtle cells were also more resistant to particulate Cr(VI)-induced genotoxicity. In human cells, at concentrations of 0.1, 0.5, and 1 $\mu\text{g}/\text{cm}^2$ lead chromate induced damage in 16, 28 and 36 percent of metaphases with damage, respectively, and 17, 35, and 46 damaged chromosomes in 100 metaphases, respectively. At 5 $\mu\text{g}/\text{cm}^2$ no metaphases were detected. In sea turtle cells, the same concentrations of lead chromate damaged chromosomes in 11, 15 and 26 percent of metaphases producing a total of 11, 17, and 30 aberrations in 100 cells (Fig. 3). Consistent with these observations, the calculated TC_{20} s for the percent of metaphases with damage and total aberrations in 100 metaphases for lead chromate in sea turtle cells were 3.7- and 3.1-fold higher than in human cells, respectively (0.67 and 0.44 $\mu\text{g}/\text{cm}^2$ (95% confidence intervals for each respectively: 0.44 to 0.94 and 0.33 to 0.56)

compared to 0.18 and 0.14 $\mu\text{g}/\text{cm}^2$ (95% confidence intervals for each respectively: 0.10 to 0.26 and 0.09 to 0.19), respectively).

The increase in total aberrations compared to percent of cells with damage in human cells reflects an increased number of human cells with multiple aberrations. This effect was noticeably less in the sea turtle cells. For example, treatment with 2.5 μM sodium chromate produced an average of 10 metaphases with multiple aberrations in human cells, compared to an average of 2 in sea turtle cells. The spectrum of damage was similar in both human and sea turtle cells for both particulate and soluble Cr(VI) with chromatid breaks and gaps being the principal type of aberration (Table 1).

3.3 Intracellular Chromium Concentrations

We considered the possibility that the difference between the species was due to differences in Cr uptake. To address this possibility, we measured intracellular Cr ion concentrations in human and sea turtle cells (Fig. 4). Intracellular Cr levels increased in a concentration-dependent manner in both human and sea turtle cells after both soluble and particulate Cr(VI) treatments. Human skin cells took up more Cr than sea turtle cells. After exposure to 0.5, 1, 2.5 and 5 μM sodium chromate human cells had intracellular Cr levels of 291, 476, 1,172 and 2,055 μM , respectively. Sea turtle cells treated with the same concentrations had intracellular Cr levels of 37, 265, 518 and 805 μM , respectively (Fig. 4A). After exposure to 0.1, 0.5, 1 and 5 $\mu\text{g}/\text{cm}^2$ lead chromate, human cells had intracellular Cr levels of 285, 781, 1,077 and 1,581 μM , respectively. After treatment with the same concentrations of lead chromate, sea turtle cells had intracellular Cr levels of 0, 343, 507 and 1,295 μM , respectively (Fig. 4B).

These Cr uptake data reveal that the explanation for the differences in cytotoxicity and genotoxicity were due to differential Cr uptake and the sea turtle cells were most likely resistant to the administered concentration because they took up less Cr. Correcting for this difference by considering the cytotoxicity and genotoxicity data based on intracellular concentration revealed a very different pattern. Specifically, soluble Cr(VI) was more cytotoxic to sea turtle cells than human cells, but its clastogenicity was similar between the two species (Fig. 5A, Fig. 6A, Fig. 6B). For example, based on intracellular concentration, the calculated LC_{50} for sodium chromate in sea turtle cells was almost 2-fold lower than in human cells (271 μM compared to 471 μM (95% confidence intervals for each respectively: 253 to 289 and 444 to 499)). By contrast, the calculated $\text{TC}_{20\text{s}}$, based on intracellular concentration, were essentially the same. Specifically 1.3-fold higher in sea turtle cells for the percent of metaphases with damage (434 μM compared to 342 μM (95% confidence intervals for each respectively: 326 to 586 and 234 to 431)) and 1.1 lower for sea turtle cells for the total aberrations in 100 metaphases (298 μM compared to 323 μM (95% confidence intervals for each respectively: 217 to 373 and 272 to 269)).

By contrast, particulate Cr(VI) induced similar amount of cytotoxicity and genotoxicity in human and sea turtle cells after correcting for difference in intracellular uptake (Fig 5B, Fig 6C, Fig 6D). Based on administered dose, discussed above, hawksbill sea turtle cells were more resistant to lead chromate-induced cytotoxicity than human cells with a calculated LC_{50} 6.7-fold higher in sea turtle cells. However, based on intracellular concentration, the

calculated LC₅₀ for lead chromate in sea turtle cells was only 1.6-fold higher in sea turtle cells than in human cells ((558 uM compared to 351 uM (95% confidence intervals for each respectively: 512 to 610 and 312 to 388)). Similarly, the calculated TC₂₀s dropped from 3.7- and 3.1-fold higher for the percent of metaphases with damage and total aberrations, respectively in sea turtle cells to essentially the same level in both species (353 and 304 uM, in sea turtles respectively (95% confidence intervals for each respectively: 211 to 471 and 236 to 365), compared to 368 and 318 uM, respectively, for these same endpoints in human cells (95% confidence intervals for each respectively: 238 to 473 and 233 to 390)).

4. Discussion

Aquatic reptiles are an underutilized model for environmental health, despite studies showing they can be effective monitors for pollution and models for human health. We have begun developing sea turtles as a possible model system to consider genotoxic outcomes. Using Cr(VI) as a representative environmental concern, we directly compared the cytotoxic and clastogenic effects of particulate and soluble Cr(VI) in primary human and sea turtle skin fibroblasts. Our data show that Cr(VI) is cytotoxic and clastogenic to skin cells from both species; however, sea turtles cells are more resistant to Cr(VI)-induced cytotoxicity and genotoxicity than human cells. Much of this difference results from sea turtle cells taking up less Cr than human cells. However, correcting for differential uptake revealed interesting differences – soluble Cr(VI) induced similar amounts of chromosome damage in the two species, but induced less cell death in sea turtle cells. By contrast, for particulate Cr(VI) the amount of cytotoxicity and genotoxicity in human and sea turtle cells was similar.

This study is the first to compare cytotoxicity in marine reptiles and human cells. Previous studies compared the cytotoxic effects of particulate and soluble Cr(VI) in North Atlantic right whale (*Eubalaena glacialis*) and sperm whale (*Physeter macrocephalus*) cells to human cells. Like the outcome in sea turtle cells, they found Cr(VI) was cytotoxic to whale cells (Li Chen et al., 2009b, 2012). However, they found conflicting results with right whale cells behaving opposite of the sea turtle cells and being similar to humans for soluble Cr(VI) and more sensitive to particulate Cr(VI) (Li Chen et al., 2009b). Sperm whale cells, however, were similar to human cells for particulate Cr(VI), but were resistant to the cytotoxic effects of soluble Cr(VI) after correcting for uptake (Li Chen et al., 2012).

The cytotoxic results in sea turtle cells are particularly intriguing when considered with its genotoxic effects. Sea turtle cells incurred similar amounts of genotoxic damage, but less cell death after soluble Cr(VI) exposure, after correcting for difference in Cr uptake. These data suggest more damaged cells would survive in sea turtle cells creating a greater opportunity for a carcinogenic outcome. The explanation for this difference is uncertain as cell death pathways in sea turtle cells have not been studied. However, these data imply the cell death pathways in sea turtle cells may be less robust than in human cells. Alternatively, these outcomes may reflect differences in crosstalk between DNA damage and cell death pathways in the two species. Further research is needed to distinguish between these possibilities.

It is interesting to note for particulate Cr(VI) the amount of cytotoxicity and genotoxicity in human and sea turtle cells was similar. The explanation for why the outcome is different for soluble Cr(VI), likely reflects the contribution of the cation (lead versus sodium) to cytotoxicity. Lead ions induce apoptosis in mammalian cells (Pulido and Parrish, 2003), although this outcome has not been studied for lead in reptile cells. However, our previous report showed low lead levels inside sea turtle cells after lead chromate exposure (Wise et al., 2014). It is also possible the difference reflects a cytotoxic impact of particle internalization on the cells. Such an outcome is consistent with our previous observations in human, whale and sea lion cells (Li Chen et al., 2009b, 2012; Wise et al., 2020).

This study is also the first to compare genotoxic outcome in marine reptiles and human cells. Previous studies, using whale cells, also compared the genotoxic effects in those cells to human cells (Li Chen et al., 2009b, 2012). As was the case with sea turtle cells, they found Cr(VI) was genotoxic to whale cells (Li Chen et al., 2009b, 2012). However, they found, primary cells from North Atlantic right whale (*Eubalaena glacialis*) and sperm whale (*Physeter macrocephalus*), were resistant to the genotoxic effects of particulate and soluble Cr(VI) after correcting for uptake (Li Chen et al., 2009b, 2012). The data in this study differs from those studies as the sea turtle cells had similar amounts of genotoxicity as human cells. This outcome supports our previous hypothesis that marine mammals may have evolved novel cellular mechanisms to protect them from genotoxic exposures.

The differences in Cr(VI) uptake between the sea turtle and human cells were also notable. Sea turtle cells took up less Cr than the human cells. The underlying mechanism for this outcome is uncertain. Cr(VI) uptake is mediated by anion transport proteins (Wise et al., 2008a). The most likely explanation, albeit untested, is that sea turtle cells have fewer anion transport proteins in their cellular membranes resulting in reduced Cr uptake. Of course, it is also possible that sea turtle cells evolved to use a different transport protein for Cr(VI) that has a lesser Cr(VI) affinity. It is interesting to note previous reports showed North Atlantic right whale, sperm whale and Steller sea lion cells also took up less Cr than human cells suggesting this phenotype is a common feature of cells in the marine environment (Li Chen et al., 2009b, 2012; Wise et al., 2010).

It would be interesting to understand these treatments in the context of sea turtles in the ocean. However, currently, there aren't a good exposure profile for sea turtles as Cr(VI) levels have not been determined in their environment. A dietary exposure could be considered, though there is limited data from potential prey species, which for hawksbill turtles is mostly marine sponges. Focusing on this exposure route, data show total Cr levels from marine sponges in the Mediterranean Sea (Perez et al., 2004) ranged from 0.15 to 2.3 ug total Cr/g tissue (converted from dry weight to wet weight with a 0.25 adjustment factor (Wise et al., 2011a,b)). Given hawksbill sea turtles eat about 1,500 g prey/day NMFS, 2014), then the sea turtles daily exposure might range from 225-3,412 ug total Cr just from prey consumption [for example: 0.15 ug Cr/g tissue $\times$ 1,500 g sponge tissue/day = 225 ug total Cr/day]. Given our study treatment range of 0.52-273 ug total Cr/day, our exposure regimen generally falls below the low end of the range of what sea turtles could potentially be exposed to in their diet. Thus, Cr seems to be a significant health hazard for sea turtles as well as humans.

In summary, our study shows sea turtle cells respond similarly to human cells to the cytotoxic and genotoxic effect of Cr(VI) indicating sea turtles can be a useful model for metal genotoxicity in human systems. There are some interesting nuances between the species with sea turtle cells possibly differing in their cell death pathways or crosstalk between cell death and DNA damage pathways. The data further suggest sea turtle cells may be more sensitive to the cytotoxic effects of lead ions. We intend to investigate these differences further and expand our comparisons to other metals and other aquatic reptiles including other sea turtles.

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Fig.1.

Hawksbill sea turtle and human skin cells exhibit similar cytotoxic responses to soluble Cr(VI), but not to particulate Cr(VI). Particulate Cr(VI) is more cytotoxic to human skin cells than hawksbill sea turtle cells. This figure shows that exposure to Cr(VI) induces cytotoxicity in human and hawksbill sea turtle skin cells in a concentration-dependent manner. (A) Soluble Cr(VI) cytotoxicity in human and hawksbill sea turtle skin cells. In sea turtle cells all concentrations were significantly different from the control except for 0.5 uM ($p < 0.02$ for 1uM, $p < 0.002$ for 2.5 uM, $p < 0.001$ for 5 uM). 0.5 uM was statistically different from 2.5 and 5 uM ($p < 0.05$ and $p < 0.005$, respectively). In human cells all concentrations were statistically different from the control ($p < 0.04$ for 0.5 uM and $p < 0.0001$ for 1, 2.5, and 5 uM). 0.5 uM was statistically different from 1, 2.5 and 5 uM ($p < 0.007$, $p < 0.0001$ and $p < 0.0001$, respectively). 1 uM was statistically different from 2.5 and 5 uM ($p < 0.0001$ and $p < 0.0001$, respectively). (B) Particulate Cr(VI) cytotoxicity in human and hawksbill sea turtle skin cells. Data represents the average of three experiments $\pm$ the standard error of the mean. In sea turtle cells all concentrations were significantly different from the control except for 0.1 and 0.5 ug/cm² ($p < 0.0003$ for 1 ug/cm² and $p < 0.0001$ for 5 ug/cm²). 0.1 ug/cm² was statistically different from 0.5, 1.0, and 5 ug/cm² ($p <$

0.02, $p < 0.0001$, and $p < 0.0001$, respectively). 0.5 ug/cm^2 was statistically different from 1 and 5 ug/cm^2 ($p < 0.04$ and $p < 0.0001$, respectively). 1 ug/cm^2 was statistically different from 5 ug/cm^2 ($p < 0.0002$). In human cells all concentrations were statistically different from the control ($p < 0.002$ for 0.1 ug/cm^2 and $p < 0.0001$ for 0.5 , 1 and 5 ug/cm^2). 0.1 ug/cm^2 was statistically different from 0.5 , 1 and 5 ug/cm^2 ($p < 0.002$, $p < 0.0001$, $p < 0.0001$, respectively). 0.5 ug/cm^2 was statistically different from 5 ug/cm^2 ($p < 0.01$).

Fig.2.

Hawksbill sea turtle cells are more resistant to soluble Cr(VI)-induced clastogenicity than human skin cells. This figure shows that soluble Cr(VI) induces a concentration-dependent increase in clastogenicity in both human and hawksbill sea turtle cells. (A) Soluble Cr(VI)-induced percent of metaphases with chromosome damage. In sea turtle cells all concentrations were significantly different from the control except 0.5 and 1 uM ($p < 0.004$ for 2.5 uM and $p < 0.0007$ for 5 uM). 0.5 uM was statistically different from 2.5 and 5 uM ($p < 0.05$ and $p < 0.005$, respectively). 1 uM was statistically different from 5 uM ($p < 0.03$). In human cells all concentrations were statistically different from the control except 0.5 uM ($p < 0.02$ for 1 uM and $p < 0.0008$ for 2.5 uM). 0.5 uM was statistically different from 2.5 uM ($p < 0.009$). (B) Soluble Cr(VI)-induced total chromosome damage. In sea turtle cells all concentrations were significantly different from the control except 0.5 uM ($p < 0.02$ for 1 uM, $p < 0.0004$ for 2.5 uM, and $p < 0.0001$ for 5 uM). 0.5 uM was statistically different from 2.5 and 5 uM ($p < 0.006$ and $p < 0.0002$, respectively). 1 uM was statistically different from 5 uM ($p < 0.001$). In human cells all concentrations were statistically different from the control except 0.5 uM ($p < 0.03$ for 1 uM and $p < 0.0002$ for 2.5 uM). 0.5 uM was statistically different from 2.5 uM ($p < 0.0005$). 1 uM was statistically different from 2.5 uM

($p < 0.006$). Data represents the average of three experiments (100 metaphases per experiment) $\pm$ the standard error of the mean. NM - no metaphases observed. *Only 290 metaphases were scored at 0.5 μ M sodium chromate in hawksbill sea turtle cells.

Fig.3.

Hawkbill sea turtle cells are more resistant particulate Cr(VI)-induced clastogenicity than human skin cells. This figure shows that particulate Cr(VI) induces a concentration-dependent increase in clastogenicity in both human and hawksbill sea turtle cells. (A) Particulate Cr(VI)-induced percent of metaphases with chromosome damage. In sea turtle cells all concentrations were significantly different from the control except 0.1 and 0.5 ug/cm² ($p < 0.002$ for 1 ug/cm² and $p < 0.0001$ for 5 ug/cm²). 0.1 ug/cm² was statistically different from 1 and 5 ug/cm² ($p < 0.03$ and $p < 0.0008$, respectively). 0.5 ug/cm² was statistically different from 5 ug/cm² ($p < 0.002$). In human cells all concentrations were statistically different from the control ($p < 0.006$ for 0.1 ug/cm² and $p < 0.0001$ for 0.5 and 1 ug/cm²). 0.1 ug/cm² was statistically different from 0.5 and 1 ug/cm² ($p < 0.008$ and $p < 0.0005$, respectively). (B) Particulate Cr(VI)-induced total chromosome damage. In sea turtle cells all concentrations were significantly different from the control except 0.1 and 5 ug/cm² ($p < 0.05$ for 1 ug/cm² and $p < 0.006$ for 5 ug/cm²). 0.1 ug/cm² was statistically different from 5 ug/cm² ($p < 0.004$). 0.5 ug/cm² was statistically different from 5 ug/cm² ($p < 0.005$). 1 ug/cm² was statistically different from 5 ug/cm² ($p < 0.05$). In human cells all concentrations were statistically different from the control except for 0.1 ug/cm² ($p < 0.0007$).

for 0.5 ug/cm^2 and $p < 0.0001$ for 1 ug/cm^2). 1 ug/cm^2 was statistically different from 0.5 and 1 ug/cm^2 ($p < 0.02$ and $p < 0.002$, respectively). Data represents the average of three experiments $\pm$ the standard error of the mean (100 metaphases per experiment). NM - no metaphases observed. *Only 254 metaphases were scored at 5 ug/cm^2 lead chromate in hawksbill sea turtle cells.

Fig.4.

Hawksbill sea turtle skin cells take up less Cr(VI) than human skin cells. This figure shows that human and hawksbill sea turtle skin cells take up Cr in a concentration-dependent manner after exposure to Cr(VI). (A) Cr uptake after soluble Cr(VI) treatment. In sea turtle cells all concentrations were significantly different from the control except 0.5 and 1 uM ($p < 0.01$ for 2.5 uM and $p < 0.003$ for 5 uM). 0.5 uM was statistically different from 2.5 and 5 uM ($p < 0.04$ and $p < 0.002$, respectively). 1 uM was statistically different from 5 uM ($p < 0.02$). In human cells all concentrations were statistically different from the control except 0.5 uM ($p < 0.05$ for 1 uM and $p < 0.0001$ for 2.5 and 5 uM). 0.5 uM was statistically different from 2.5 and 5 uM ($p < 0.002$ and $p < 0.0001$, respectively). 1 uM was statistically different from 2.5 and 5 uM ($p < 0.005$ and $p < 0.0001$, respectively). 2.5 uM was statistically different from 5 uM ($p < 0.0009$). (B) Cr uptake after particulate Cr(VI) treatment. In sea turtle cells only 5 ug/cm² was significantly different from the control ($p < 0.0003$). 0.1 ug/cm² was statistically different from 5 ug/cm² ($p < 0.0001$). 0.5 ug/cm² was statistically different from 5 ug/cm² ($p < 0.002$). 1 ug/cm² was statistically different from 5 ug/cm² ($p < 0.004$). In human cells all concentrations were statistically different from the

control except 0.1 and 0.5 ug/cm² ($p < 0.02$ for 1 ug/cm² and $p < 0.002$ for 5 ug/cm²). 0.1 ug/cm² was statistically different from 1 and 5 ug/cm² ($p < 0.05$ and $p < 0.003$, respectively). 0.5 ug/cm² was statistically different from 5 ug/cm² ($p < 0.05$). Data represents the average of three experiments $\pm$ the standard error of the mean.

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Fig.5.

Difference in Cr uptake indicate that hawksbill sea turtle cells are more sensitive to soluble Cr(VI)-induced cytotoxicity, but not to particulate Cr(VI)-induced cytotoxicity. This figure shows Cr(VI)-induced cytotoxicity in human and hawksbill sea turtle skin cells as a function of intracellular Cr ion levels. (A) Soluble Cr(VI)-induced cytotoxicity in human and hawksbill sea turtle cells as a function of intracellular Cr ion levels. (B) Particulate Cr(VI)-induced cytotoxicity in human and hawksbill sea turtle cells as a function of intracellular Cr ion levels. Data represents the average of three experiments $\pm$ the standard error of the mean.

Fig.6.

Difference in Cr uptake can explain the observed difference between human and hawksbill sea turtle skin cells to Cr(VI)-induced clastogenicity. This figure shows that exposure to Cr(VI) induces a concentration-dependent increase in clastogenicity in human and hawksbill sea turtle cells as a function of intracellular chromium concentration. (A) Soluble Cr(VI)-induced percent metaphases with chromosome damage as a function of intercellular Cr(VI) concentration. (B) Soluble Cr(VI)-induced total chromosome damage as a function of intercellular Cr(VI) concentration. (C) Particulate Cr(VI)-induced percent metaphases with chromosome damage as a function of intercellular Cr(VI) concentration. (D) Particulate Cr(VI)-induced total chromosome damage as a function of intercellular Cr(VI) concentration.

Table 1

Spectrum of chromosome damage in hawksbill sea turtle and human skin cells after exposure to soluble and particulate Cr(VI).

Treatment	Chromosome Aberration Type								Total aberrations observed
	Chromatid Break	Chromatid Gap	Isochromatid Break	Isochromatid Gap	Acentric Fragment	Double Minute	Dicentric	Chromatid Exchange	Centromere Spreading
<i>Hawksbill sea turtle skin cells</i>									
Sodium Chromate (uM)									
0	1±0.3	2±0.6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	3±0.3
0.5	4±1.4	7±2.0	2±0.9	1±0.6	0±0.0	0±0.0	0±0.0	0±0.0	10±1.2
1	5±3.1	6±1.2	3±1.0	2±1.2	0±0.0	0±0.0	0±0.3	0±0.0	16±2.9
2.5	10±0.7	7±3.8	5±2.6	3±1.2	0±0.0	0±0.0	0±0.3	0±0.0	26±2.6
5	17±0.5	15±2.0	3±1.0	4±0.0	0±0.0	0±0.0	0±0.0	0±0.0	39±0.5
Lead Chromate (ug/cm ²)									
0	2±1.0	1±0.7	0±0.3	1±0.3	0±0.0	0±0.0	0±0.0	0±0.0	4±0.3
0.1	5±0.5	5±1.0	0±0.0	1±0.0	0±0.0	0±0.0	0±0.0	0±0.0	11±1.0
0.5	8±1.0	8±1.9	0±0.3	1±0.3	0±0.0	0±0.0	0±0.0	0±0.0	17±0.9
1	17±6.7	7±1.9	3±0.6	2±0.6	0±0.0	0±0.0	0±0.3	0±0.0	30±5.7
5	31±5.9	15±2.0	6±1.9	2±0.8	1±0.6	0±0.0	0±0.0	1±0.6	56±8.1
<i>Human skin cells</i>									
Sodium Chromate (uM)									
0	0±0.3	1±0.3	1±0.7	0±0.3	0±0.0	0±0.0	0±0.0	0±0.0	2±1.0
0.5	9±0.9	8±1.7	1±0.7	1±0.3	1±0.7	0±0.0	0±0.0	0±0.0	18±3.5
1	16±4.3	11±1.0	1±1.0	1±0.4	1±0.4	0±0.0	0±0.0	0±0.4	31±6.7
2.5	33±3.7	13±2.5	5±0.6	2±1.8	1±0.6	1±0.7	0±0.5	1±1.0	64±3.5
5	NM	NM	NM	NM	NM	NM	NM	NM	NM
Lead Chromate (ug/cm ²)									
0	1±0.3	1±0.9	0±0.0	0±0.0	0±0.0	1±0.7	0±0.0	0±0.0	3±1.3
0.1	7±0.7	8±1.0	0±0.3	0±0.0	0±0.3	0±0.3	0±0.3	0±0.0	17±0.3
0.5	18±2.3	12±1.2	3±1.5	1±0.3	1±0.6	0±0.0	0±0.0	1±0.3	35±4.5
1	30±3.6	12±2.2	2±0.8	1±1.0	0±0.0	1±0.3	0±0.0	0±0.3	45±3.7

Treatment	Chromosome Aberration Type									Total aberrations observed
	Chromatid Break	Chromatid Gap	Isochromatid Break	Isochromatid Gap	Acentric Fragment	Double Minute	Dicentric	Chromatid Exchange	Centromere Spreading	
5	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM

Values represent the number of occurrence of each type of chromosome aberrations. NM - no metaphases.