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Concentrations of the Genotoxic Metals, Chromium and Nickel, in Whales, Tar Balls, Oil Slicks, and Released Oil from the Gulf of Mexico in the Immediate Aftermath of the Deepwater Horizon Oil Crisis: Is Genotoxic Metal Exposure Part of the Deepwater Horizon Legacy?

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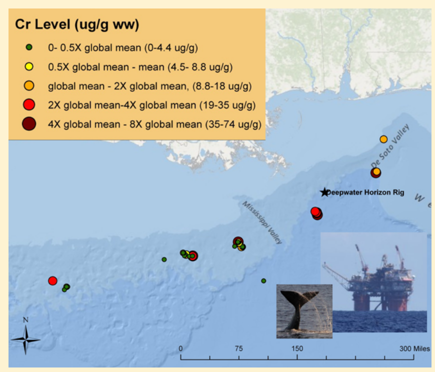
ABSTRACT: Concern regarding the Deepwater Horizon oil crisis has largely focused on oil and dispersants while the threat of genotoxic metals in the oil has gone largely overlooked. Genotoxic metals, such as chromium and nickel, damage DNA and bioaccumulate in organisms, resulting in persistent exposures. We found chromium and nickel concentrations ranged from 0.24 to 8.46 ppm in crude oil from the riser, oil from slicks on surface waters and tar balls from Gulf of Mexico beaches. We found nickel concentrations ranged from 1.7 to 94.6 ppm wet weight with a mean of 15.9 ± 3.5 ppm and chromium concentrations ranged from 2.0 to 73.6 ppm wet weight with a mean of 12.8 ± 2.6 ppm in tissue collected from Gulf of Mexico whales in the wake of the crisis. Mean tissue concentrations were significantly higher than those found in whales collected around the world prior to the spill. Given the capacity of these metals to damage DNA, their presence in the oil, and their elevated concentrations in whales, we suggest that metal exposure is an important understudied concern for the Deepwater Horizon oil disaster.



INTRODUCTION

The Gulf of Mexico is the ninth largest body of water on Earth and boasts a variety of rich and diverse ecosystems. The coastal habitats exhibit large, diverse communities of nesting seafowl, sea turtles, and fish nurseries, while the offshore habitats support a variety of deep sea corals, fish spawning grounds, rich sport and commercial fisheries, and residential cetacean species. Despite the apparent differences in these ecosystems, they are intricately intertwined and depend on each other for sustainability; for example, the deep sea corals require organic debris from the surface for food, and pelagic sea turtles need the clean sandy beaches for nesting. In addition, the Gulf of Mexico provides certain ecosystem services that are essential for human health and survival. Ecosystem services are simply defined as contributions from the environment that support, sustain, and enrich human life.[1] Many people have attempted to assign a cost estimate to the services provided by the environment for the purposes of policy development and implementation, but this has not yet been done for the Gulf of Mexico. Nonetheless, there is clear and important commercial value in the Gulf of Mexico ecosystem from commercial and sport fishing, tourism, and rich cultural heritage.

There are 840 offshore drilling units worldwide, and 110 units are in the Gulf of Mexico.[2] Between 1979 and 2005 there were six oil spills in the Gulf of Mexico that stand out for the amount of oil released, the duration of the spill, or the resulting environmental impact.[3] On April 20, 2010, an explosion on the Deepwater Horizon oil rig led to the deaths of 11 workers and released over 779 million liters of crude oil over the course of 87 days. The resulting size of this spill was estimated to cover as much as 75,000 km^2 and to directly impact over 1046 km of coastline.[4,5]

This crisis was by far the worst oil spill in a marine environment in U.S. history. In response to the crisis, efforts focused on getting rid of the oil as quickly as possible. Measures taken included application of over 1.8 million gallons of chemical dispersants sprayed on the ocean surface and injected near the well head, skimming oil from the surface, burning surface oil, and protecting coastal shorelines with containment

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Table 1. Quality Assurance and Quality Control Data for Analysis of Tissue Samples

sample	element	LOD[a][μg/g (ppm[b])]	blank	duplicate RPD[c] (%)	LCS,[d] % recovery	spike, % recovery	SRM,[e] % recovery
tissue	Cr	0.10	BDL[f]	8.6	103.4	95.9	111.4
tissue	Ni	0.09	BDL	8.5	102.0	95.5	70.3
tar ball	Cr	0.67	BDL	[g]	91.6	94.3	na[h]
tar ball	Ni	0.22	BDL	4.7	92.1	91.4	na
oil slick	Cr	0.57	BDL	[g]	100.8	100.1	na
oil slick	Ni	0.57	BDL	5.7	99.5	97.5	na

[a]LOD = limit of detection. [b]ppm = parts per million. [c]RPD = relative percent difference. [d]LCS = laboratory control sample. [e]SRM = standard reference material (DOLT-4; DORM-3). [f]BDL = below detection limit. [g]All duplicate measurements were below the project quantitation limit. [h]na = not applicable.

booms. Despite the wide variety of efforts to remove the oil, only 25% of the entire spill is believed to have been recovered or removed by direct recovery from the wellhead (17%), skimmers (3%) or burning (5%).[6]

In the aftermath of the crisis, there has been significant federal, scientific, and public concern regarding the toxicological impacts of the hydrocarbons in the oil and the possible toxicity of the chemical dispersants.[7−17] In contrast, minimal attention has been paid to the potential threat of carcinogenic metals that are known to be in crude oil.[18−22] Metals are known to accumulate in marine animal tissues after oil spills.[23,24] A recent report shows that oil which came from the well (MC252 crude oil) contained measurable amount of metals including aluminum (Al), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), nickel (Ni), vanadium-(V), and zinc (Zn).[25] Each of these metals was also present in oil mousse collected from the sea surface with the addition of manganese (Mn).[25] Of this particular set of metals, two, Ni and Cr, are known to damage DNA and cause cancer in humans and animals.[26,27] Thus, because of the threat these two metals pose to human and marine animal health, we assessed their concentrations in marine mammals, the closest relatives to humans in the Gulf of Mexico.

To assess the threat of oil-associated metals to humans and marine species, we studied the two populations of whales considered to be residential in the northern Gulf of Mexico. Specifically, the populations are Bryde's whales (*Balaenoptera edeni*) and sperm whales (*Physeter macrocephalus*) with approximately 15 and 1600 individuals, respectively.[28] Both species live, reproduce, and feed in areas exposed to the oil, and anecdotal reports indicate that workers on the Deepwater Horizon rig often watched sperm whales from the platform. Hence, these populations of whales are at high risk for exposure to the spill and to Cr and Ni in the oil. Although the exposure route of whales to heavy metals is not well understood, studies show that they can accumulate in whale tissue and cause toxicity.[29−36] Thus, the purpose of this study was to measure the concentrations of two carcinogenic metals, chromium and nickel, that are commonly present in crude oil and to compare these measured concentrations with previous measurements taken during a global study in sperm whales.

■ MATERIALS AND METHODS

Sample Collection. We collected skin biopsies from whales in the northern Gulf of Mexico in the immediate aftermath of the spill over a 4 month period from August to November 2010. Our research platform was the research vessel *Odyssey*, a 93-foot motor-sailer ketch. The *Odyssey* is equipped to acoustically track sperm whales using an underwater hydrophone array and RainbowClick software. Efforts to find and track whales acoustically were maintained constantly around the clock while at sea. In addition, the *Odyssey* has three outlook platforms to search for and track whales visually; on top of the pilot house (approximately 10 feet off the deck), halfway up the main mast (approximately 30 feet off the deck), and near the top of the main mast (approximately 50 feet off the deck). Whale watch consisted of 1−2 h shifts searching for whales from one of these outlooks from sunrise to sunset, weather permitting. When a whale was spotted, one biopsier would walk to the end of the *Odyssey*'s "whale boom", a 30-ft pole with a deer stand attached to the starboard bow, while a second, backup biopsier would be positioned in the bowsprit. The "whale boom" enabled a biopsier to get closer to a whale while keeping the boat at a respectful distance from the whale. The backup biopsier only released an arrow if the primary biopsier missed, did not make an attempt, or was incapable of making an attempt (e.g., if the whale moved too close). Males were classified as subadult or adult based on a visual estimate of the length of the whale; adult males are much larger than subadults or females.[37] Females could not be similarly classified because of the overall smaller size compared to adult males. Females and subadult male sizes could not be visually distinguished and gender was determined in these groups using the genotyping methods described herein.

Biopsies. All biopsies from Gulf of Mexico whales were collected between August and November 2010, after the well was capped and the oil had ceased spewing. Biopsies were collected from the left flank of free-ranging whales using a crossbow and specialized arrows that have a hydrostatic buoy behind a 50-mm stainless steel cylindrical biopsy dart.[33,38] Sampling was carried out simultaneously with photoidentification of the dorsal fin and flukes of sperm whales to minimize duplication. All whales appeared to be healthy, did not appear to have oil on their skin, and were not encountered near oil slicks. Previously we demonstrated that metals are not released from the biopsy darts into the samples.[33]

After biopsies were retrieved, samples were removed from the tip and divided into several pieces for contaminant analysis (skin for metals, blubber for organics), genetic analysis (skin), and development of cell lines (interface between skin and blubber). Tissue samples for contaminant and genetic analysis were frozen at −20 °C within a few minutes of collection.

Genotyping. Gender was determined by PCR amplification reactions by amplifying the SRY (male determining factor) according to published methods.[33] The keratin gene was used as an amplification control for all samples; hence, male samples showed both the keratin band (∼311 bp) and SRY band (∼152 bp), whereas females only showed the keratin band. Primer sequences were the following:

- SryPMF: 5′ CATTGTGTGGTCTCGTGATC

- SryPMR: 5′ AGTCTCTGTGCCTCCTCGAA
- KF: 5′ AGATCAGGGGTTCATGTTTCTTTGC
- KR: 5′ TTTACAGAGGTACCCAAGCCTAAG

Oil Samples. Oil samples were collected during June of 2010, while the oil was still flowing, including one sample from the broken riser itself. Tar balls were collected from beaches in Louisiana, Alabama, and Florida. Oil from slicks was collected in waters in Barataria Bay, between Mendicant Island and Queen Bass Island, Louisiana.

Inductively Coupled Plasma Mass Spectrometry. Whale, tar ball, and oil slick samples were analyzed for total Cr and total Ni using inductively coupled plasma mass spectrometry (ICPMS) according to our published methods using a Perkin-Elmer/Sciex ELAM ICPMS.[32] Interference check solutions were analyzed with all sample runs to compensate for any matrix effects which might interfere with sample analysis. Standard quality assurance procedures were employed (Table 1). Instrument response was evaluated initially, after every 10 samples, and at the end of each analytical run using a calibration verification standard and blank. For ease of comparison, all data are presented as ppm wet weight. Visual inspection of each whale sample also suggested no oil on the skin, and each sample was washed extensively with deionized water to remove any external contaminants. To further confirm that the concentrations were from an internal exposure and not any oil adhered to the skin, we checked the metal concentrations in the blubber in a whale with high exposure and found both Ni and Cr in the blubber also at elevated concentrations (data not shown), indicating that internal exposure had occurred.

The riser sample was measured by a method for metals in organic matrix by ICPMS by the company Intertek Commodities. The purpose of the method is to determine the metal concentration in naphtha, diesel, gasoline, and other related hydrocarbon products by ICPMS. All standards and samples were diluted with semiconductor grade xylene (Alpha Aesar). A four-point curve was generated by using organometallic certified reference standards Multi-Element Metallo-Organic CRM, Custom Standard 701 and Matrix: 75 cSt Hydrocarbon Oil (VHG Laboratories, Manchester, NH). A midpoint check was analyzed at the beginning and end of the sample analysis. The acceptable midpoint recovery range was 80−120% of the accepted value. A blank was analyzed prior to the sample, and a duplicate was included. The RSD was <20% for any result that is greater than the reporting limit that was in a 10% batch.

Mapping. The whale samples were mapped based on GPS coordinates collected at the time of sampling using ArcGIS version 10.1 [Environmental Systems Research Institute (ESRI), Redlands, CA]. The specific map used was the Ocean basemap provided in the program, which was developed for the program using data from GEBCO (General Bathymetric Chart of the Oceans), NOAA (National Oceanic and Atmospheric Administration), National Geographic, DeLorme, and ESRI.

Statistics. Means and standard errors were calculated for total groups and within subgroups. Mean values were compared using *t* tests. Because the distributions of values were skewed, a normalizing logarithmic transformation was used for statistical testing. The Pearson product-moment correlation coefficient was used for assessing the association between concentrations in skin biopsies and distance from the location of the spill. *P*-values less than 0.05 were regarded as statistically significant, and no adjustment was made for multiple comparisons. The statistical analyses were all conducted in SAS.[39]

RESULTS

Ni is a known human carcinogen and genotoxicant and is generally understood to be in crude oil.[26] Ni was present in all oil samples types collected (Table 2). Ni concentrations ranged from 0.24 to 8.46 ppm in tar balls collected from Gulf of Mexico beaches, and 2.29 to 3.70 ppm in oil slicks collected from coastal waters from Louisiana, and was detected at 2.18 ppm in a sample of crude oil from the Deepwater Horizon riser.

Table 2. Concentrations of Cr and Ni in 2010 Tar Balls and Oil

sample type	location	Cr (ppm[a])	Ni (ppm)
tar ball 1	Pensacola, FL	0.49	2.36
tar ball 2	Pensacola, FL	0.36	1.24
tar ball 3	Pensacola, FL	ND[b]	2.02
tar ball 4	Mobile, AL	0.40	0.24
tar ball 5	Grand Isle, LA	2.32	4.94
tar ball 6	Grand Isle, LA	4.75	8.46
oil slick 1	29°17′56″ N, 89°57′35″ W	ND	2.29
oil slick 2	29°17′56″ N, 89°57′35″ W	ND	3.70
crude oil	Deepwater Horizon riser	<0.05	2.18

[a]ppm = parts per million (measured as mg/kg). [b]ND = not detectable.

Consistent with the oil data, all whales sampled had detectable skin concentrations of Ni (Figure 1). Whale skin Ni concentrations ranged from 1.7 to 94.6 ppm with a mean of 15.9 ppm (Figures 1A and 2A). Although this population of sperm whales is considered resident in the Gulf of Mexico, they can move around large distances within the Gulf.[28] Thus, it is notable that the higher Ni concentrations were commonly found in whales nearest the accident, with a correlation of −0.579 between Ni concentration and distance from the spill (Figure 1B).

Previously, from 2000 to 2005, we conducted a global study of Ni in sperm whales using the same vessel and sampling protocols as we used in this Gulf of Mexico study. In the previous study, we measured Ni concentrations in 298 whales from 17 regions around the globe. The global skin Ni average in those whales was 2.4 ppm (Figure 2A). In contrast, the average skin Ni concentration in the Gulf whales was 15.9 ppm, 6.6 times higher than the global average ($p < 0.0001$) (Figure 2A). The mean Ni concentrations in these Gulf whales were significantly higher than each of the other regions except for the Bahamas and Kiribati; however, only one whale was sampled in Kiribati (Figure 2B). Adding the Gulf data to our previous study data indicates a new global mean of 4.0 ppm.

Mature adult sperm whale males typically remain solitary and migrate to the poles to feed and to the Gulf to breed. By contrast, females, juveniles, and subadult males retain social groups and are believed to comprise the majority of the residential population in the Gulf.[28] Thus, we compared Ni concentrations by age and gender for sperm whales sampled from the Gulf of Mexico to those samples around the globe (Figure 2C). Interestingly, the data show that sperm whales age classes considered resident in the Gulf of Mexico (i.e., females and subadult males) had higher mean Ni concentrations than the same age and gender from the global study. By contrast, the age class that is considered to be transient (i.e., adult males)

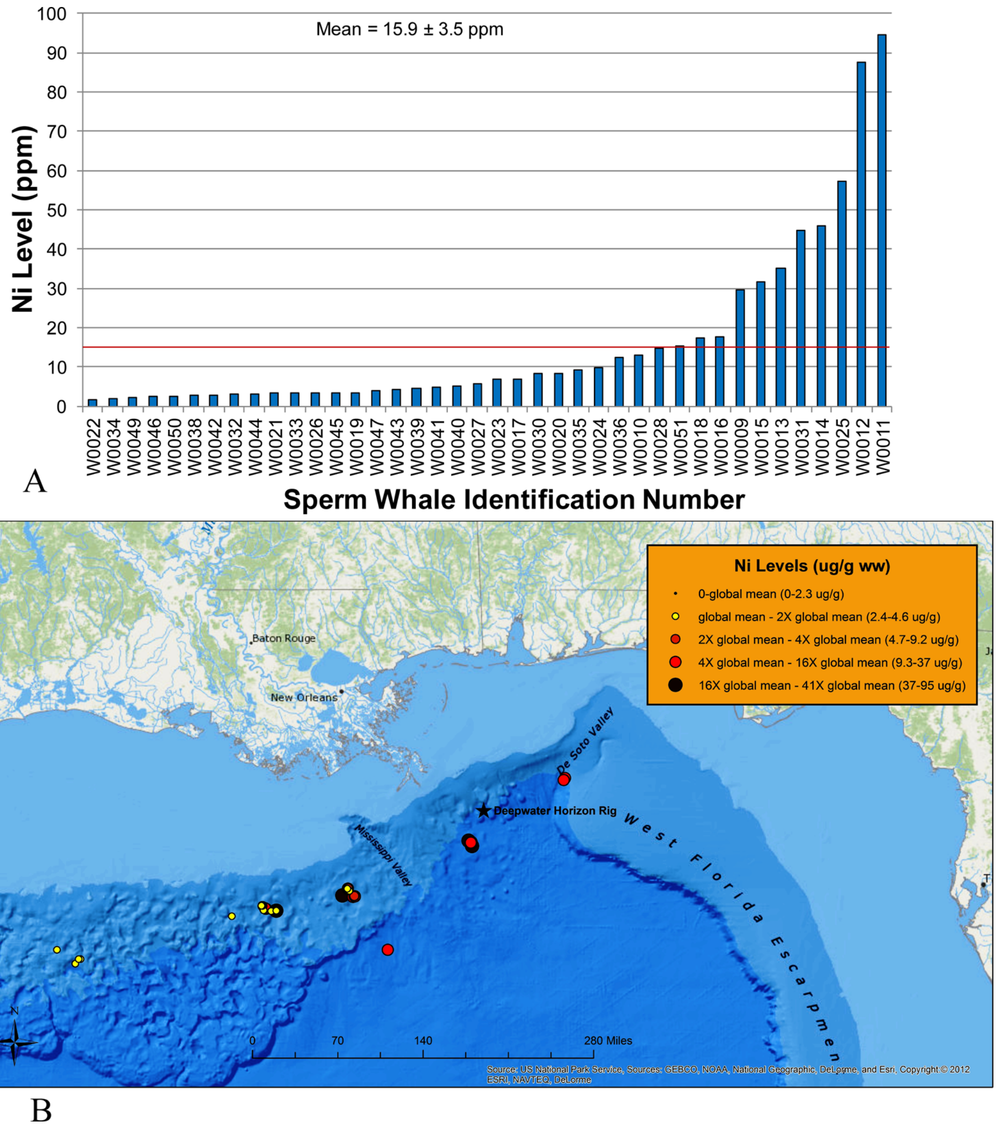

Figure 1. Sperm whales sampled in the Gulf of Mexico have elevated Ni concentrations, and animals with higher concentrations occur closer to the accident epicenter. This figure shows the concentration of Ni in the skin of 40 Gulf of Mexico sperm whales and a map of the location of the Bryde's and sperm whales sampled in 2010 in the aftermath of the Deepwater Horizon Accident. (A) Individual data measurements shown by whale. The red line indicates the mean concentration observed in these whales. All data are measured as μg/g wet weight and expressed as ppm. (B) Map showing the location of individual whales by color coded Ni concentrations compared to the global mean of our previous study. Note that the majority of the highest concentrations were found in areas that were covered with oil. Green = concentrations that range from 0 to the global sperm whale mean; yellow = concentrations that range from the global sperm whale mean to twice the global mean; orange = concentrations that range from 2 times the global mean to 4 times the global mean; red = concentrations that range from 4 times the global sperm whale mean to 16 times the global mean; dark red = concentrations that range from 16 times the global sperm whale mean to 41 times the global mean.

was much lower, with mean concentrations similar to adult males from the global voyage.

Cr is also a known human carcinogen and genotoxicant, and it also is generally understood to be in crude oil.[27] Cr was detected in all but one tar ball sample at concentrations of 0.36 to 4.75 ppm (Table 2). Cr concentrations in the two oil slicks and the riser were below our detection limit (Table 2), but this outcome is not surprising given that crude oil is not a homogeneous mixture, and only three samples were analyzed. We found that each of the sampled whales had detectable skin concentrations of Cr (Figure 3A). Concentrations of Cr in sperm whale skin ranged from 2.0 to 73.6 ppm with a mean of 12.8 ppm (Figures 3A and 4A). Similar to the outcome for Ni, the higher Cr concentrations were commonly found in whales nearest the accident with a correlation of −0.543 between Cr concentration and distance from the spill (Figure 3B).

Comparing Cr concentrations to our global data set (Figure 4A), the Gulf whales had a concentration 1.4-times higher than the global average (12.8 ppm versus 9.3 ppm, respectively), which was statistically significant (p = 0.012). The mean Cr concentrations in these Gulf whales were significantly higher than eight of the other regions (Figure 4B). Considering the concentrations by age classes considered resident in the Gulf of Mexico, as was the case with Ni, the Cr concentrations were highest in whales that are most likely resident in the Gulf of Mexico (Figure 4C). Adding the Gulf data to our previous study data indicates a new global mean of 9.7 ppm.

Finally, we also found Ni and Cr in the one Bryde's whale we were able to sample. While it is only one data point, it does

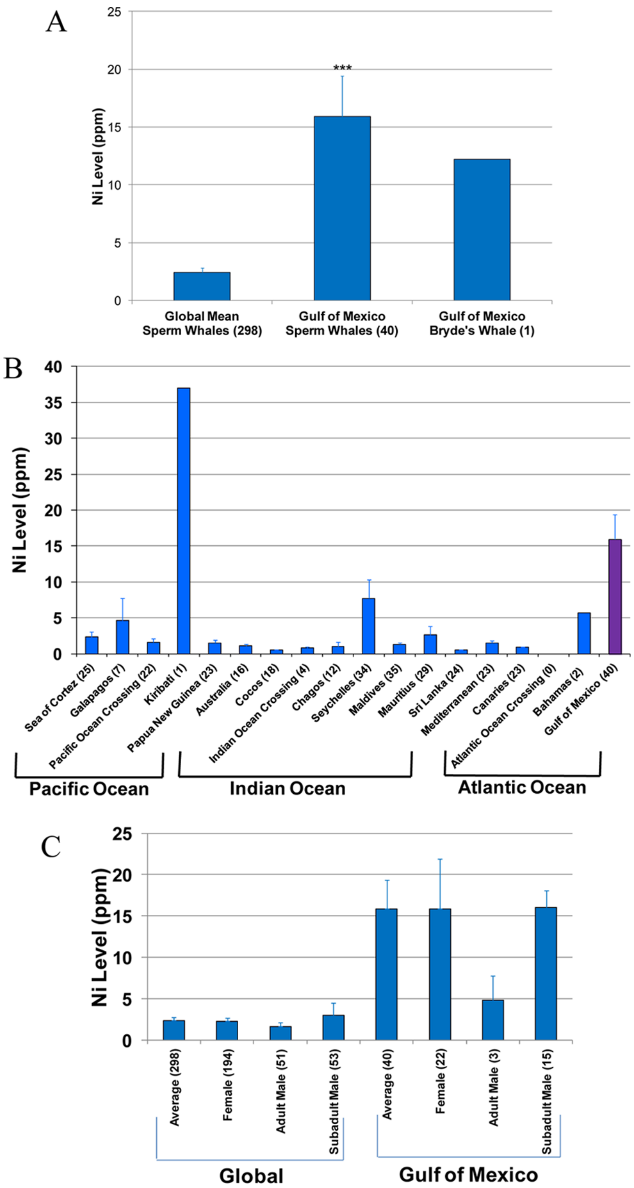

Figure 2. Ni skin concentrations in Gulf of Mexico whales are higher than in sperm whales from around the globe. This figure shows a comparison of the mean concentrations of 40 sperm whales and one Bryde's whale sampled in the Gulf of Mexico in 2010 with mean concentrations from 298 sperm whales sampled around the globe between 2000 and 2005. All data are measured as μg total Ni/g tissue wet weight and expressed as ppm. (A) The mean Ni skin concentration in Gulf of Mexico whales compared to overall mean concentration for whales in the global set. Mean Ni concentrations were 15.9 ± 3.5 ppm for Gulf of Mexico whales and 2.4 ± 0.4 ppm for the global whale set. The mean value for the Gulf of Mexico was significantly different from the global mean ($p < 0.0001$). (B) The mean Ni skin concentration in Gulf of Mexico whales (purple bar) compared to individual mean concentrations for each region in the global set (blue bars). The mean in the Gulf of Mexico was statistically significantly higher than the means for all regions except Kiribati and the Bahamas ($p < 0.05$) (no concentrations for Ni were avalable for the Atlantic Crossing). (C) Mean concentrations of Ni in sperm whale skin samples from the Gulf of Mexico and the global means in sperm whale skin samples by gender and approximate age. Age was evaluated by approximate size of the whale. Adult females, adult males, and subadult males all had statistically higher means in the Gulf of Mexico as compared to the rest of the world ($p < 0.0001$, $p < 0.0001$, and $p = 0.011$, respectively).

represent ~6.7% of the resident Gulf population (estimated 15 individuals) and is, therefore, valuable. The concentrations for Cr and Ni in this whale were 17.2 and 12.2 ppm, respectively, concentrations similar to the sperm whales. Altogether these data support the hypothesis that the Gulf of Mexico whales are experiencing higher concentrations of Cr and Ni exposure.

DISCUSSION

The 2010 Gulf of Mexico oil crisis was a major catastrophe and a reminder of human dependency on this important resource. Yet, despite the Gulf's essentiality to the health and well being of many U.S. citizens, and despite the fact that they are known to be present in and to impact on the Gulf, pollutants are markedly understudied in this key water body. As air breathing mammals that nurse their young, marine mammals are the best representative of humans in the water and are often used as sentinel species for human health. Studies have determined there are two residential populations of whales in the northern Gulf of Mexico, sperm whales and Bryde's whales. In this study we present the first consideration of the concentrations of two known human metal carcinogens, Cr and Ni, in these two populations of whales from the Gulf of Mexico. The data show that, relative to other parts of the world, both metals are significantly elevated in these whales.

We found remarkably high Ni concentrations in the Gulf whales (1.7 to 94.6 ppm Ni wet weight). Ni is not commonly studied in whales, though two studies have reported Ni concentrations in sperm whales, both in the North Sea. The concentrations we observed were much higher than those in either study.[34,35] The Holsbeek study considered muscle, liver, and kidney tissue from seven sperm whales that were stranded in the North Sea.[35] All of the samples were taken within 24 h of death. Ni concentrations ranged from undetectable to 2.5 ppm Ni dry weight. Our samples were measured as wet weight so to compare them, if we assume typical moisture content of 75%,[40] the Holsbeek values convert to undetectable to 0.63 ppm Ni wet weight. In other words, their highest concentration was less than half our lowest concentration. The second sperm whale study considered liver tissue from a single whale that was stranded in the North Sea.[34] This sample was collected immediately after death, and the Ni concentration was 0.39 ppm Ni wet weight. Thus, this sample was less than one-quarter of our lowest concentration. Skin was not measured in either study so direct organ comparisons are not possible.

We also found high Cr concentrations in the Gulf whales (2.0 to 73.6 ppm Cr wet weight). Cr is also not commonly studied in whales, though the same two studies that considered Ni in sperm whales also considered Cr.[34,35] For Cr, the Holsbeek study reports a range of undetectable to 0.9 ppm Cr dry weight, which converts to undetectable to 0.2 ppm Cr wet weight. The study by Law et al.[34] reported a concentration of 0.79 ppm Cr wet weight. Thus, as with the case with Ni, both studies' highest concentrations were lower than our lowest concentration (here just 10–40% of our lowest concentration).

Considering other whale species, the Gulf sperm whale metal concentrations are also much higher than previously reported in other whales. Three studies considered skin Ni and/or Cr concentrations in bottlenose dolphins.[41−43] One study considered Ni but not Cr and reported a mean Ni skin concentration (converted to wet weight) of 0.52 ppm in two dolphins sampled in the Western Atlantic Ocean.[41] One study considered Cr but not Ni and reported a mean Cr skin concentration of 0.14 ppm wet weight in 40 dolphins sampled

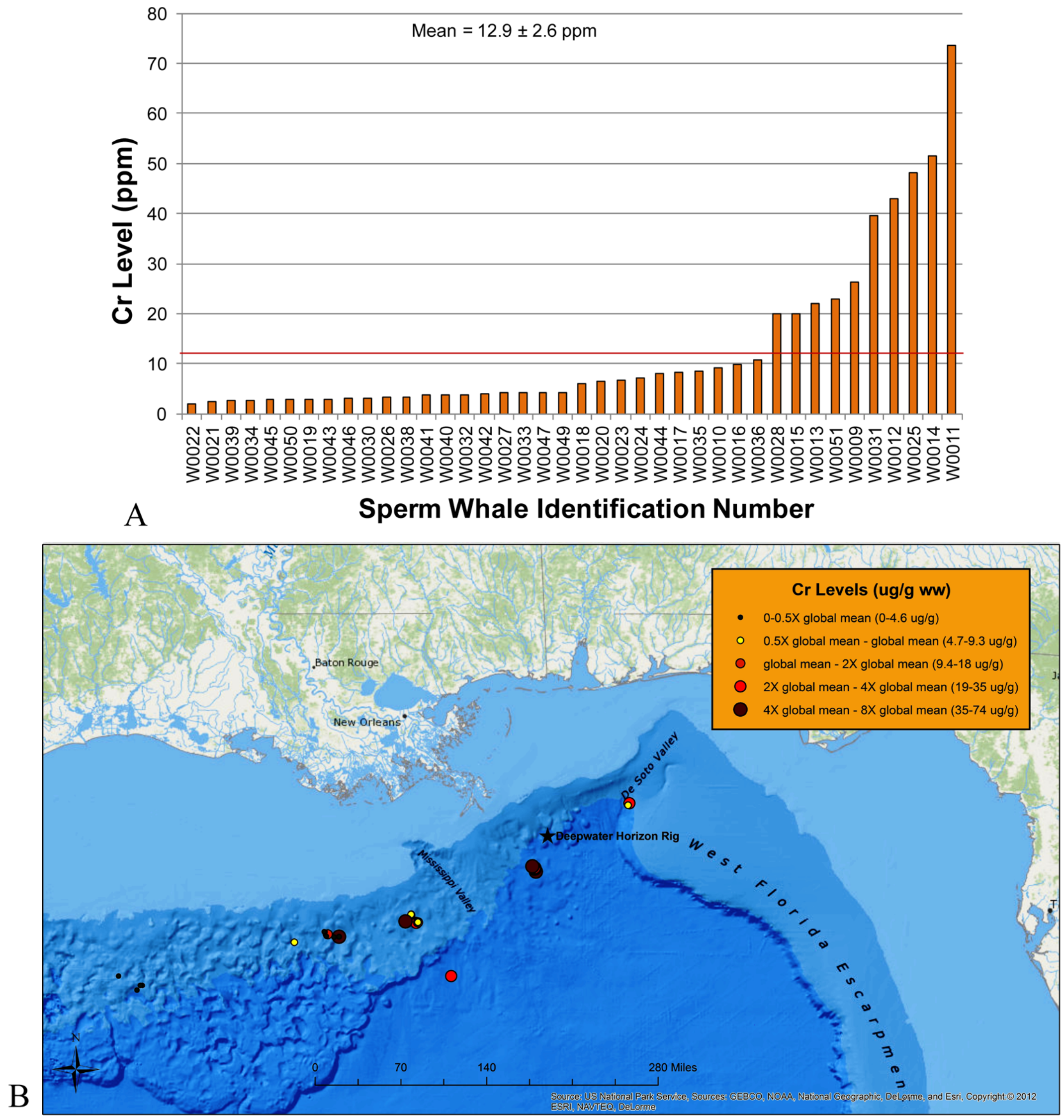

Figure 3. Sperm whales sampled in the Gulf of Mexico have elevated Cr concentrations, and animals with higher concentrations occur closer to the accident epicenter. This figure shows the concentration of Cr in the skin of 40 Gulf of Mexico sperm whales and a map of the location of the Bryde's and sperm whales sampled in 2010 in the aftermath of the Deepwater Horizon accident. (A) Individual data measurements shown by whale. The red line indicates the mean concentration observed in these whales. All data are measured as μg total Cr/g tissue wet weight and expressed as ppm. (B) Map showing the location of individual whales by color-coded Cr concentrations compared to the global mean of our previous study. Note that the majority of the highest concentrations were found in areas that were covered with oil. Green = concentrations that range from 0 to 0.5 times the global sperm whale mean; yellow = concentrations that range from 0.5 times to the global sperm whale mean; orange = concentrations that range from the global sperm whale mean to 2 times the global mean; red = concentrations that range from 2 times the global sperm whale mean to 4 times the global mean; dark red = concentrations that range from 4 times the global sperm whale mean to 8 times the global mean.

in Florida.[42] The third study considered both Cr and Ni and reported mean Ni skin concentrations (converted to wet weight) of 0.02 and 0.01 ppm Ni in 44 dolphins sampled in South Carolina and 39 dolphins sampled in Florida, respectively, and mean Cr skin concentrations of 0.23 and 0.22 ppm Cr in some these same animals (74 dolphins in South Carolina and 67 dolphins in Florida, respectively).[43] Another study measured Ni and Cr liver concentrations in 11 animals from 11 different dolphin and whale species (i.e., one animal per species) that did not include sperm whales. The highest Ni concentration was 1.0 ppm wet weight, and the highest Cr concentration was 1.7 ppm.[44]

The explanation for the much higher Ni and Cr concentrations in the Gulf whales is uncertain. Given that the concentrations are higher than those we found in our global study, which was conducted using the exact same protocols on free-ranging whales, one can rule out explanations based on between-study variation, such as differences in post-mortem decomposition. Instead, the data strongly suggest a significant exposure to Ni and Cr occurring in the Gulf of Mexico. This suggestion is further supported by our observations that these metals appear to be higher in the animals thought to be resident in the Gulf (females and subadult males) compared to those that are thought to seasonally migrate into the Gulf (adult males), although more robust numbers are needed to fully draw this conclusion.

The major input of Cr and Ni into the environment is from anthropogenic sources, and crude oil is one possible source.[48] It is notable that our geospatial location analysis of the whale samples showed that most of whales with the higher

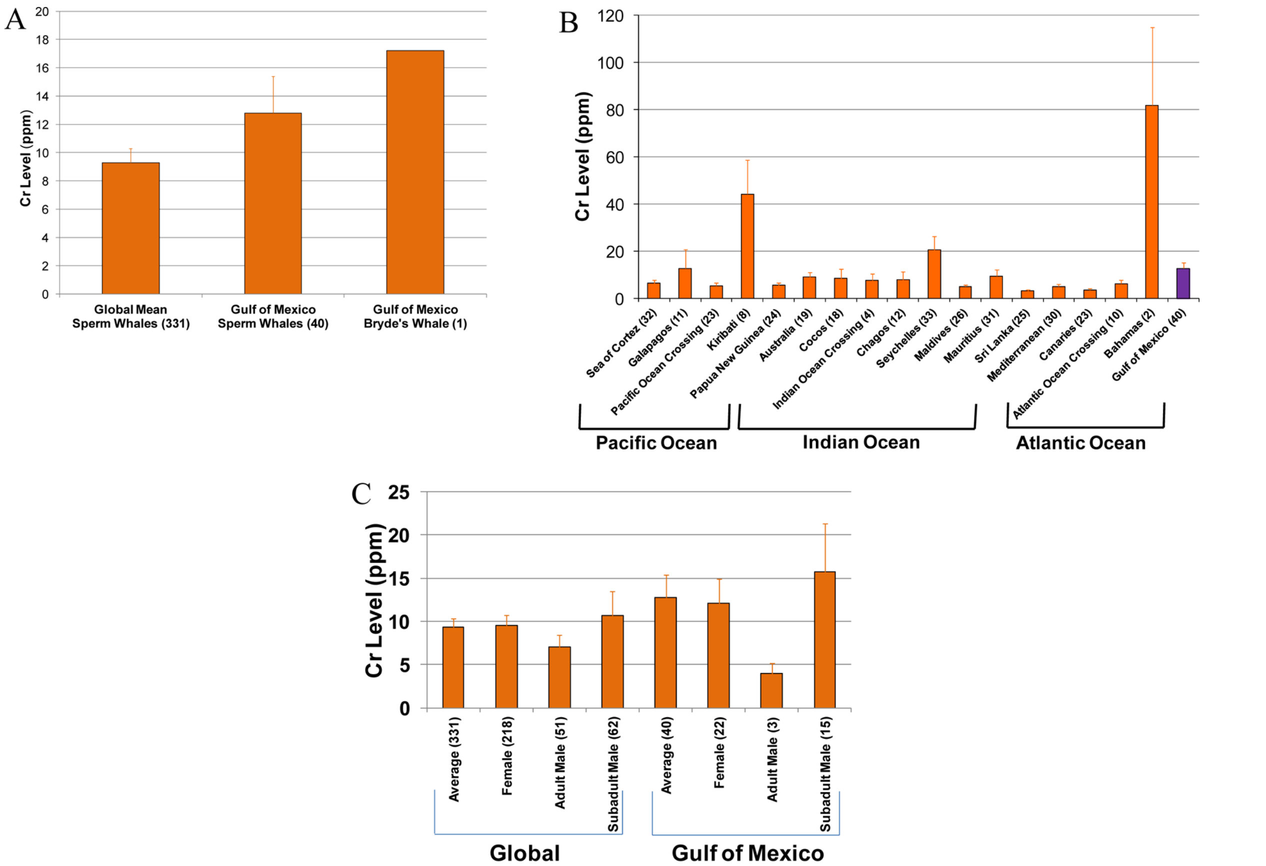

Figure 4. Cr skin concentrations in Gulf of Mexico sperm whales compared to sperm whales from around the globe. This figure shows a comparison of mean concentrations from 40 sperm whales and one Bryde's whale sampled in the Gulf of Mexico in 2010 with mean concentrations from 331 sperm whales sampled around the globe between 2000 and 2005. All data are measured as μg/g wet weight and expressed as ppm. (A) The mean Cr skin concentration in Gulf of Mexico whales compared to overall mean concentration for whales in the global set. Mean Cr concentrations were 12.8 $\pm$ 2.6 ppm for Gulf of Mexico whales, and Cr concentrations were 9.3 $\pm$ 1.0 ppm for the global whale set. The mean in the Gulf of Mexico was statistically significantly higher than the global mean (p = 0.012). (B) The mean Cr skin concentration in Gulf of Mexico whales (purple bar) compared to individual mean concentrations for each region in the global set (orange bars). Gulf whales were higher than those from all regions except for the Bahamas, Kiribati, or Seychelles. The mean in the Gulf of Mexico was significantly higher than the means for eight regions ($p < 0.05$ for Canaries, Cocos, Maldives, Mediterranean, Pacific Crossing, Papua New Guinea, Sea of Cortez, and Sri Lanka). (C) Mean concentrations of Cr in sperm whale skin samples from the Gulf of Mexico and the global means in sperm whale skin samples by gender and approximate age. Age was evaluated by approximate size of the whale.[37] Adult females and subadult males both had higher means in the Gulf of Mexico, but not significantly so (p = 0.052 and p = 0.085, respectively). The mean for the three adult males sampled in the Gulf of Mexico was nonsignificantly lower than the corresponding mean for the rest of the world (p = 0.607).

concentrations of metals were sampled in areas that were heavily contaminated with oil. Also, to provide some context, we measured Ni and Cr in Gulf oil products (e.g., tar balls, oil slicks, and a sample of oil from the Deepwater Horizon riser) that were collected from the Gulf of Mexico while the oil was still flowing and did detect them. Our data showing the presence of Ni and Cr in these oil products are consistent with previous data showing Cr and Ni in oil products from the crisis. For example, in two tar balls, collected in 2010 from Gulf beaches in Alabama, the U.S. Environmental Protection Agency reported (in μg/g) Cr concentrations of 0.06 ppm and undetectable (compared to our levels of 0.36−4.75 ppm) and Ni concentrations of 0.32 ppm for both tar balls (compared to our levels of 0.24−8.46).[45] Similarly, in a sample of weathered oil they reported concentrations of undetectable for Cr and 0.079 ppm for Ni, which is similar to our levels of undetectable for Cr and 2.29−2.70 in our oil slick samples, which were most likely weathered.[45]

At first glance our values seem high, but we believe they reflect the heterogeneity of the oil and the possible impact of weathering of the oil samples. For example, Liu et al. measured the amount of metals in crude oil from the well (Macondo 252 crude oil) and in oil mousse (a mousse being a blend of oil and seawater in which they do not actually combine) from the surface of the water in two locations in the Gulf.[25] In those samples, they report Cr and Ni concentrations (in μg/g) of 9.4 and 1.5 ppm, respectively, in the one crude oil sample and ranging from 7.8 to 9.4 ppm and 4.2−7.7 ppm, respectively, in the two surface water mousse samples. Our measured values for products from the Gulf are within the range of these and the other samples, reflecting the heterogeneity of the oil.

It is possible that some of our oil product measurements are skewed by environmental factors such as seawater, dispersants, and weathering. Given that the oil was collected in the water, there undoubtedly was some amount of seawater present in the crude oil and the oil slick, though no seawater was present in the tar balls. However, recent seawater measurements (presented as nmol/kg) show Cr to be at concentrations of 0.00017 ppm or less and Ni to be at 0.00014 ppm or less.[46] A contribution of this amount is insignificant relative to the concentrations we found; thus, the metals are not likely coming from the seawater, though the seawater, itself, may have reduced our concentrations some by causing a small amount of sample dilution.

The oil products were all collected in June 2010 when the oil was flowing and dispersants were being applied on a regular basis. Thus, the dispersants might have contributed some amount of metal to our samples, if it were present, which we have no way of knowing. However, published values for Ni and Cr in dispersants are 0.14 and 0.03 ppm (measured as mg/kg).[47] These concentrations are also well below our measured values for most of our samples.

The other possible factor is weathering. Aside from the sample collected from the riser, the oil slicks and tar balls had traveled some distance from the well head and, therefore, had undergone some degree of weathering. Weathering may increase Ni but not Cr metal concentrations by aggregating dissolved metals and clay minerals.[25] Thus, if weathering were playing a role, one would expect higher Ni concentrations in the samples further from the well, so higher levels in tar balls than in the oil slicks and higher levels in the oil slicks than from the riser. In general, our data are consistent with weathering impacting our measured Ni concentrations, as our crude oil level of 2.18 ppm was lower than three of the tar balls and both of the oil slicks, but other tar balls were lower so it is difficult to conclude. The original weathering study[25] was based on three samples compared to crude so there may be some complexity caused by the heterogeneity of the oil. Regardless of the weathering aspects, our oil product data and literature clearly show Cr and Ni were present in the oil released during this crisis.

Of course, while our data cannot directly show that the Ni and Cr in the whales came from the oil spill, the data are consistent with a hypothesis that the oil from the crisis could be contributing to the high concentrations in the whales. Other sources of Ni and Cr to the whales might be industries releasing Ni- and Cr-containing waste and exhaust into the Gulf environment or boat paint containing Cr as an antifouling agent. Moreover, our data are consistent with a recent report by Steffy et al., showing the Gulf Crisis significantly affected Cr and Ni concentrations on the sea floor of the Gulf.[47]

Another important consideration is the exposure route to these metals: inhalation, ingestion, or dermal absorption. Considering exposure by ingestion, the whales could have ingested water or prey containing metals. Generally, water is not considered a likely source of contamination because metals do not accumulate in the marine water column,[45] but, in the midst of the oil crisis, ingestion of oil on the surface or dispersed in the water column could have occurred. However, both Ni and Cr are poorly absorbed across the mammalian gastrointestinal tract and do not biomagnify,[26,27] making ingestion an unlikely source of exposure. Moreover, the two whale species we sampled feed at different trophic concentrations; sperm whales prey on giant squid and large fish deep under the surface, whereas Bryde's whales prey on krill and small fish at the surface. Thus, the fact that the single Bryde's whale that we sampled had concentrations of Ni and Cr similar to the high concentrations observed in sperm whales tends to argue against the food chain as the major route of exposure.

Alternatively, the exposure could have been dermal. The whales could have swum through the oil and incurred a skin exposure. Various studies with laboratory animals have demonstrated that both Ni and Cr have poor absorption across mammalian skin,[26,27] and whales have much thicker skin than humans or laboratory animals, suggesting dermal absorption would be even more difficult. Furthermore, whales slough their external skin continuously, and it is likely any external oil would be removed in that sloughing process.[37] We found no visible oil in the water where we encountered whales and observed none on the whale skin. Hence, we believe dermal absorption of the Ni and Cr is also highly unlikely.

In contrast to ingestion or dermal exposures, inhalation is a very plausible route of exposure. Inhalation is known to be a primary exposure route in humans and other mammals for Ni and Cr particulates.[26,27] Whales breathe approximately 100-times more air than humans per hour and hold their breath for long periods while they dive.[49] The length and depth of their dives allows ample time for particulates in the air to settle onto the epithelial layer of their lungs and will likely increase the amount of metals they absorb. The concentrations of Cr and Ni in the offshore air in the Gulf of Mexico are unknown, nor are their sources known. It is notable that extensive burnoff was conducted to remove oil during the Gulf spill, and both metals are known to be released by fossil fuel combustion.[26,27] A study in Nigeria showed elevated concentrations of heavy metals (including Ni and Cr) in the environment and animals around oil platforms and suggests gas flares from oil platforms as one likely source.[50] There are over 100 rigs in the Gulf, and the combined effect of their flares could also be a major source to the Gulf air.

It is important to note that for Cr, there can be a valence issue. There are two biologically stable valence states for Cr, hexavalent [Cr(VI)], and trivalent [Cr(III)]. Of the two, Cr(VI) is the more potent toxicant, though Cr(III) is also genotoxic, and some studies show it may also cause cancer.[51] The difference in potency is considered to be a difference in uptake with the Cr(VI) form more readily absorbed compared to the Cr(III) form.[51] However, the Cr(VI) form does not react with DNA, and once inside the cell, Cr(VI) is immediately reduced to Cr(III), which can react with DNA; Cr(III) is the persistent form in biological tissues.[51] Hence, for the study of tissues such as whale tissue, total Cr is measured because little if any Cr(VI) is thought to be remain after its reduction to Cr(III). However, given the high concentrations of Cr in the whales and the propensity for each form of Cr to induce genotoxicity, the original form of the exposure may not matter.

In summary, our data show that Cr and Ni are present at high concentrations in whales in the Gulf of Mexico. Both metals are known to damage DNA, which raises a health concern not previously been considered for these species in the Gulf environment. The source of the metals to the Gulf environment are uncertain, but our data give some support to the hypothesis that the burning of surface oil during the Deepwater Horizon accident in the Gulf of Mexico may have been a significant source of metal exposure to humans and whales.

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Notes

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Environmental Health Sciences (NIEHS), the

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■ ABBREVIATIONS USED

Ni	nickel
Cr	chromium
PCR	polymerase chain reaction
ICPMS	inductively coupled plasma mass spectrometry
RSD	relative standard deviation
GPS	global positioning system

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