Chemosphere 75 (2009) 1461-1467



Contents lists available at ScienceDirect

Chemosphere



journal homepage: www.elsevier.com/locate/chemosphere

A global assessment of chromium pollution using sperm whales (*Physeter macrocephalus*) as an indicator species

John Pierce Wise Sr.^{a,b,c,d,*}, Roger Payne^{b,d}, Sandra S. Wise^{a,b,c,d}, Carolyne LaCerte^{a,b,c,d}, James Wise^{a,d}, Christy Gianios Jr.^{a,d}, W. Douglas Thompson^{b,c}, Christopher Perkins^e, Tongzhang Zheng^f, Cairong Zhu^f, Lucille Benedict^g, Iain Kerr^{b,d}

^aWise Laboratory of Environmental and Genetic Toxicology, University of Southern Maine, Portland, ME 04104, United States

^b Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, ME 04104, United States

^c Department of Applied Medical Sciences, University of Southern Maine, 96 Falmouth St., P.O. Box 9300, Portland, ME 04104-9300, United States

^d Ocean Alliance, 191 Weston Rd., Lincoln, MA 01773, United States

^e Center for Environmental Sciences and Engineering, University of Connecticut, Storrs, CT, United States

^f Division of Environmental Health Sciences, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT, United States

^g Department of Chemistry, University of Southern Maine, Portland, ME 04104, United States

ARTICLE INFO

Article history: Received 5 November 2008 Received in revised form 12 February 2009 Accepted 14 February 2009 Available online 25 March 2009

Keywords: Sperm whale Chromium Chromate Tissue levels Marine mammal Physeter macrocephalus

ABSTRACT

Chromium (Cr) is a well-known human carcinogen and a potential reproductive toxicant, but its contribution to ocean pollution is poorly understood. The aim of this study was to provide a global baseline for Cr as a marine pollutant using the sperm whale (*Physeter macrocephalus*) as an indicator species. Biopsies were collected from free-ranging whales around the globe during the voyage of the research vessel The *Odyssey*. Total Cr levels were measured in 361 sperm whales collected from 16 regions around the globe detectable levels ranged from 0.9 to 122.6 μ g Cr g tissue⁻¹ with a global mean of 8.8 \pm 0.9 μ g g⁻¹. Two whales had undetectable levels. The highest levels were found in sperm whales sampled in the waters near the Islands of Kiribati in the Pacific (mean = 44.3 \pm 14.4) and the Seychelles in the Indian Ocean (mean = 19.5 \pm 5.4 μ g g⁻¹). The lowest mean levels were found in whales near the Canary Islands (mean = 3.7 \pm 0.8 μ g g⁻¹) and off of the coast of Sri Lanka (mean = 3.3 \pm 0.4 μ g g⁻¹). The global mean Cr skin levels in humans without occupational exposure. The whale levels were more similar to levels only observed previously in human lung tissue from workers who died of Cr-induced lung cancer. We conclude that Cr pollution in the marine environment is significant and that further study is urgently needed.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Ocean pollution is emerging as a global concern for both human health and the health of the ocean ecosystem. The data available about the extent and impact of pollutants is heavily focused on organic contaminants. Much less is understood about the threat posed by inorganic metals. In particular, chromium (Cr) is a metal of increasing concern regarding environmental health. Cr is released into the marine environment by both natural and anthropogenic sources. The primary natural source of Cr is continental dust flux (US Department of Health, 1993). Anthropogenic sources release more Cr to the environment than natural sources, and include industrial, commercial and residential fuel combustion (natural gas, coal, and oil), emissions from metal industries, and wastewaters from industries such as electroplating operations, leather tanning industries, and textile manufacturing (US Department of Health, 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 of Cr found in marine waters, and can have residence times ranging from 4.6 to 18 years (Pettine and Millero, 1990; US Department of Health, 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 (Georgescu et al., 1988; Aboul Dahab, 1989; Kamala-Kannan et al., 2008).

Marine air measurements are infrequent, however a few measurements have been done. Total Cr levels in Baltimore harbor and Hawaii were reported as 0.226 and 0.067 μ g m⁻³, respectively (Bowen, 1979; IARC, 1990). Total Cr levels in areas bordering marine environments have been reported in Kolkata, India

^{*} Corresponding author. Address: Department of Applied Medical Sciences, University of Southern Maine, 96 Falmouth St., P.O. Box 9300, Portland, ME 04104-9300, United States. Tel.: +1 207 228 8050; fax: +1 207 228 8518.

E-mail address: John.Wise@maine.edu (J.P. Wise Sr.).

^{0045-6535/\$ -} see front matter @ 2009 Elsevier Ltd. All rights reserved. doi:10.1016/j.chemosphere.2009.02.044

(0.006–0.07 μ g m⁻³), Hudson County, NJ (0.005–0.007 μ g m⁻³), Sydney, Australia (0.0002–0.0013 μ g m⁻³), and from six cities in Egypt (0.019–6.84 μ g m⁻³) (Lioy et al., 1992; Borai et al., 2002; Li et al., 2002; Karar and Guota, 2007). From the six cites studied in Egypt, Tabbin, Ramsis and Shoubra had the highest levels of chromium; 5.266, 4.231, and 6.842 μ g m⁻³ respectively (Borai et al., 2002). The lack of data is due in major part to the inability to determine the initial Cr speciation that enters the environment, and the complexity of the analyses needed in different environmental media. Although *in situ* analytical techniques are being produced, much work still needs to be done before reliable results are available (Khlystov and Ma, 2006; Elci et al., 2008; Jena and Raj, 2008).

Cr(VI) levels are not generally measured in marine pollution studies concerning marine organisms. The major reason for this lack of measurement is that in biological systems Cr(VI) is rapidly reduced within minutes to trivalent Cr (Cr(III)) and consequently Cr(VI) levels cannot be accurately measured directly (De Flora and Wetterhahn, 1989). Instead, studies that have sought to measure Cr in marine organisms, typically measure total Cr levels. These studies have found total Cr levels to be generally undetectable or low (<1 μ g g⁻¹) in whales (Byrne et al., 1985; Holsbeek et al., 1999; Tilbury et al., 2002). However, we recently observed in a small pilot study that total Cr levels in the skin of North Atlantic right whales (Wise et al., 2008) are much higher (mean 7.1 μ g total Cr g tissue⁻¹) than those previously reported for any marine mammal species. Because mammals absorb Cr(III) poorly, these data suggest that marine Cr(VI) exposure may be a significant concern for this species (Wise et al., 2008). These right whale levels were 24-fold higher than the 0.31 μ g g⁻¹ level previously reported in the skin of humans who were not exposed to industrial levels of Cr (Schroeder et al., 1970).

Between 2000 and 2005, the research vessel *Odyssey* collected biopsies from Pacific, Indian, and Atlantic Ocean and Mediterranean Sea sperm whales. Sperm whales have a global distribution and feed high on oceanic food chains. Because this species in an apex mammalian predator in the ocean, it reflects what might be expected for humans who depend on the ocean for food and in that sense may serve as a sentinel for human health as well. Here we present the first toxicological dataset from that global voyage, focusing on total Cr levels in these whales. The data indicate that Cr is indeed a global pollutant with some regions relatively low and others reaching levels that are alarmingly high, and are only seen in occupationally exposed workers.

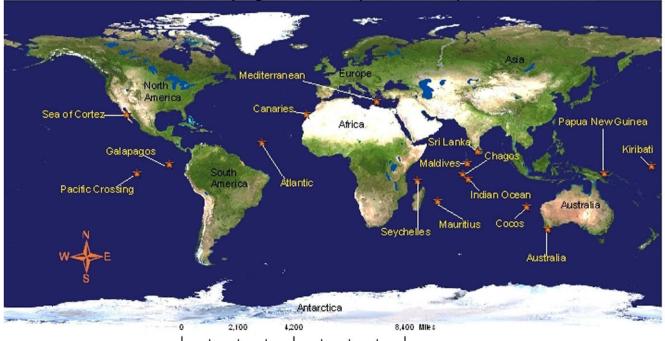
2. Materials and methods

We measured Cr levels in 361 sperm whales collected from 16 regions around the globe (Fig. 1). We considered 217 adult females and 144 male sperm whales (53 adults and 91 subadults). Table 1 shows the distribution of the whales by region.

2.1. Biopsies

During the voyage of the research vessel *Odyssey* biopsies were collected from free-ranging sperm whales using standard methods (Brown et al., 1991). Sampling was carried out simultaneously with photo-identifications of individual whales to minimize duplication. The behaviors of all whales sampled appeared to be healthy. Samples were taken from the whale's flank, a location that has been shown to elicit the fewest reactions (Brown et al., 1991). We used a 50 mm stainless steel cylindrical biopsy dart. Samples were removed from the biopsy dart and divided into two pieces at the interface between skin and blubber. These two pieces were stored separately for later genetic and metal analysis. All tissue samples were frozen at -20 °C within a few minutes of collection. The samples were also shipped frozen to the Wise Laboratory.

To ensure that there was no contamination of the sample by the biopsy dart, these tools were rinsed extensively and cleaned between each use. In addition, to confirm that no Cr was leaching out of the tips, we tested a piece of bowhead whale tissue. This tissue was sampled with biopsy darts of the same make and



Sampling Locations for Sperm Whale Biopsies

Fig. 1. The route of the voyage of the Odyssey. This figure shows the 16 regions where whales were sampled during the voyage of the Odyssey. The voyage started in the Sea of Cortez and continued westward, ultimately ending in Massachusetts.

compared to a sample prepared with a ceramic knife. There were no differences in total Cr levels between these samples, and furthermore, the measured total Cr levels were low indicating that the darts did not contaminate the tissue (data not shown). We also attempted to leach Cr from these instruments with a 2% HNO₃ solution and found the leachate to be nondetectable for total Cr (data not shown).

2.2. Genotyping

Gender was determined by genotyping based on published methods (Richard et al., 1994). DNA was extracted from a piece of whale skin using standard methods (Alijanabi and Martinez, 1997). Gender was determined by PCR amplification reactions in which the SRY (male determining factor) gene was amplified according to published methods (Richard et al., 1994). The keratin gene was used as an amplification control for all samples. Male samples showed both the keratin band (~311 bp) and SRY (male) band at ~152 bp. Female samples showed only the keratin band at ~311 bp. Primer sequences were the following:

- SryPMF: 5'CATTGTGTGTGTGGTCTCGTGATC
- SryPMR:5'AGTCTCTGTGCCTCCTCGAA
- KF: 5'AGATCAGGGGTTCATGTTTCTTTGC
- KR: 5'TTTACAGAGGTACCCAAGCCTAAG

2.3. Inductively coupled plasma mass spectroscopy

Whale skin samples were analyzed for total Cr using inductively coupled plasma mass spectrometry (ICPMS) according to our published methods using a Perkin–Elmer/Sciex ELAN ICPMS (Wise et al., 2008). Interference check solutions were analyzed with all sample runs to compensate for any matrix effects which might be interfering with sample analysis. Standard quality assurance procedures were employed (Table 2). Instrument response was

Table 1

Distribution of the whales across regions

evaluated initially, after every 10 samples, as well as at the end of each analytical run using a calibration verification standard and blank. All data are presented as μ g Cr g tissue⁻¹ wet weight.

2.4. Statistics

Mean values were compared using analysis of variance. Differences for individual pairs of means were assessed via *t*-tests, with the Bonferroni correction for multiple comparisons. When Cr was not detected in a specimen, a value of one-half the detection limit was used in the analysis. Since the distributions of values were skewed, a normalizing logarithmic transformation was used for the statistical testing. The statistical analyses were all conducted in SAS (SAS Institute, 2004).

3. Results

3.1. Chromium level comparisons by region

Cr was present in all but two whales (Table 3). Detectable levels ranged from 0.9 to 122.6 μ g Cr g tissue⁻¹ with a global mean level equal to 8.8 ± 0.9 μ g g⁻¹. Cr concentrations from some ocean regions were higher than others (Table 3; *F*(15,315) = 3.74; *p* < 0.0001). The highest levels were found in sperm whales sampled in waters near the Islands of Kiribati in the Pacific (mean = 44.3 ± 14.4) and the Seychelles in the Indian Ocean (mean = 19.5 ± 5.4 μ g g⁻¹). The lowest mean levels were seen in whales near the Canary Islands (mean = 3.7 ± 0.8 μ g g⁻¹) and off of the coast of Sri Lanka (mean = 3.3 ± 0.4 μ g g⁻¹).

3.2. Chromium level comparisons by gender

We also considered the whale Cr levels by gender (Figs. 2 and 3). Measurable levels in female whales with detectable levels of

Ocean/Sea	Region	# Of female whales Adult	# Of male whales		Total number of whales
			Adult	Subadult	
Pacific	Sea of Cortez	21	12	0	33
	Galapagos	0	3	8	11
	Pacific Crossing	17	6	0	23
	Kiribati	9	0	0	9
	Papua New Guinea	14	5	5	24
	Australia	10	0	9	19
	Cocos	18	0	0	18
Indian	Indian Ocean Crossing	0	4	0	4
	Chagos	0	0	0	12
	Seychelles	25	4	6	35
	Maldives	18	4	5	27
	Sri Lanka	26	1	0	27
	Mauritius	29	2	1	32
Mediterranean	Mediterranean	14	8	11	33
Atlantic	Canaries	17	2	4	23
	Atlantic Crossing	8	0	2	10

Table 2

Mean quality assurance and quality control data for Cr analysis.

Element	LOD ^a (ppm)	Blank (ppm)	Duplicate (RPD) (%)	LCS recovery (%)	Spike recovery (%)	SRM ^b recovery (%) DORM-2
Cr	0.07	BDL ^c	13.5	101.8	99.8	105.9

^a LOD = Limit of detection.

^b SRM = Standard reference material.

^c BDL = Below detection limit.

Table 3

Global distribution	of chromium	lovole in co	arm whaloc ^a

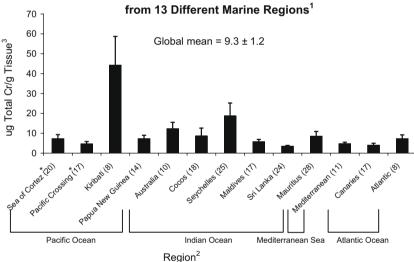
Region ^b	Ν	Minimum ^c	Maximum	Mean	Standard error
Sea of Cortez ^d	32	1.5	41.0	6.5	1.3
Galapagos	11	1.9	91.0	12.9	7.9
Pacific Crossing ^d	23	1.6	22.5	5.5	1.1
Kiribati	8	2.8	101.9	44.3	14.4
Papua New Guinea	24	1.7	23.0	5.7	1.1
Australia	19	1.1	32.5	9.2	2.0
Cocos	18	1.1	75.6	8.6	4.0
Indian Ocean Crossing	4	2.3	13.1	7.7	2.8
Chagos	12	0.9	44.3	8.0	3.5
Seychelles	35	0.9	122.6	19.5	5.4
Maldives	26	2.0	19.7	5.2	0.8
Sri Lanka	25	1.1	9.3	3.3	0.4
Mauritius	31	0.9	63.2	9.6	2.7
Mediterranean	30	1.0	28.7	5.2	1.0
Canaries	23	1.0	17.6	3.7	0.8
Atlantic Crossing	10	1.5	19.4	6.3	1.7

Two hundred and seventeen adult female sperm whales and 114 male sperm whales were sampled.

^b Specific regions are named for the nearest land body or ocean region and are illustrated in Panel A (along with number of whales sampled).

All data are presented in µg total Cr g tissue⁻¹ wet weight.

^d This region had one whale with undetectable Cr levels. ½ the detection level was used in the analysis (1.28 µg g⁻¹ for Pacific Crossing; 2.33 µg g⁻¹ for Sea of Cortez).



Skin Chromium Levels in 217 Adult Female Sperm Whales

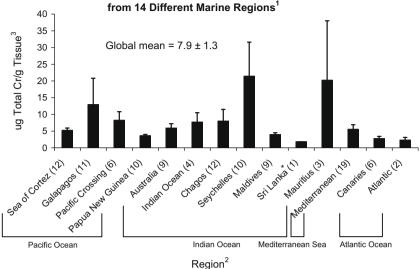
Fig. 2. Global distribution of chromium levels in female sperm whales. This figure shows the global distribution of mean Cr levels in 217 adult female sperm whales grouped by sampling region. ¹No female whales were found in the Chagos, Galapagos or Indian Ocean regions; ²Specific regions are named for the nearest land body or ocean region and are illustrated in Fig. 1; ³All data are presented in µg total Cr g tissue⁻¹ wet weight ± standard deviation; ^{*}This region had one whale with undetectable Cr levels. ½ the detection level was used for this whale in the analysis.

Cr ranged from 0.9 to 122.6 $\mu g g^{-1}$ (two female whales had undetectable levels of Cr). The global mean for all female whales was $9.3 \pm 1.2 \ \mu g \ g^{-1}.$ All male whales had detectable levels with a range of 0.9–94.6 $\mu g\,g^{-1}$ and a global mean 7.9 \pm 1.4 $\mu g\,g^{-1}.$ Overall, the mean level for females was slightly higher than males, although this difference was not significantly different (F(1,329) = 0.50;p = 0.48), indicating that sex was not a confounding factor. We found no conclusive evidence that increased size and age gave higher contaminant burdens; in fact, the lowest mean levels occurred in the much larger and older adult males (7.0 ± 1.4) compared to levels in subadult males (8.7 ± 2.2) , though these differences were also not statistically significant (F(1,112) = 0.27;p = 0.61).

Whales were not evenly distributed by region as only female whales were found in the waters around Kiribati and the Cocos islands. Similarly, only male whales were found in waters around the Chagos archipelago, the Galapagos islands and during the Indian Ocean crossing. All other regions had a mix of males and females at the times the Odyssey was present and collecting samples.

Considering gender by region, the highest Cr levels for females were found in whales sampled in the waters near the Islands of Kiribati in the Pacific (range of 2.8-101.9; mean = 44.3 ± 14.4 - $\mu g g^{-1}$) and the Seychelles in the Indian Ocean (range of 1.5– 122.6; mean = $18.7 \pm 6.5 \ \mu g \ g^{-1}$). The lowest mean levels were seen in whales near the Canary Islands (range of 1.0-17.6; mean = 4.0 \pm 1.0 $\mu g \: g^{-1})$ and off of the coast of Sri Lanka (range of 1.1–9.3; mean = $3.4 \pm 0.5 \ \mu g \ g^{-1}$). The variation among regions was statistically significant (F(12,204) = 4.31; p < 0.0001). Pairwise *t*-tests showed that female whales in Kiribati had significantly higher Cr levels than all other regions (p < 0.05) except for the Atlantic Ocean and the Seychelles. The levels in the Seychelles were significantly higher than those in the Canaries (p < 0.05).

The highest Cr levels in male sperm whales were found in those animals sampled in the waters near Mauritius (range of 2.3–55.9;



Skin Chromium Levels in 114 Male Sperm Whales from 14 Different Marine Regions¹

Fig. 3. Global distribution of chromium levels in male sperm whales. This figure shows the global distribution of mean Cr levels in 114 subadult male sperm whales grouped by sampling region. ¹No male whales were found in the Cocos or Kiribati regions; ²Specific regions are named for the nearest body or ocean region and are illustrated in Fig. 1; ³All data are presented in µg total Cr g tissue⁻¹ wet weight ± standard error; ^{*}Standard error not applicable – only 1 sample.

mean = 20.2 ± 17.8 µg g⁻¹) and the Seychelles in the Indian Ocean (range of 0.9–94.6; mean = 21.4 ± 10.2 µg g⁻¹). The lowest mean levels were seen in whales near the Canary Islands (range of 1.3–5.1; mean = 2.8 ± 0.6 µg g⁻¹) in the Atlantic (range of 1.5–3.0; mean = 2.3 ± 0.8 µg g⁻¹). The one adult male whale measured off of the coast of Sri Lanka had a level of 1.8 µg g⁻¹. There were no statistical differences among samples from male whales that were collected from the different regions (F(13,100) = 0.91; p = 0.55). There was no statistically significant variation across regions among either adult (F(10,40) = 1.67; p = 0.12) or subadult males (F(9,53) = 1.26; p = 0.28).

4. Discussion

Chromium is one of the least studied marine pollutants. This study reports the global distribution of chromium in sperm whales, an apex marine predator. As such it is the first global study of the distribution of a major pollutant. Previous marine pollution studies have largely focused on opportunistically obtained samples in a few well-researched areas of the Northern hemisphere. Thus, it has been unclear if levels observed reflected regional problems or a more global concern for the pollutants involved. Using sperm replace with whale as an indicator species, we find that total Cr levels are dramatically high in these animals in several locations around the world.

Within our detectable range of 0.9–122.6 μ g g⁻¹ we found a global mean level of 8.8 ± 0.9 μ g g⁻¹ (with two whales showing undetectable levels). These levels are higher than those reported in a previous study of seven sperm whales from the North Sea (Holsbeek et al., 1999). Six of the whales in that study stranded alive and all of the samples were taken within 24 h of death. That study reported detectable Cr levels from muscle, kidney and liver that ranged from 0.03 to 0.36 μ g g⁻¹ Cr wet weight. If we assume that these samples had 70% moisture the values originally reported would convert to 0.1–1.2 μ g g⁻¹ dry weight with several samples having undetectable levels). Unfortunately, skin was not measured in this previous study so direct organ comparisons are not possible.

Considering other whale species, the levels we observed in sperm whales are also much higher than those previously reported in other whales. In subsistence-hunted whales, liver, kidney and brain tissues of gray whales ranged from 0.2–0.29 μ g g⁻¹ (Tilbury et al., 2002) and blubber, liver, kidney, muscle and spleen tissues of bowhead whales ranged from 0.01 to 0.55 μ g g⁻¹ (Byrne et al., 1985). Considering other studies of whale skin Cr, mean levels in minke whales were 0.68 and 0.62 μ g g⁻¹ calculated wet weight (values converted from dry weight assuming 70% moisture); for males and females respectively and less than 1.1^{*} μ g g⁻¹ wet weight^{*} in Dall's porpoise (Kunito et al., 2002; Yang et al., 2002). In bottlenose dolphins, mean skin levels were also lower; reported as 0.14 μ g g⁻¹ wet weight (Bryan et al., 2007) and 0.23 μ g g⁻¹ calculated wet weight (Stavros et al., 2007).

The explanation for these differences is uncertain. There could be some loss due to postmortem decomposition, but this cannot explain all of the differences as Holsbeek et al. took all of their sperm whale samples within 24 h of death and the bowhead and gray whale samples were obtained during a subsistence hunt. It could be that the values reflect different accumulation of Cr in whale organs as we considered skin levels whereas previous studies considered internal organs. This explanation seems unlikely as rodent studies indicate that Cr accumulates more in liver and kidney than in skin (ATSDR, 2000). Thus, for this possibility to be true whales would have had to evolve a different mechanism for Cr accumulation; of course it could be possible, but at this time there is no evidence for such an occurrence.

It could be that Cr levels have escalated in the years between these studies and ours. Cr levels may have been low then, but may now be on the increase. However, more work returning to the same sites over a period of years is needed to determine if this increase is occurring. Perhaps the best explanation is that the data reflect regional differences (e.g. lower levels in the North Sea compared to the regions we sampled), such a possibility is consistent with our observations that there are regional differences in Cr skin levels in free-ranging sperm whales and wide interindividual variation in Cr levels. This explanation is also consistent with the fact that our reported levels are consistent with elevated levels reported in skin biopsies from free-ranging North Atlantic right whales collected in the Bay of Fundy off the east coast of North America (mean of 7.1 μ g g⁻¹; range of 4.9–10 μ g g⁻¹) (Wise et al., 2008). The possibility of regional differences may also explain the fact that the adult males had lower levels than adult females or subadult males. Sperm whales are one of the most sexually dimorphic species with adult males much bigger than females (Whitehead, 2003). The males are also thought to feed in near-polar waters and to migrate into equatorial waters only to breed (Whitehead, 2003). The fact that their levels are lower, when considered with their migration patterns suggest that their chromium exposure may be reduced due to the significant time spent in near-polar waters. Of course, more studies of polar Cr levels are needed to determine if this possibility is true.

The major input of Cr(VI) into the environment is anthropogenic sources (ATSDR, 2000). There are a number of anthropogenic inputs including factories making Cr containing compounds such as paints, dyes and inks, tanneries and direct input from boat paint containing chromates which are often used as antifouling agents. Thus, it is interesting to note that the highest levels were seen in Kiribati. This group of islands is in the remote Pacific with no known industrial discharges. Point sources for these whales are difficult to determine, in part because the whales migratory routes are largely unknown. However, the range of female whales is believed to rarely exceed 3000 km (Whitehead, 2003) and all of the whales sampled near Kiribati were female.

If we assume a 3000 km range for these Kiribati animals, then the whales could be exposed to atmospheric and industrial discharges of chromium from the Australia state of Queensland on the northeastern corner of the Australian continent. Consistent with this possibility, the National Pollutant Inventory for the Australian government shows that the majority of Australia's Cr(VI) emitting industries are present in this location (National Pollutant Inventory, 2008). It estimates that in 2006-2007 these industries emitted 11 tons of Cr(VI) so such an exposure scenario is plausible if the Kiribati whales reach these areas, but more work is needed to determine if it is in fact occurring. We also sampled sperm whales near Australia. Some of these samples were also high in Cr, reaching levels as high as 32.5 μ g g⁻¹, but the 9.2 μ g g⁻¹ mean was much lower than the Kiribati whales. However, it should be noted that these Australian sperm whales were sampled along the Western coast of Australia more than 3000 km from the northeastern Australian coast. Western Australia has Cr industry present, but its emissions are much lighter with only 2 tons of Cr(VI) emitted in 2006-2007 (National Pollutant Inventory, 2008). Of course, it is also possible that exposures could have come from other regions the whales migrated through (e.g. Papua New Guinea), but we were unable to locate Cr release data for other regions.

These high levels raise concerns about the potential impact of Cr on the health of the whales. The consequences of such high levels for the whales are uncertain as Cr toxicity has not been studied in sperm whales. In fact, the only study of Cr toxicity in any whale species showed that Cr(VI) is cytotoxic and genotoxic to Northern right whale lung and testes cells (Wise et al., 2008). Cr(VI)-exposed human cells show similar effects (Wise et al., 2002) suggesting that Cr may have similar toxic effects in marine mammals and humans.

The major health concern in humans with respect to Cr(VI) is cancer (IARC, 1990). It is unknown if Cr causes cancer in sperm whales, but it is notable that the Cr levels we found in sperm whales are similar to levels reported for lung tissue from workers with Cr(VI)-induced lung cancer. Specifically, the range of total Cr levels in such workers was $0.4-132 \ \mu g \ g^{-1}$ with a median level of 20.4 in their lungs (Tsuneta et al., 1980). Considering all of the whales that we sampled from every region, almost 8% of them had Cr levels above the median level of these exposed workers. Moreover, the skin levels in Kiribati whales, which had a range of 2.8-101.9 $\ \mu g \ g^{-1}$ and a median of 20.4 are remarkably close to these workers. To be clear, we are not saying that because the whale levels which resemble those seen in these workers that

the whales are suffering from Cr-induced cancer, but rather that these similarities suggest the whale levels are quite high.

Of course, this comparison considers lung versus skin levels. Skin total Cr levels are rarely measured in human autopsies, but in one case they were. In that case the worker had lung levels ranging from 33 to 45.6 μ g g⁻¹ and a skin level of 0.05 μ g g⁻¹ (Mancuso, 1997) much lower than the skin level in the whales. Similarly, the mean sperm whale skin Cr level was 28-times higher than the 0.31 μ g g⁻¹ ± 0.099 mean level reported in humans without occupational exposure (Schroeder et al., 1970) suggesting that whale skin levels are indeed high and reflective of significant exposure.

Of course these data do not necessarily mean that sperm whales have Cr-induced cancer. Sperm whales are an endangered species largely due to over-hunting of the males (Whitehead, 2003). If Cr(VI) were to increase the incidence of cancer in this species it might impair the recovery of this species. Because of their ability to travel vast distances in deep ocean waters, it is difficult to assess the occurrence of cancer in their population and it is currently unknown whether this is a concern for this species. Cancer is a concern for other cetaceans with a more limited range, such as the St. Lawrence Estuary Beluga whales (Martineau et al., 2002), but Cr(VI) has not been studied in this species.

A second possible threat Cr exposure poses to the whales is that it could affect their reproductive fitness. Cr(VI) is known to target the reproductive system in mammals. It can accumulate in the testes and induce testicular toxicity including decreasing sperm counts and altering reproductive behaviors among other effects (Witmer et al., 1989, 1991; Bataineh et al., 1997; Al-Hamood et al., 1998). It is unknown if sperm whales are having issues with reproduction, but another whale, the North Atlantic right whale, does have an underlying reproductive problem, which may be either caused by or exacerbated by exposure to environmental contaminants (Kraus et al., 2001). It is interesting to note that this population of whales has a similar mean Cr level as the sperm whales (Wise et al., 2008). More work is needed to determine if Cr is impacting the reproductive success in these whales.

It is possible that whales normally have such high Cr levels due to some novel adaptation of whale physiology. We believe this outcome is unlikely as there is no evidence to support it. In addition, we found that detectable levels of Cr spanned almost a 200-fold range and that many whales had relatively low levels (<1 ppm), which would then suggest that they might be Cr deficient. A Cr deficient state has not been readily achievable in humans or rodents (Keen, 1996). Moreover, Cr levels in bowhead, gray and other sperm whales were also much lower (<0.55 ppm), which is not consistent with an ability for cetaceans to greatly accumulate Cr. Instead, the levels are more consistent with a conclusion they come from environmental sources and not a novel adaptation. Of course, further work is needed to fully support this conclusion.

Of course, it was not possible to determine the valence of Cr that the whales were originally exposed to from these samples, because Cr(VI) is immediately metabolized to Cr(III) after exposure (De Flora and Wetterhahn, 1989). However since Cr(VI) is the major valence of Cr in marine waters (Pettine and Millero, 1990), and Cr(III) is poorly absorbed by mammals (ATSDR, 2000), it seems likely that a significant portion of the exposure most likely involved Cr(VI). The data from the Australian National Pollutant Inventory shows that substantial amounts of Cr(VI) can be emitted each year. It also was not possible to assess when exposure occurred. Considered together the collective data suggest that significant regional exposures to Cr occur and that Cr levels may be increasing over time. More work is needed to determine the exposure route, point sources, and the potential health effects, but these data suggest that Cr exposure may be a significant concern for the health of marine organisms, particularly whales.

Acknowledgments

We thank all those who served as staff and crew during the Voyage. We thank Drs. Gregory Buzard and Hong Xie for technical assistance. We thank the many supporters of the Ocean Alliance and the Maine Center for Toxicology and Environmental Health for financial support. Work conducted under NMFS permit #1008-1637-00 (J. Wise, PI) and permit #751-1614 (Iain Kerr, PI).

References

- Aboul Dahab, O., 1989. Chromium biogeochemical cycle in Abu Kir Bay, East of Alexandria, Egypt. Estuar. Coast Shelf. Sci. 29, 327–340.
- Al-Hamood, M.H., Elbetieha, A., Bataineh, H., 1998. Sexual maturation and fertility of male and female mice exposed prenatally and postnatal to trivalent and hexavalent chromium compounds. Reprod. Fertil. Dev. 10, 179–183.
- Alijanabi, S.M., Martinez, I., 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 25, 4692–4693.
- ATSDR, 2000. Toxicological profile for chromium. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, Georgia.
- Bataineh, H., Al-Hamood, M.H., Elbetieha, A., Bani Hani, I., 1997. Effect of long-term ingestion of chromium compounds on aggression, sex behavior and fertility in adult male rat. Drug Chem. Toxicol. 20, 133–149.
- Borai, E.H., El-Sofany, E.A., Abdel-Halim, A.S., 2002. Speciation of hexavalent chromium in atmospheric particulate samples by selective extraction and ion chromatographic determination. TrAC Trends Anal. Chem. 21, 741–745.
- Bowen, H.J.M., 1979. Environmental Chemistry of the Elements. Academic Press, London.
- Brown, M.W., Kraus, S.D., Gaskin, D.E., 1991. Reaction of right whales (*Eubalaena glacialis*) to skin biopsy sampling for genetic and pollutant analysis. Reports of the International Whaling Commission, Special Issue, vol. 13, pp. 81–89.
- Bryan, C.E., Christopher, S.J., Balmer, B.C., Wells, R.S., 2007. Establishing baseline levels of trace elements in blood and skin of bottlenose dolphins in Sarasota Bay, Florida: implications for non-invasive monitoring. Sci. Tot. Environ. 388, 325–342.
- Byrne, C., Balasubramanian, R., Overton, E.B., Albert, T.F., 1985. Concentrations of trace metals in the bowhead whale. Mar. Pollut. Bull. 16, 497–498.
- De Flora, S., Wetterhahn, K.E., 1989. Mechanisms of chromium metabolism and genotoxicity. Life Chem. Rep. 7, 169–244.
- Elci, L., Kartal, A.A., Soylak, M., 2008. Solid phase extraction method for the determination of iron, lead and chromium by atomic absorption spectrometry using Amberite XAD-2000 column in various water samples. J. Hazard. Mater. 153, 454–461.
- Georgescu et al., 1988 as cited in Aboul Dahab, O., 1989. Chromium biogeochemical cycle in Abu Kir Bay, East of Alexandria, Egypt. Estuar. Coast. Shelf. Sci. 29, 327–340.
- Holsbeek, L., Joiris, C.R., Debacker, V., Ali, I.B., Roose, P., Nellissen, J.P., et al., 1999. Heavy metals, organochlorines and polycyclic aromatic hydrocarbons in sperm whales stranded in the Southern North Sea during 1994/1995 winter. Mar. Pollut. Bull. 38, 304–313.
- International Agency for Research Cancer, 1990. Chromium, Nickel and Welding IARC Monographs on the Evaluation Carcinogenic Risks to Humans, vol. 49. Lyon: International Agency for Research on Cancer.
- Jena, B.K., Raj, R., 2008. Highly sensitive and selective electrochemical detection of sub-ppb level chromium(VI) using nano-sized gold particle. Talanta 76, 161–165.
- Kamala-Kannan, S., Batvaru, B., Lee, K.J., Kannan, N., Krishnamoorthy, R., Shanthi, K., Jayaprakash, M., 2008. Assessment of heavy metals (Cd, Cr and Pb) in water, sediment and seaweed (*Ulva lactuca*) in the Pulicat Lake, South East India. Chemosphere 71, 1233–1240.

- Karar, K., Guota, A.K., 2007. Source apportionment of PM₁₀ at residential and industrial sites of an urban region of Kolkata, India. Atmos. Res. 84, 30–41.
- Keen, C.L., 1996. Teratogenic effects of essential trace metals: deficiencies and excesses. In: Chang, L.W. (Ed.), Toxicology of Metals. CRC Press Inc., New York, pp. 977–1001.
- Khlystov, A., Ma, T., 2006. An on-line instrument for mobile measurements of the spatial variability of hexavalent and trivalent chromium in urban air. Atmos. Sci. 40, 8088–8093.
- Kraus, S.D., Hamilton, P.K., Kenney, R.D., Knowlton, A.R., Slay, C.K., 2001. Reproductive parameters of the North Atlantic right whale. J. Cetacean Res. Manag (special issue) 2, 213–236.
- Kunito, T., Watanabe, I., Yasunaga, G., Fujise, Y., Tanabe, S., 2002. Using trace elements in skin to discriminate the populations of minke whales in southern hemisphere. Mar. Environ. Res. 53, 175–197.
- Li, Y., Pradhan, N.K., Foley, R., Low, G.K.C., 2002. Selective determination of airborne hexavalent chromium using inductively coupled plasma mass spectrometry. Talanta 57, 1143–1153.
- Lioy, P.J., Freeman, N.C.G., Wainman, T., Stern, A.H., Boesch, R., Howell, T., Shupack, S.I., 1992. Microenvironmental analysis of residential exposure to chromium laden wastes in and around New Jersey homes. J. Risk Anal. 12, 287–299.
- Mancuso, T.F., 1997. Chromium as an industrial carcinogen: Part II. Chromium in human tissues. Am. J. Ind. Med. 2, 140–147.
- Martineau, D., Lemberger, K., Dallaiire, A., Labelle, P., Lipscomb, T.P., Michel, P., Mikaelian, I., 2002. Cancer in wildlife, a case study: beluga from the St. Lawrence estuary, Quebec, Canada. Environ. Health Perspect. 110, 285–292.
- National Pollutant Inventory of Australia, 2008. Available: http://www.npi.gov.au/cgi-bin/npidbsearch.pl [accessed June 23, 2008].
- Pettine, M., Millero, F.J., 1990. Chromium speciation in seawater: the probable role of hydrogen peroxide. Limnol. Oceanogr. 35, 730–736.
- Richard, K.R., McCarry, S.W., Wrighy, L.M., 1994. DNA sequence from the SRY gene of the sperm whale (*Physeter macrocephalus*) for use in molecular sexing. Can. J. Zool. 72, 873–877.
- SAS Institute Inc. 2004. SAS/STAT 9.1 User's Guide. SAS Institute Inc., Cary, NC.
- Schroeder, H.A., Nason, A.P., Tipton, I.H., 1970. Chromium deficiency as a factor in atherosclerosis. J. Chron. Dis. 23, 123–142.
- Stavros, H.C., Bossart, G.D., Hulsey, T.C., Fair, P.A., 2007. National Oceanic and Trace element concentrations in skin of free-ranging bottlenose dolphins (*Tursiops truncatus*) from the southeast Atlantic coast. Sci. Tot. Environ. 388, 300–315.
- Tilbury, K.L., Stein, J.E., Krone, C.A., Brownell Jr., R.L., Blokhin, S.A., Bolton, J.L., Ernest, D.W., 2002. Chemical contaminants in juvenile gray whales (*Eschritius robustus*) from a subsistence in Arctic feeding grounds. Chemosphere 67, 555–564.
- Tsuneta, Y., Ohsaki, Y., Kimura, K., Mikami, H., Abe, S., Murao, M., 1980. Chromium content of lungs of chromate workers with lung cancer. Thorax 35, 294–297.
- United States Department of Health and Human Services, 1993. Toxicological Profile for Chromium(VI). Agency for Toxic Substances and Disease Registry, 92-08, Atlanta.
- Whitehead, H., 2003. Sperm Whales, Social Evolution in the Ocean, first ed. University of Chicago Press, Chicago.
- Wise Sr., J.P., Wise, S.S., Little, J.E., 2002. The cytotoxicity and genotoxicity of particulate and soluble hexavalent chromium in human lung cells. Mutat. Res. 517, 221–229.
- Wise Sr., J.P., Wise, S.S., Kraus, S., Shaffiey, F., Grau, M., Li Chen, T., et al., 2008. Hexavalent chromium is cytotoxic and genotoxic to the North Atlantic right whale (*Eubalaena glacialis*) lung and testes fibroblasts. Mutat. Res. 650, 30–38.
- Witmer, C.M., Park, H.S., Shupack, S.I., 1989. Mutagenicity and disposition of chromium. Sci. Tot. Environ. 86, 131–148.
- Witmer, C.M., Harris, R., Shupack, S.I., 1991. Oral bioavailability of chromium from a specific site. Environ. Health Perspect. 92, 105–110.
- Yang, J., Miyazaki, N., Kunito, T., Tanabe, S., 2002. Trace elements and butyltins in a Dall's porpoise (*Phocoenoides dalli*) from the Sanriku coast of Japan. Chemosphere 63, 449–457.