A Journey of Discovery

Circumnavigating the Globe ~ March 2000 to August 2005

The Voyage of the Odyssey

Ocean Alliance

Executive Summary

Conserving the Ocean Environment

www.oceanalliance.org

The Voyage of the *Odyssey*

The First Expedition

March 2000 – August 2005

A Pioneering Global Research Voyage to Collect Baseline Data on Contaminants in the World's Oceans

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Contained here is an Executive Summary of the 180 page full report of the Voyage of the Odyssey.

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OCEAN ALLIANCE

Introduction to Ocean Alliance

Ocean Alliance works to protect whales and reduce global ocean pollution by conducting groundbreaking scientific research and working for change through public education and outreach.

Ocean Alliance, Inc., a 501(c)3 organization, was founded in 1971 by biologist Roger Payne. Led by Dr. Payne and CEO Iain Kerr, Ocean Alliance collects a broad spectrum of data on whales and ocean life relating particularly to toxicology, bioacoustics, behavior, and genetics. Working from that data, we give information about ocean pollution and the health of marine mammals and other ocean life to policy makers, politicians, nongovernmental organizations, educators, and students. Our data is the basis of many conservation success stories. Our major scientific partner is the Wise Laboratory of Environmental and Genetic Toxicology, at the University of Southern Maine, which conducts state-of-the-art research aimed at understanding how environmental contaminants affect the health of humans and marine animals. The Wise Laboratory's mission is accomplished through the pursuit of a number of key objectives, including innovative and multidisciplinary research in toxicology and molecular epidemiology to increase understanding of disease in humans and marine organisms, particularly in relation to cancer, asthma, and reproductive/developmental effects.

Ocean Alliance Research Programs

► Tracking Sperm Whales – Sentinels of Ocean Health

We learned to track sperm whales day and night by listening for the vocalizations they make. We towed an array of underwater microphones (called hydrophones) behind the *Odyssey* at all times. The sounds the hydrophones pick up are then broadcast inside the pilot house and displayed visually through a computer program invented by Douglas Gillespie of the International Fund for Animal Welfare. The program, called Rainbow Click, assigns a different color to the clicks of each different sperm whale, determines a bearing to them, and displays those clicks without ocean noise, enabling us to find and follow the individual whale. The crew of the *Odyssey* has become so skilled at doing this that if we hear just one "click train," we can almost always find the group, and having done so, we can stay with it for days as long as at least one of the whales in the group keeps clicking.

► Right Whale Program

Ocean Alliance runs the longest continuous study of any baleen whale species in which specific individual whales are tracked and identified over time. The subject of this study is one of the most endangered of the great whales, the southern right whale (*Eubaleana australis*). Ocean Alliance right whale researchers, led initially by Roger Payne and now by Victoria Rowntree, have studied one of the world's largest populations of right whales (approximately 2,000 individuals) on their calving grounds in Argentine waters since 1970.

► Humpback Whale Songs

Ocean Alliance is best known for Roger Payne's 1968 discovery (with Scott McVay) that the intricate and impressive vocalizations produced by humpback whales (*Megaptera novaengliae*), particularly on their winter assembly grounds, are produced in repeated, rhythmic patterns and are therefore, by definition, songs. Roger Payne and other researchers at Ocean Alliance have gathered recordings from humpback whale populations throughout the world, a collection that now contains more than 1,500 recordings from 14 geographic regions. It may be the largest collection of humpback recordings anywhere and is now being curated by Cornell University's Macaulay Library of Natural Sounds. The Ocean Alliance library also contains recordings from right whales, sperm whales (*Physeter macrocephalus*), and several other species, which, together with the humpback recordings, total more than 6,000 hours of sounds.

Ocean Alliance Education Programs

Ocean Alliance has developed several novel education programs that provide a foundation for our conservation efforts. These programs are designed to reach people of all ages with an emphasis on students, participants in whale watches, and multimedia resources. The goals of our programs are to build awareness of whales and their ocean environment and to encourage the participation of an informed public in promoting lasting, effective conservation policies.

► Ocean Encounters

Our education program took place hand in hand with our scientific research. We believe that learning and discovery happen best when they happen together, and when students can participate in a program of genuine scientific research. Thus, our education approach focused on the research we were doing, and on conserving whales and the rest of ocean life. We also taught classes about human impacts on the marine environment. The most satisfactory aspect of this approach was that people's eyes were opened to the realization that they could make a real difference.

Our education team often taught from the *Odyssey* in remote locations where we were able to share the experience of the voyage with students back in the United States via the Internet, as well as in the regions we were visiting. We also developed and disseminated educational materials directly from the *Odyssey*.

From March 2000 to August 2005, we identified, developed and implemented four education programs that made up what we called **Ocean Encounters** (discussed in greater detail in the full report).

1. A **Voyage of the *Odyssey* Website** hosted by PBS Online. We wrote education-based stories called Odyssey Logs, short video documentaries based on the scientific research we were doing as well as on the crew's experiences. We also provided transcripts as well as photographs and audio reports. Numerous schools linked, and continue to link, to our website. Teachers use the content with students of a variety of ages and in various subjects. In addition, Roger Payne and sometimes guest scientists produced pieces for the "Voice from the Sea" section. For more on this see, www.pbs.org/odyssey.

2. The *Odyssey* was equipped to conduct live videoconferences via satellite from anywhere on the planet. Thus was born our **Ocean Encounters Education Initiative**, in which we partnered with museums, aquariums, zoos, and schools around the world. Our technology permitted students to speak directly with scientists aboard the *Odyssey* and to participate directly in ongoing research. The Education and Media Team aboard the *Odyssey* developed interactive DVDs and education kits for teachers to use in the classroom; these kits were based on the stories produced for the website.

3. The **Young Scientist Program** was a five-week education program specifically aimed at enhancing science literacy. It used videoconferencing via satellite, DVDs, and the website. The Education Team aboard the *Odyssey* designed lesson plans, group activities, and homework assignments aimed at exposing eighth-grade children to the challenge and excitement of collecting scientific data in the field. They reviewed existing information on whale abundance, weather, ocean productivity, and bottom topography, and from their findings helped us plan our route. Whenever possible we included and followed their suggestions and reported to them about what we encountered.

4. Odyssey Education: In all of the 121 ports we visited we gave presentations about work we were doing. Whenever possible, we brought students, educators, NGOs, policy makers, representatives of government agencies, local scientists, and journalists from all media on board the *Odyssey* and gave them the full experience of working on a research vessel in the midst of a global expedition. Our ultimate aim was to empower and encourage people to improve the health of the world's oceans both locally and internationally.

In ports we also produced reports based on our research in that region. These documents listed the routes of the *Odyssey*, the animals we had seen, as well as other information on the local marine environment.

► School Programs

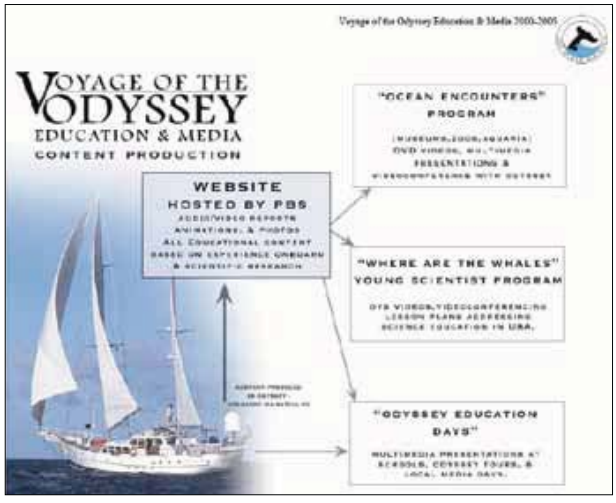
The Pacific Life WHALE Education Kit is Ocean Alliance's major resource for school programs. The award-winning kit is a multimedia, hands-on education program designed to promote an appreciation and understanding of whales, science, and the ocean environment. In addition to the education kit, Ocean Alliance organized several school programs associated with the Voyage of the *Odyssey* including Odyssey Education Days, Ocean Encounters, and the Young Scientist Program.

► Whale Watch Education

Ocean Alliance has created a public outreach program that enlists new friends of ocean life by taking advantage of the charisma of whales and the listening of captive audiences aboard whale watch vessels. Ocean Alliance works with Cape Ann Whale Watch in Gloucester, Massachusetts (www.seethewhales.com), to provide training and education programs through the CETA (Cetacean Education Through Awareness) program.

► Multimedia Ocean Education

Ocean Alliance has harnessed the power of all screens, including the Internet, to increase public appreciation for whales and their environment. Ocean Alliance staff members have participated in more than 30 ocean documentaries – many based largely on Ocean Alliance work. Several of the films have been hosted by Roger Payne including the Discovery Channel's films *In the Company of Whales* and *Finite Oceans*. He also narrated their miniseries *Ocean Planet*, co-wrote and co-directed the IMAX film *Whales*, and is the subject of the recent PBS film, *A Life Among Whales*. On the Internet, Ocean Alliance collaborated with the Public Broadcasting Service to produce a website that tracks the Voyage of the *Odyssey*. And through a series of online audio pieces, "A Voice from the Sea," Roger Payne discusses the fate of the world's oceans, connecting it to daily experiences aboard the *Odyssey*.



Voyage of the *Odyssey* – A Description

The first Voyage of the Odyssey *was a five-and-a-half-year scientific circumnavigation of the globe to document the health of the world's oceans and to provide data for efforts by ourselves and others to reduce the flow of contaminants into the world's oceans.*

Despite mounting evidence that pollution is threatening the oceans, no global scientific expedition had been undertaken before the Voyage of the *Odyssey* to document the full nature and extent of this invisible threat. (This is perhaps for good reason – it is a daunting task.) But Ocean Alliance decided pollution was a problem of such importance that continuing to ignore it was a greater folly than trying to do something about it, even though doing so exposed us to major risks.

The decision to go forward paid off, and this quest on a Homeric scale succeeded. The Voyage of the *Odyssey* is the first worldwide study of chemical contaminants in a single ocean species (in this case, the sperm whale), carried out with consistent data-collecting protocols. Because sperm whales are long-lived apex predators, their tissues record the chronic impact of pollutants within them. The 955 pencil-eraser-sized tissue samples that we collected from sperm whales during the five and a half years of the Voyage provide both a record of contaminants and a resource for detecting their life-altering effects.

Voyage of the *Odyssey* Goals

The primary goals of this pioneering Voyage were to:

- establish an archive of samples from whales from the major ocean basins of the world;
- measure contaminant burdens in these samples, especially for metals, persistent organohalogens and flame retardants, and to establish a baseline against which future studies can be compared to determine changes in the levels of these and other pollutant levels in the oceans;
- generate data on critical biomarkers of contaminant exposure, specifically, cytochrome P450 1A1;
- compare and contrast data from the above goals with the existing database on contaminants and their effects on vertebrates around the globe.

The Voyage of the *Odyssey* team also worked with partners around the world to pursue several related goals, including:

- to increase awareness through innovative education programs about the threats from chemical contamination, illegal fishing and over exploited fisheries, interference from ships and sonar, and other abuses;
- to promote the conservation and sustainable management of critical marine habitat by holding workshops in local communities; and
- to build a foundation for future long-term global research by forming research relationships with scientists in other countries.

The offshore component of the Voyage of the *Odyssey* was not only successful but in many cases exceeded our expectations principally as a result of the efforts and dedication of four remarkable people: Bob Wallace, Genevieve Johnson, Chris Johnson, and the late Rebecca Clark. The Ocean Alliance and the ocean's wildlife live in their debt.

– Iain Kerr, CEO

The Voyage spanned a remarkable 87,000 nautical miles (139,000 km), and visited 121 ports in 22 countries. While much of the *Odyssey* crew's time was spent at sea collecting tissue biopsies for toxicological analysis, the Ocean Alliance team seized opportunities to collect specimens and data for studies on genetics, acoustic behavior, photo identification of individual whales, stable isotopes (to determine where sperm whales – particularly adult males – are feeding), and evaluation of habitat. When on land, the Ocean Alliance team partnered with local educators, scientists and government officials to help advance conservation efforts in local marine habitats and to share education materials.

The success of the Voyage of the *Odyssey* can be attributed to the many dedicated crew members who poured their sweat, energy, tears, and intellect into this mission. Several spent over six months on the *Odyssey*. The full report gives information about them and about the leaders of the expedition, scientists, educators and researchers who, along with 190 additional crew members, observers, scientists, and interns contributed boundless energy and knowledge to the effort. Countless more individuals and organizations in the places we visited welcomed us to their home countries. We, as well as the world, owe them our greatest thanks.

The Reasons for a Global Survey of Ocean Pollution

The contamination of the world's oceans is a rapidly expanding global problem. According to 1998 reports from the U.S. Environmental Protection Agency and the U.N. Environment Programme, between 1930 and 2000, the global production of human-made chemicals increased exponentially from 1 million to 440 million short tons per year. Because the oceans are downhill from everything on earth, they are the final destination for every contaminant that can be moved by water or wind.

Poisonous chemicals contaminate ocean food chains throughout the world. Pesticides poison our food and leach into waterways; many industrial emissions poison the air and damage our lungs, kill wildlife, and impair the ability of our children to learn. We are facing a slow graying of the human future caused by the inexorable accumulation of persistent organic pollutants (POPs). Because they are metabolized so slowly POPs increase in concentration as they move up food pyramids.

In the 1980s more than 900 dolphins died off the East Coast of the United States from immune system disorders, some of which may have been caused by pollutants in their habitat. In the seemingly crystal-clear water off the Bahamas, Atlantic spotted dolphins (*Stenella frontalis*) show signs of pox, skin lesions, tumors, and birth defects. Beluga whales (*Delphinapterus leucas*) in the Gulf of the St. Lawrence river are so full of chemical pollutants that when they die and their bodies wash ashore, they have to be treated as toxic waste.

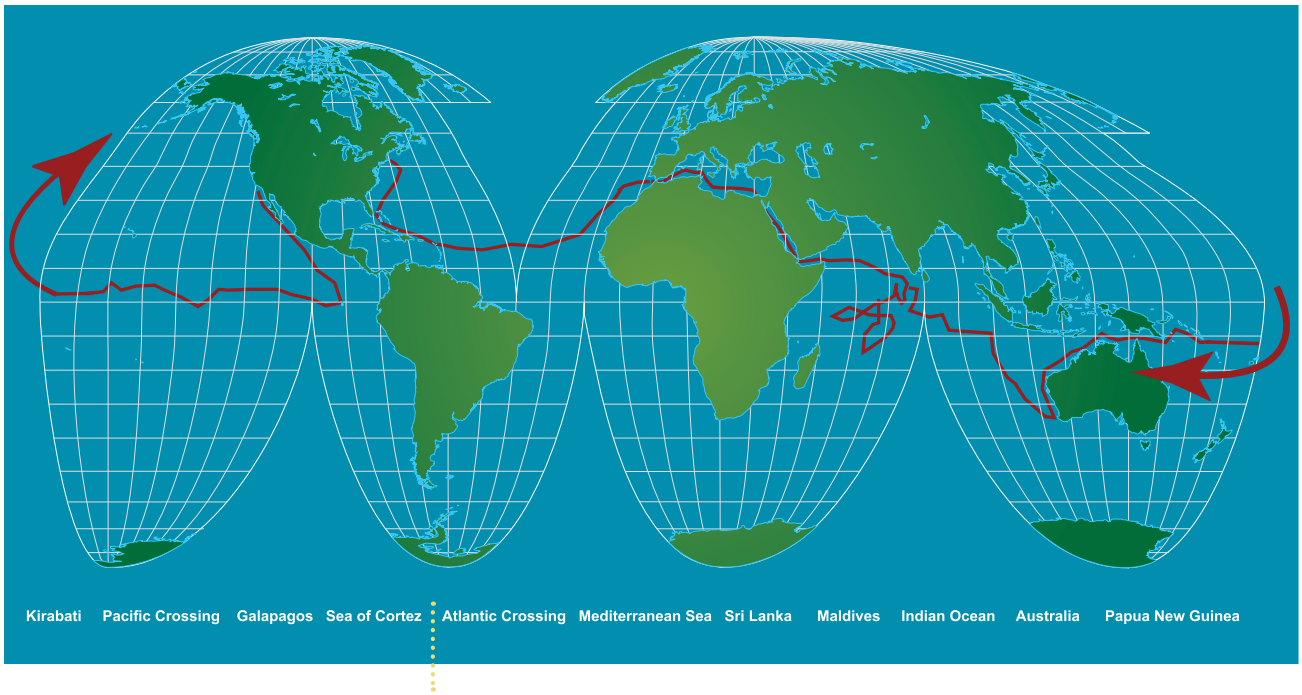
Yet the lack of any baseline data on the concentrations of these substances has made it all but impossible to persuade governments to take the steps necessary to reduce the manufacture, use, and improper disposal of POPs in the environment.

The Voyage of the *Odyssey* program was established to fill this gap and collect the first-ever global dataset on the distribution, concentrations, and effects of toxic pollutants in the world's oceans. Ocean Alliance has accomplished this by collecting 955 sperm whale tissue samples worldwide. In doing so, we have used sperm whales as our bioassay – as a global indicator of contaminant loading in a top predator. We chose to study sperm whales because they, like us, are top predators – and therefore can be expected to offer an instructive reflection of the problems from contaminants that we also face. We have collected samples from every ocean (including polar oceans because that is where adult males feed), advanced the field of marine mammal toxicology, and created an unrivaled sample set of information and other resources for future collaborative research. The Voyage has made it possible to draw direct comparisons between contaminant concentrations in all oceans, and has provided the first worldwide assessment using the same protocols of the risks facing humans and wildlife from pollutants in the sea.

Here we offer a primer on POPs and toxic metals as well as a summary of results from the Voyage of the *Odyssey*. In the coming months, additional analyses will be conducted and published in the peer-reviewed literature, expanding on the details and ecological impact of the data presented here. The scientific results from Ocean Alliance's Voyage of the *Odyssey* are sobering, but the fact that they now exist also offers a reason for hope. The dozens of scientists who worked aboard the *Odyssey* have, through their collective efforts, greatly expanded our knowledge of whales, pollution, and the health of the ocean environment. This is the story of what their research revealed along the way and what the initial analysis of their extensive data set tells the world.

Collecting pencil-eraser-sized tissue samples from the side platform using a crossbow.

Part 1: An Atlas of the Voyage



Summary Statistics

- 87,000 nautical miles traveled (160,000 kilometers)
- 22 countries visited
- 121 ports entered
- Approximately 190 crew, scientists, observers, and interns hosted
- 955 sperm whale samples collected, producing 4,500 tissue subsamples
- 20 other whale and dolphin species sampled
- Over 200 fish, squid, and non sperm whale samples collected
- 3,000 hours of acoustical data recorded
- More than 30,000 photographs taken

It seemed outlandish to attempt such a voyage: only governments or large oceanographic institutions had attempted such global research expeditions. Perhaps with good reason since such ambitious voyages are costly and fraught with unanticipated problems. But although Ocean Alliance is small we are not easily daunted. Our courage had paid off before, so we took the plunge. Thanks to dedicated teams both on land and at sea, the Voyage succeeded.

– Roger Payne

The Voyage of the *Odyssey* also visited 22 eco regions. In the full report we provide regional reports of our findings.

Notable Voyage Accomplishments

During their five and a half years at sea aboard the Odyssey, Ocean Alliance staff and crew made several notable advances in marine science and conservation.

The Voyage of the *Odysse*y began as a vision shared by Drs. Roger Payne and Iain Kerr back in 1987. Twenty years after that idea, the *Odyssey* completed an 87,000-mile (139,000-kilometer) journey that circled the world. While much remains to be learned about the condition of ocean ecosystems, the observations and scientific research conducted aboard the *Odyssey* constitute a major contribution to marine science and conservation. Chief among these was Ocean Alliance's success in gathering what is the largest global tissue sample collection based on the same protocols from a single marine species – a resource that is now yielding critical information about ocean contamination as well as its impacts.

Notable Voyage Accomplishments

- Using sperm whales as global indicators of contamination by collecting 955 tissue samples from that species
- Documenting that sperm whales are returning to former whaling grounds – in two instances the visits by the Voyage of the *Odyssey* were the first made by whale scientists to old whaling grounds since the cessation of whaling
- Making a rare live sighting of a Longman's beaked whale
- Finding sperm whales off Aldabra atoll, a remote island in the Seychelles chain whose surrounding waters have received relatively little study
- Observing whales in the waters of the Chagos Archipelago, where the *Odyssey* was the first nongovernmental research vessel to be allowed to resupply
- Being the first such vessel to be granted a permit to conduct research in the waters surrounding Diego Garcia and the first to report whales in this area
- Observing a female humpback and her calf bow-riding on the *Odyssey* bow wave over a three-hour period – it is rare to see such behavior over such an extended period from a baleen whale and/or her calf

Progress in Marine Conservation

Odyssey educators and scientists partnered with many people and organizations to achieve the following conservation results:

- Helped establish a million-square-mile (2.6 million-square-kilometer) Marine Mammal Sanctuary in Papua New Guinea waters
- Documented an illegal shark-finning operation in the Pacific
- Shared Voyage findings with the Scientific Committee of the International Whaling Commission
- Bolstered support for the IWC, Indian Ocean Whale Sanctuary with research findings from the Voyage
- Captivated, educated, and informed thousands of people worldwide with the message of the Voyage

Home from the sea; the crew along with those who supported the voyage from the land; (in the center) Iain Kerr, Chris and Genevieve Johnson opening champagne to celebrate the Odyssey's safe return home.

Part 2: Reaching Out to People of the World

Outreach Work Included

Our PBS-sponsored website (www.pbs.org/odyssey)

- produced over 450 audio and video reports from the *Odyssey*;
- broadcast 75 Voice From the Sea pieces, written by Roger Payne;
- attracted 1.3 million website visitors who viewed 4 million pages;
- received several awards and citations including:
  - The 2003 Scientific American Sci/Tech Web Award
  - The USA Today Hot Site of the Day
  - Yahoo's Yahoo! Pick of the Day

Our education team

- gave presentations to approximately 40,000 people worldwide;
- contributed to two museum exhibits featuring whales and the Voyage

Voyage of the *Odyssey* Education Program

Everywhere the Voyage went the *Odyssey* crew, educators, and scientists reached beyond their primary mission of documenting ocean pollution to engage in education, conservation, and ocean policy initiatives. As a result, the Voyage of the *Odyssey* is remembered in many of the countries it visited not just as an expedition of fundamental scientific importance, but also for instilling a fascination for whales in the hearts of thousands of children, teachers, and ordinary citizens.

Goals and Philosophy

Children are naturally drawn to the wild world, and whales arouse their sense of curiosity and awe. The Voyage of the *Odyssey* education program builds on the natural human affinity for wildlife. The specific education goals were as follow:

- To promote awe, curiosity, and concern about whales and the ocean environment
- To underscore the need to preserve the oceans as healthy, integrated ecosystems
- To educate people about how they can make a difference in the conservation of whales and the world's oceans

To achieve these goals, Ocean Alliance believes that an effective way to shape people's behavior is to enlist them directly in doing science so that they can gain firsthand experience of the natural world around them. The *Odyssey* educators used several specific strategies to engage their audiences. Specifically, they sought to do the following:

- Utilize sperm whales as a charismatic species to capture the attention of children and to connect them directly to the work of the Voyage, and to the excitement of doing science
- Use global exploration as a way to engage students and promote an interest in the oceans, its whales, and ocean ecosystems
- Develop education activities and materials that are dynamic and hands-on, and which can be disseminated by means of multimedia technology
- Build partnerships with educational organizations in all countries whose ports the *Odyssey* visited

Voyage of the *Odyssey* Education Programs and Materials

The Ocean Alliance education team, led by Genevieve and Chris Johnson, promoted four major education programs and developed original education materials that were designed to share the experiences of the Voyage of the *Odyssey* with students in the United States, as well as in the 22 countries they visited, and via the Internet, with the entire world. The programs employed innovative techniques to bring the experience of global exploration to teachers through firsthand contact, multimedia programs, and classroom lessons in marine ecology – all via the Internet.

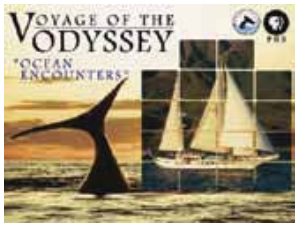
► PBS Website for the Voyage of the *Odyssey*

(www.pbs.org/odyssey)
An award-winning educational website tracking the Voyage of the *Odyssey* was produced through a collaboration with the Public Broadcasting System. The website provided a venue for sharing the experience of the Voyage in real time with approximately 1.3 million people. The photography and video footage featured on the website, except where noted, was shot by *Odyssey* multimedia coordinator Chris Johnson, who produced video and audio accounts of the Voyage during the five and a half years at sea. The logs and scripts were written primarily by crew member Genevieve Johnson (often with help by Roger Payne). Together with Payne's *Voice from the Sea* series, more than 500 accounts of Voyage activities were produced.

► *Odyssey* Education Days

Odyssey Education Days were a core activity of the Voyage of the *Odyssey* education programs. Wherever possible, the *Odyssey* educators welcomed local schools, members of the public, and the media aboard the *Odyssey* to learn firsthand about how an active ocean research vessel operates.

► Ocean Encounters Program



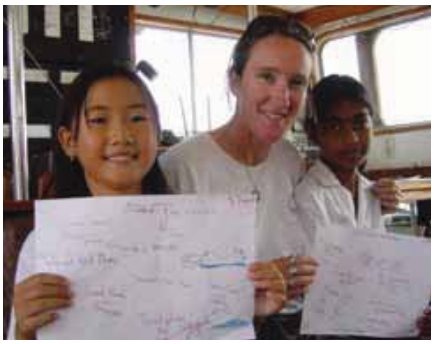
The Ocean Encounters Program brought the experiences of the Voyage of the *Odyssey* to students and teachers around the world through DVDs, multimedia presentations, and satellite video conferences. The program packaged the best education content from the PBS Voyage of the *Odyssey* website and used partnerships with museums, aquariums, and schools to help share these materials directly with teachers and students.

► Young Scientist Program

The Young Scientist Program engaged students in the United States in creative scientific exploration of whale ecology through a five-week lesson plan in which students used real data and other resources to answer the question, Where are the whales? The program was a pilot project that operated in conjunction with a 12th-grade class at the Lincoln Public School in Massachusetts.

► Education Materials

Some of the countries visited by the *Odyssey* education team lacked direct access to the Internet and other IT resources. To help address this gap, Ocean Alliance distributed the Pacific Life Whale Education Kit free of charge to thousands of educators across the globe. The kit contained several films and audio resources, books, lesson plans, and an educational whale poster.



Part 3: Scientific Results from the Voyage of the *Odyssey*

Ocean Alliance and Toxicology: A Brief History

In 1997 Ocean Alliance started what it called its Global Ecotox Program. In 1999 it changed that name to the Ocean Alliance Toxicology Program. In the same year it made measuring contaminant concentrations throughout the world's oceans the principal mission of a global "Voyage of the *Odyssey*." When the Voyage concluded successfully in the fall of 2005, Ocean Alliance chose marine toxicologist Dr. John Wise to manage the analyses of the 955 biopsy samples the Voyage had collected from sperm whales worldwide, and also to head the Ocean Alliance Toxicology Program.

A **baseline**, in this toxicological context, measures the concentrations of pollutants in the environment at specific times and places. Baselines tell us initial concentrations. Establishing a baseline is an essential first step and allows us then to determine with later measurements whether levels of a pollutant are rising or falling. By itself a baseline doesn't tell us whether levels are of concern or are significant health threats, but when considered with experimental studies, it can.

The original goals of our toxicology program were as follows:

1. To establish an archive of skin and blubber biopsy samples from sperm whales from all oceans
2. To measure the contaminant burdens present in our samples, especially for EDCs (endocrine-disrupting chemicals) and other persistent organohalogens.
3. To establish a baseline against which future studies can be compared, in order to monitor changing ocean pollutant levels in the world's oceans
4. To generate data on critical biomarkers of contaminant exposure, specifically cytochrome P450 1A1 (an enzyme produced by animals as a result of exposure to chemical contaminants)
5. To compare and contrast our data with existing databases on contaminants and their effects on vertebrates around the globe

Organohalogens (organic molecules with attached halogen atoms such as chlorine or bromine) are part of a greater group of chemicals called persistent organic pollutants (POPs). We chose organohalogens as our initial focus because most are synthetic chemicals that are pervasive, and have potential toxic effects such as immunosuppression, disruption of neurological functions, reproductive abnormalities, and cancer. Also, POPs accumulate in fatty tissues (e.g., blubber), which makes measuring them in skin/blubber biopsies feasible and practical. Furthermore, as they play no known positive physiological role in animals, the presence of most organohalogens in animal cells indicates that the animal has been exposed to industrial chemicals. The principal organohalogens that we targeted for study are recognized toxicants and include DDT, DDE, dioxin, and several PCBs (polychlorinated biphenyls). Although the toxicity of these substances was well established by others prior to our Voyage, the extent to which they had reached into the marine environment was unknown. It was to document their concentrations near-shore as well as in remote ocean regions that Ocean Alliance launched the Voyage of the *Odyssey*.

A New Toxic Threat Emerges

About the time that the Voyage was drawing to a close, we began to recognize a new and impending threat from an even more ubiquitous suite of organohalogens, the polybrominated diphenyl ethers or PBDEs. These compounds are used as flame retardants and are now found worldwide in many consumer products. Structurally, they are very similar to PCBs except that the halogen atom is a bromine instead of a chlorine. Near the end of the Voyage, studies by others began to emerge showing that concentrations of PBDEs were dramatically increasing in human mothers' breast milk, particularly in U.S. mothers. There were also disturbing reports that these substances may be neurotoxic to a developing brain. Because we had waited until the end of the Voyage to settle on what compounds to analyze, Ocean Alliance was able to shift its principal focus on organohalogens to these key and newly recognized toxic PBDEs, the distribution of which are virtually unknown. Regulation is now coming into place for the more familiar toxicants such as PCBs while PBDEs are mostly unregulated.

As the Voyage neared its end, Ocean Alliance's partner Dr. John Wise of the Wise Laboratory of Environmental and Genetic Toxicology at the University of Southern Maine, was fully engaged in the analysis of our samples. Dr. Wise and his team are pioneers in marine toxicology. Their studies include an emphasis on the toxic effects of chemicals in cells grown in culture. This means that not only are we able to establish baseline concentrations of pollutants in the sea, but we now can measure directly the toxic effects of the pollutant concentrations we observed on sperm whale cells grown in culture. This will provide a toxicity context to our measured baseline of pollutants. The latter step will occur later, but for now, under Dr. Wise's leadership, we are continuing to analyze the pollutant concentrations in the samples we brought back from the Voyage.

The Four Phases of the Voyage of the *Odyssey*

The Voyage of the *Odyssey* consists of four overlapping phases:

Phase 1 was the *collection phase* — the actual Voyage itself. It was complete when sample collection stopped at the end of the Voyage.

Phase 2 is the *curation phase.* This phase began when all samples were shipped to the Wise Laboratory, where they were organized into state-of-the-art storage systems and painstakingly hand-inventoried, with each sample clearly identified and stored in a specific location. At the same time, all of the storage data were accumulated into the toxicological database (ultimately they will be added to the database from the entire Voyage). This phase is 75% complete.

In total, we collected 955 skin biopsies from sperm whales. Each of these biopsies was then cut into up to seven individual pieces as illustrated in Figure 1. DNA was then extracted from one piece of skin and the isolated DNA aliquoted (divided) into three test tubes. Thus, considering just the sperm whale biopsy collection, we returned with approximately 20,000 physical samples (955 x 7 x 3 = 9,550). Clearly, it is a large volume of material to maintain and track along with the sometimes extensive supporting data that goes with each sample. Thus we certainly achieved one of our first goals of the Voyage: **establish an archive of skin and blubber biopsy samples from sperm whales from all oceans.**

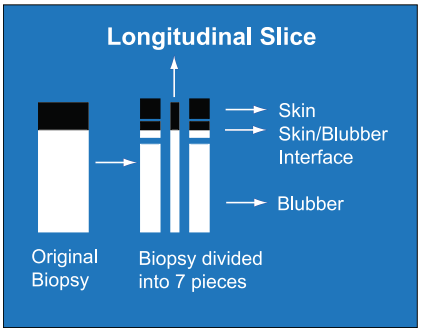


Figure 1. *Diagram of the biopsy sectioning. This figure shows how the sperm whale biopsies were divided into seven pieces. Note that there are two skin pieces, two interface pieces, two blubber pieces, and one longitudinal section.*

Phase 3 is the *analysis phase.* In this phase, samples are analyzed for contaminant levels. This report gives Phase 3 results to date.

Phase 4 is the *contextualization phase.* It is here that the data are mapped and put into context with what is already known about such contaminants. There is much work to do in the upcoming years to analyze and contextualize all of the data from the Voyage. However, this phase has begun with a number of scientific breakthroughs. The most significant of which is the first scientific evidence of the widespread presence of a hitherto unrecognized global ocean pollutant: chromium. The Voyage of the *Odyssey* found it in high concentrations throughout the world in sperm whale biopsies. Scientific papers on the ubiquitous presence of chromium in sperm whales globally have been published. Because chromium has such serious consequences, this is an important discovery of alarming concentrations of chromium in ocean life (Wise *et al.*, 2009).

The Sperm Whale Biopsy Collection

It is important to understand the spectrum of the samples we collected as there are differences in age and gender among the regions. Ideally, we would have collected an evenly distributed sampling from the various regions. For example, we collected 955 biopsies after visiting 18 regions. The ideal distribution would therefore be about 50 samples per region with half of them males and half of them females of about the same age. The whales, of course, had no concern for our ideal distribution. Consequently, we visited some regions with a great many whales (the highest number was 202 in the Sea of Cortez) and others that had fewer than 50 or even none (the lowest number was the Red Sea, where we encountered no sperm whales). Table 1 and Figure 2 show the variability and location of the distribution of the whales by study region. You will notice that the total number of females and the total number of males do not add up to the total number of whales. This is because we have not yet identified the gender of 63 samples.

This variability in the number of samples in each region has two important effects. The first is that for some regions (e.g., Kiribati) for which we have few samples, we can perform only a limited number of analyses. In order to make statistically significant comparisons between regions with low numbers and regions with higher numbers, we have to perform the same analyses on all samples. Thus, for regions with few samples we must choose our analyses carefully. The second effect is that we cannot measure every contaminant in every region. This is because after the first 25 or so measurements from one region, little scientific information would be gleaned from making more measurements from that region because, statistically speaking, the other regions with which it would be compared have too few samples to allow for strong comparisons.

Accordingly, to achieve the most statistically reliable results, our target number for each region is 25 adult females and 25 males, though some regions fall short of that goal. However, the fact that we had to limit our analysis in this manner means that in the regions with more individuals, we were able to consider more contaminants. We have also been able to save some samples for future comparative studies as well as for other researchers to use later when looking for hitherto undiscovered toxic agents that may pose problems.

Table 1. *Distribution of sperm whales across regions*

Ocean/sea	Region	# of female whales	# of male whales	Total # of whales
Pacific	Sea of Cortez	163	34	202
	Galapagos	0	34	34
	Pacific Crossing	33	27	68
	Kiribati	17	2	19
	Papua New Guinea	79	14	110
Indian	Australia	24	11	38
	Cocos	22	0	22
	Indian Ocean Crossing	0	5	5
	Chagos	0	12	12
	Seychelles	68	12	83
	Maldives	44	21	69
	Sri Lanka	100	2	110
	Mauritius	58	3	63
Red	Red Sea	0	0	0
Mediterranean	Mediterranean	17	21	41
Atlantic	Canaries	27	12	44
	Atlantic Crossing	11	2	13
	Bahamas	10	7	22
Total		**673**	**219**	**892**

While 955 biopsies were collected, 63 samples have so far proven resistant to gender identification by laboratory analysis.

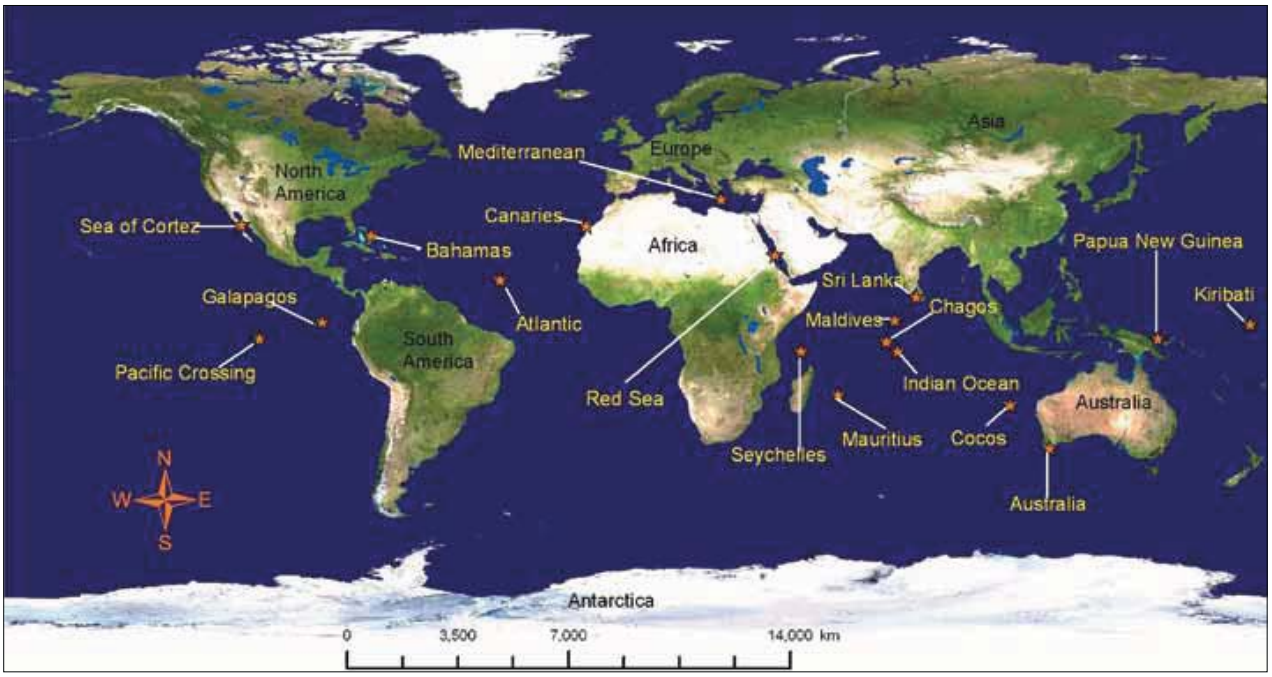


Figure 2. *Sampling locations of sperm whale biopsies. The 18 regions visited during the Voyage of the* Odyssey. *The Voyage started in the Sea of Cortez and continued westward, ending in Massachusetts. Whales were found and sampled in all regions except in the Red Sea. See the map on page 6 for the route of the Voyage.*

The Analysis Plan

The purpose of the initial analysis plan was to determine the levels of organohalogens in our samples as well as whether CYP1A1 is a biomarker of exposure to pollutants. We conducted pilot studies on samples we had collected in Pacific regions in which we considered PCBs, DDT, DDE, hexachlorobenzene (HCB), and CYP1A1. The results of those analyses are presented in the full report**.** In sum, the data showed that these organohalogens were present in the Pacific region at concentrations similar to those reported for the Pacific Ocean near the West Coast of the United States. Thus, a level baseline for these organohalogens was achieved for the Pacific Ocean in partial fulfillment of Goal 2 of the Voyage. *To measure the contaminant burdens present in our samples, especially for EDCs (endocrine disrupting chemicals) and other persistent organohalogens, and to establish a baseline against which future studies can be compared in order to monitor changing ocean pollutant levels.*

The data also showed that CYP1A1 was a useful biomarker in experimental conditions, but that this biomarker fell short in actual field studies with biopsies. CYP1A1 levels rose when slices of a whale biopsy were exposed directly to a laboratory reagent (thus demonstrating that it does respond, and proportionally, to varying dosages of chemical contaminants). This field study was the first of its kind and offered us great hope that CYP1A1 levels in sperm whale skin could be used as a biomarker for levels of organohalogen exposure. But when we analyzed the data from other whale biopsies, we found that this biomarker is not sensitive enough for the exposures the whales were experiencing. Thus, although we were pleased that we met Goal 3 of the Voyage *(to generate data on critical biomarkers of contaminant exposure, specifically cytochrome P450 1A1)*, we were disappointed that this biomarker would not be suitable for further study.

The second stage of the analysis plan involved metals. As the Voyage was ending, Dr. Wise encouraged us to consider metal levels since many metals are highly toxic and some are both toxic and essential. Dr. Wise's suggestion was echoed by Dr. David Evers of the Biodiversity Institute. With their help a pilot study was made of 10 samples, each of which was analyzed for 18 metals. The pilot study indicated that metals were indeed present in the whale samples at significant concentrations. Dr. Evers then led a second pilot study of about 50 samples, each of which was analyzed for eight metals. This study confirmed the findings of the first ten samples.

At this stage it became clear that there were many potential directions and contaminants to be considered and that an experienced leader was needed to guide the toxicology program. We decided that Dr. Wise was the appropriate choice to help us prioritize our goals, and a strong partnership was formed. We decided to continue to create a global baseline for a representative organohalogen. However, we shifted our focus to PBDEs (polybrominated flame retardants) because of the emergent threat they represent and the critical need for data on their effects and existing concentrations. Particularly germane to our decision to focus on PBDEs was that PCBs, DDT, DDE, and dioxins have already been widely banned, whereas PBDEs are under current discussion and almost nothing is known of their global distribution. Thus, if analyzed for PBDEs, samples from the Voyage could not only meet our goal of establishing a baseline for a persistent organic pollutant, but also simultaneously help those responsible for creating future regulations concerning these chemicals. We are pleased to report that with a generous grant from the National Fish and Wildlife Foundation, we have begun these analyses for several Pacific regions, one region in the Indian Ocean, and one in the Atlantic (other regions will be considered eventually, but this is the limit of our funding at present). We expect these analyses to be completed later this year. At present we are indeed finding PBDEs in our samples.

We also decided to complete the baseline analysis of metals for the world, but to work with a fuller spectrum of 18 metals rather than the more limited set of eight metals. These data are now complete and are in the process of being contextualized and written up for publication. (Fifteen of the eighteen abstracts submitted to and accepted by the Society for Marine Mammalogy for presentation in their 2009 Biennial Conference in Quebec, were on these results.) They are presented in more detail in the full report.

Dr. Wise also introduced us to the emerging threat of nanoparticles, particularly silver and gold nanoparticles. Silver nanoparticles are already in extensive use as bacteriocides in consumer products that range from baby pacifiers to washing machines. They are being disposed of directly into wastewater streams and will likely be reaching the marine environment soon if they have not already. We are pleased to report that because we collected our samples before these nanoparticles were being marketed in any major way, our data will provide a global baseline for silver and gold nanoparticle levels *prior* to their widespread use. This baseline will be critically useful in helping us and others to monitor the impact that these new classes of active substances are having on marine life. (These baselines are also described in the full report.)

Treating new biopsy samples in Odyssey's *pilot house lab space.*

The Data Reports

(Full data available upon request. Contact Iain Kerr at iaink@oceanalliance.org)

Data Report I: Organohalogen Analysis I (PCBs, DDT, and HCB)

What Are These Chemicals?

Halogens are nonmetal elements consisting of fluorine, chlorine, bromine, iodine, and astatine. An organohalogen is a chemical that has a halogen as part of a molecule the remainder of which is carbon based. In environmental toxicology, perhaps the most infamous organohalogens are the organochlorines. These carbon-based chemicals have at least one covalently bound chlorine atom. Their principal uses are as solvents, pesticides, and electrical insulators. In our study, we focused on three groups of them: polychlorinated biphenyls (PCBs), which were used primarily as electrical insulators, DDT and hexachlorobenzene (HCB). The latter two are pesticides.

What Samples Did We Use?

The PCB, DDT, and HCB analysis was done by Dr. Cristina Fossi at the University of Sienna in Italy. The Fossi study included 50 individual whales that we selected on the basis of their CYP1A1 levels (discussed later). They came from five Pacific Ocean regions (10 individual samples per region).

Data Report II: CYP1A1 Analysis

Because of the potential high cost of organic contaminant analysis and our desire to rapidly achieve a global dataset reflecting pollutant levels in the whales, we chose to measure the levels of CYP1A1 in whale biopsies. The chief scientist of the Voyage at the time, Celine Godard-Codding, also had a specific research interest in this biomarker. See full report for results.

Data Report III: Organohalogen Analysis II (PBDEs)

These Chemicals and their Health Effects

Polybrominated diphenyl ethers (PBDEs) are organohalogens that are structurally like PCBs, except that instead of chlorines, they have bromines. PBDEs are predominantly used as flame retardants in many products including clothes, furniture, electronics, cars, plastic packaging, and building materials.

The health effects of these compounds are just being established. The most common concern focuses on their potential for affecting the developing nervous system and possibly for disrupting the endocrine system.

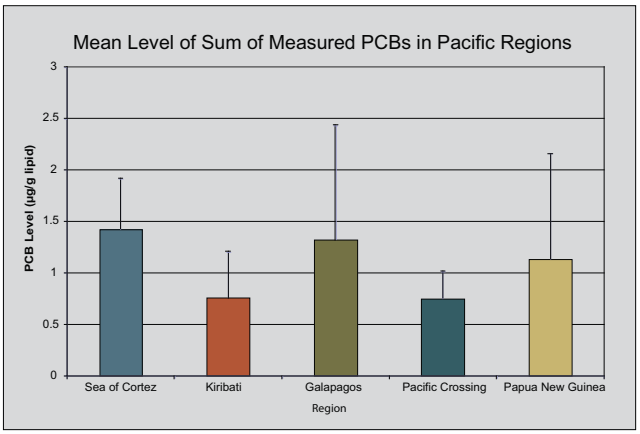


Figure 3. *Mean level of sum of measured PCBs in Pacific Ocean region's sperm whale biopsies. Highest levels were seen in whales from the Sea of Cortez and the Galapagos.*

Data Report IV: Toxic Metals

What Are Toxic Metals and Why Should We Care about Them?

Toxic metals are metals that poison the body and have no known biological role. As a group they can poison all of the organ systems of the body, though individually the scope of their toxicity is, in most cases, more limited. Some of their organic forms (such as methylmercury or tetraethyl lead) are even more toxic than they are as elements, but others (such as arsenobetaine) are less toxic. Because toxic metals are elements, they cannot be destroyed, although they can bioaccumulate, and some biomagnify. One aspect of toxic metals is that they can mimic essential metals and interfere with the uptake or function of that essential metal. For example, cadmium, lead, and other toxic metals can interfere with the uptake of iron and cause iron deficiency.

Chromium is one toxic metal that can be either toxic or essential. In its hexavalent form, it is clearly a toxic metal. In its trivalent form, it is classified as an essential metal; however, more recent data are challenging that classification and finding that although it may have activity as a drug, it may not be essential. Chromium is currently unique among essential metals in that it is the only one for which there is no recognized disease state that results from its deficiency. The absence of such a state further supports the argument that although it has pharmacological activity it may not have essential activity. We have included it here with the toxic metals because with the levels we find – whether in its trivalent or hexavalent form – there is reason for concern.

Although we measured all toxic metals, the full report presents the baselines and some brief context with respect to the uses and health effects of chromium, mercury, aluminum, arsenic, cadmium, lead, gold, silver, and titanium. We also provide the context of other published studies of sperm whales, other whales, and other marine mammals. We are still in the process of reviewing existing literature studies, but we have summarized what we have found so far. We have yet to find data for some pollutants, suggesting that ours may be the first. Of course, there may be publications that we have not yet uncovered, and new data are being reported all the time. Thus, contextualizing our data is an ongoing process, and we will be expanding it to include the remainder of the toxic metals we measured (barium, strontium, and tin) as well as the essential metals such as copper, iron, magnesium, manganese, nickel, zinc, and selenium. This summary focuses on chromium, mercury and selenium.

Our study was one of the very few to measure levels in free-ranging, apparently healthy whales, and thus of necessity we measured skin levels. The other studies considered dead animals and usually focused on the liver and other internal organs. They rarely considered skin, and therefore some of the differences in levels that we found may be due to the difference in organs studied. We discuss the overall implications of these data later in this summary (also see page 152 of the full report). Our context is presented later as we discuss each ocean and sea separately. A finer breakdown of locations beyond major oceans and seas can be found in the individual metals sections.

Aluminum

Aluminum and Its Uses

The metal element aluminum (Al) is used extensively in packaging, transportation, water treatment, cooking utensils, and many other applications.

What Are the Health Effects of Aluminum?

The most common health effects for people exposed to aluminum are neurotoxicity and respiratory toxicity. Aluminum may also have detrimental effects on reproduction and development (ATSDR, 2006).

What Is the Aluminum Baseline?

We measured aluminum (Al) levels in 298 sperm whales. Aluminum was present at detectable levels in all but one whale. Detectable levels ranged from 6.9 to 1,870 µg Al/g tissue with a global average level equal to 132.8 +/- 10.6 µg/g (ppm). Considered by ocean, the average Pacific Ocean aluminum level in sperm whale skin was 102 +/- 12.9 µg/g; the average Indian Ocean aluminum level was 153.4 +/- 15.6 µg/g; and the average Atlantic Ocean aluminum level was 161 +/- 29.7 µg/g.

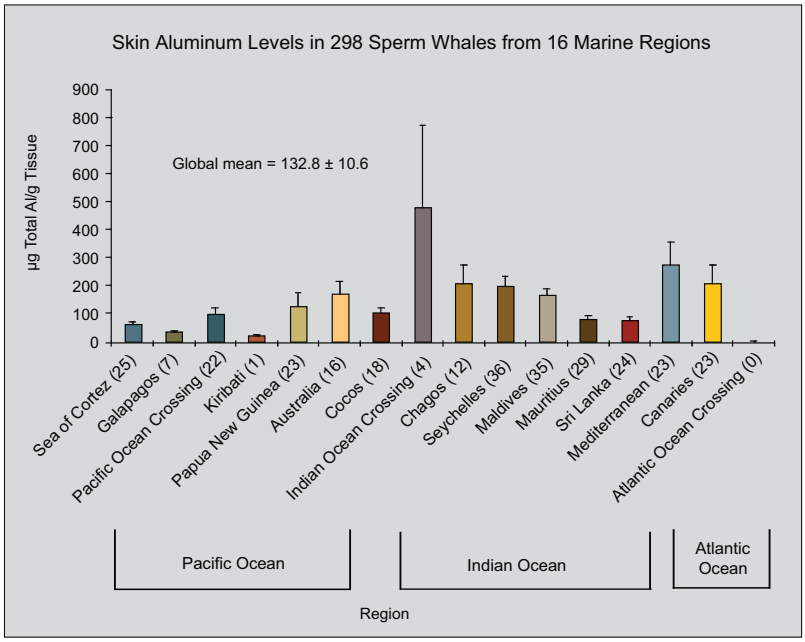


Figure 4. *Global distribution of mean Al levels in sperm whales grouped by sampling region. Specific regions are named for the nearest land body or ocean region. Data are shown as µg total Al/g tissue (ppm) +/- standard error. Half the detection level was used for samples with undetectable levels. Numbers in parentheses indicate the number of samples analyzed for aluminum.*

Aluminum concentrations were higher in some ocean regions than in others (Figure 4). The highest average aluminum level was found in sperm whales sampled during the Indian Ocean Crossing (478 +/- 296 µg/g). The lowest average aluminum level (23.1 µg/g) was found in a whale from Kiribati; however, that result came from just one whale. The lowest average aluminum level for a group of whales was found in whales off the coast of Sri Lanka in the Indian Ocean (36.5 +/- 1.5 µg/g).

Considering aluminum levels by individual whales, the highest aluminum level (1,870 µg/g) was found in a whale from the Mediterranean Sea. The lowest aluminum level (6.9 µg/g) was found in a whale from the waters around the Cocos Islands in the Indian Ocean.

How Do Our Aluminum Levels Compare with Those in Studies of Other 'Large Whales'?

We were able to locate only one study of aluminum in other 'Large Whales.' That report found an average liver aluminum level of 4.2 µg/g in gray whales from the Pacific Ocean, based on five individual whales (Tilbury et al., 2002). That level is much lower than our global average of 132.8 µg/g, based on 298 whales. It is also much lower than any of the averages we found in our skin biopsies (Figure 4). As mentioned above, our lowest average aluminum level was 23.1 µg/g from a whale from waters near Kiribati in the Pacific Ocean. The lowest average aluminum level for a group of whales was 36.5 µg/g found for whales from the Galapagos in the Pacific Ocean. The lowest individual aluminum skin level we found was 6.9 µg/g in a whale from the Cocos area in the Indian Ocean, which is closer to, but still not lower than, the level noted in this previous report. The one whale with a nondetectable measurement had a detection limit greater than 6.9 µg/g which leaves the Cocos whale still the lowest whale. The differences between our data and the published levels most likely reflect differences between liver and skin accumulation and in regional exposures to aluminum. Some may also reflect species differences.

How Do Our Aluminum Levels Compare with Those in Studies of Dolphins, Porpoises, and Other Marine Mammals?

We were able to locate only one study of aluminum in skin tissue from dolphins, porpoises, and other marine mammals. That study reported average skin aluminum levels of 2.52 and 0.93 µg/g (data converted from dry weight to wet weight assuming 70% moisture) for two groups of bottlenose dolphins off the Coast of the United States in the Atlantic Ocean (Stavros et al., 2007). These levels are much lower than what we observed in the Atlantic Ocean off the Canary Islands (Figure 4), and thus, may reflect local differences.

We located two studies of aluminum in liver tissue from dolphins, porpoises, and other marine mammals. One report found a range of 5 to 157 µg/g from the dugong (Dugong dugon) in the Pacific Ocean (Haynes et al., 2005). Those levels are consistent with the levels we found in the Pacific Ocean regions, which ranged from 9.16 to 223.29 µg/g wet weight. The second study, from the Wise Laboratory, reported average liver levels of 1.02 and 1.23 µg/g in Steller sea lion pups from the western and eastern Alaska populations, respectively (Holmes et al., 2008). The average level is much lower than what we observed (Figure 4) and may reflect a difference in age (adults versus pups) and diet (e.g., dugons are vegetarians).

What Do These Aluminum Levels Mean?

First and foremost, these levels indicate that sperm whales are exposed to aluminum. Second, they indicate that aluminum levels are high. In fact, they were much higher than those of any other toxic metal in every region in which we measured them. Third, the data indicate that aluminum levels are more significant in the Atlantic and Indian Oceans and in the Mediterranean Sea than in the Pacific Ocean. Finally, the data indicate that aluminum can and has reached even the remotest ocean locations, though we need to conduct more work to determine whether it is carried to these regions by air, by water, or only within the whales' tissues.

It is difficult to assess how high the internal body aluminum levels actually are because these levels were measured in skin tissue and it is uncertain how skin levels reflect levels elsewhere in the body. Skin tissue levels are usually lower than those in other vital organs such as the liver, kidneys, and lungs; thus, the expectation is that the aluminum level inside the body is probably much higher. The key aluminum levels will be in those organs susceptible to toxicity from exposure to it. It is also difficult to assess whether the aluminum levels we observed are toxic. Aluminum does not serve any known normal function in mammalian physiology. Therefore, its presence in a whale's body cannot be considered positive. Aluminum is at best neutral and without effect, and at worst leads to some level of toxicity. The fact that aluminum can induce reproductive and developmental effects raises concerns about its effects on whale reproduction. The fact that levels may be higher in the lung tissue raises concerns about its effects on whale respiration. However, we need to investigate these concerns more fully before we can draw any firm conclusions.

We are of course doing just that. Some of our ongoing and future work is aimed at understanding how skin aluminum levels relate to levels in internal organs. We are also continuing to work to try to understand how aluminum causes toxicity in whales and humans and how much exposure is too much. The full report discusses where the aluminum may be coming from.

Chromium

Chromium and Its Uses

The metal element chromium is used extensively in stainless steel; for corrosion resistance; in paints, dyes, and inks; and in the tanning of leather.

What Are the Health Effects of Chromium?

The most common health effects for people exposed to chromium are lung cancer and respiratory toxicity. Chromium can also affect the immune system, induce toxic effects in reproduction and development, and damage the kidneys (ATSDR, 2000).

What Is the Chromium Baseline?

We measured chromium (Cr) levels in 331 whales. Chromium was present in all but two. Detectable levels ranged from 0.9 to 122.6 ug Cr/g tissue with a global average level equal to 8.8 +/- 0.9 µg/g (ppm). Ocean by ocean averages of chromium in sperm whales are as follows: Pacific Ocean average chromium level was 9.63 +/- 1.46 µg/g; Indian Ocean average chromium level was 9.94 +/- 1.67 µg/g; and Atlantic Ocean average chromium level was 4.48 +/- 0.75 µg/g.

Chromium concentrations were higher in some ocean regions than in others (Figure 4). The highest average chromium level was found in sperm whales sampled in waters near the islands of Kiribati in the Pacific Ocean (42.3 +/- 12.8 µg/g). The lowest average chromium level was found in whales off the coast of Sri Lanka in the Indian Ocean (3.3 +/- 0.4 µg/g).

Considering chromium levels in individual whales, the highest chromium level (122.6 µg/g) was found in a whale from the Seychelles (Indian Ocean). The lowest chromium level was found in a whale from our Pacific Crossing. It had an undetectable level (<1.28 µg/g) of chromium. The other whale with an undetectable level had a higher detection limit (<2.8 µg/g) and was found in the Sea of Cortez. (The levels are different because the detection limit varies with each batch of samples.)

How Do Our Chromium Levels Compare with Those in Studies of Other 'Large Whales'?

Chromium levels vary 187-fold among whales throughout the world's oceans, based on liver levels. Most studies measured levels in dead whales and focused on internal organs. The liver was commonly used. Considering sperm whales, the published average liver chromium level was 0.038 µg/g based on seven individual whales. This level is much lower than our global average chromium skin level (8.8 µg/g) based on 331 individual sperm whales. It is also lower than our detection limit. We had only two whales with levels below our detection limit, indicating that overall our chromium levels were much higher than those noted in this earlier report. The published average came from sperm whales in sites in the Atlantic that we did not visit. Considered as an Atlantic average, this level is still much lower than the average levels we found in sperm whales for the Atlantic Ocean (4.48 µg/g) and for both Atlantic regions, which ranged from 3.7 to 6.9 µg/g (Figure 5).

Of course, we measured sperm whale skin levels, and the published studies measured liver levels. In free-ranging whales, skin is better for measuring metal levels because internal organs are unavailable and metals accumulate in skin more than in blubber. The liver is a major site of metal accumulation, and levels are expected to be higher (often much higher) than skin levels. This is true for chromium in rodents, which suggests that liver levels may be much higher in the whales we sampled. However, older data in humans suggest that chromium levels are higher in skin than in the liver (Shroeder et al., 1970). If sperm whales accumulate chromium the way humans apparently do, then it is not surprising that our sperm whale skin levels are much higher than published liver levels. We would prefer to compare skin levels to skin levels, but in dead animals skin is not often considered, and and we have not yet found any other sperm whale skin chromium data.

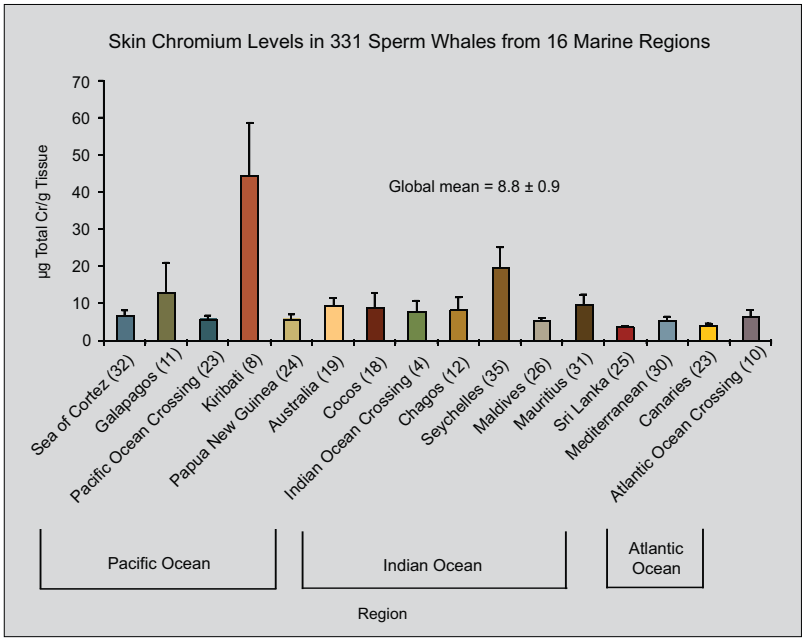


Figure 5. *Global distribution of mean chromium (Cr) levels in sperm whales grouped by sampling region. Specific regions are named for the nearest land body or ocean region. Data are shown as µg total Cr/g tissue (ppm) +/- standard error. Half the detection level was used for samples with undetectable levels.*

Considering other 'Large Whale' species, one study reported an average skin chromium level of 7.1 µg/g from free-ranging North Atlantic right whales based on seven individual whales. This level is similar to our global sperm whale chromium average of 8.8 µg/g (Figure 5). These right whales were from a site in the Atlantic that we did not visit. Considered as an Atlantic average, this level is higher, but not dramatically different from the average levels the *Odyssey* found in sperm whales for the Atlantic Ocean (4.48 µg/g) and for both Atlantic regions, which ranged from 3.7 to 6.9 µg/g (Figure 5). A second study reported average chromium levels of 0.678 and 0.618 µg/g in skin tissue from 122 male and 39 female minke whales, respectively, in the Antarctic Ocean (Kunito et al., 2002; levels are converted from the published dry weight to wet weight assuming 70% moisture). These minke whale levels are much lower than our average chromium levels (Figure 5) and likely reflect differences between species and in regional exposures to chromium.

Other published studies did not consider skin levels and focused on levels in the liver and other internal organs. The highest average liver chromium level (2.1999 µg/g) was measured in minke whales from the Antarctic Ocean, and the lowest average liver level (0.25 µg/g) was found in a long-finned pilot whale from the Atlantic Ocean. Our average skin chromium levels ranged from 3.3 to 44 µg/g, which are also higher than those noted in these earlier reports (Figure 5).

Considering chromium levels by ocean, average chromium liver levels in other 'Large Whale' species from the Pacific Ocean ranged from 0.29 to 0.36 µg/g, based on three to five individual whales. Our average level for the Pacific Ocean was much higher (9.63 µg/g), based on 98 individual whales, as were our average levels for specific regions in the Pacific, which ranged from 5.5 to 44 µg/g (Figure 5). Published average chromium liver levels in other 'Large Whale' species from the Atlantic Ocean ranged from 0.018 to 1.7 µg/g in individual whales, based on one individual whale for each species. Our average level for the Atlantic Ocean (4.48 µg/g) was much higher, based on 33 individual whales, as were our average levels for specific regions in the Atlantic, which ranged from 0.018 to 1.7 µg/g (Figure 5). The published average chromium liver level for the Mediterranean Sea was 0.43 µg/g based on one individual whale. Our average skin chromium level for the Mediterranean (5.2 µg/g) was also higher and based on 30 individual whales (Figure 5). We were unable to locate reports of 'Large Whale' chromium levels in the Indian Ocean.

We believe our data are more robust than previous published studies because of the greater numbers of animals we sampled and the fact that our subjects were alive. The differences between our data and the published levels most likely reflect differences between liver and skin accumulation and differences in regional exposures to chromium, and some may also reflect species differences. It is also possible that chromium levels have increased since these earlier reports. It is interesting to note that our average chromium skin level for whales from the Atlantic Crossing (6.28 µg/g, Figure 5) is nearly the same as the levels from 'Large Whales' sampled off the coast of Maine (7.1 µg/g), and both were skin samples. These data suggest that our numbers are accurate and that skin levels may simply be higher than liver levels in whales.

How Do Our Chromium Levels Compare with Those in Studies of Dolphins, Porpoises, and Other Marine Mammals?

We were able to locate three studies of chromium in skin tissue from dolphins, porpoises, and other marine mammals. The first study reported a skin chromium level of 0.14 ug/g wet weight from a single Dall's porpoise from the Pacific Ocean (Yang et al. 2006). A second study reported average skin chromium levels of 2.1 and 0.775 µg/g (data converted from dry weight to wet weight assuming 70% moisture) for two groups of bottlenose dolphins (74 and 67 individuals each, respectively) off the coast of the United States in the Atlantic Ocean (Stavros et al., 2007). The third study reported an average skin mercury level of 0.14 µg/g wet weight in 40 bottlenose dolphins off the coast of the United States in the Atlantic Ocean (Bryan et al., 2007). We located two studies of chromium in liver tissue from dolphins, porpoises, and other marine mammals. Fewer studies of chromium levels have been measured in dolphins, porpoises, and other marine mammal species, but again, liver was the more common organ studied. The highest average liver chromium level (2.07 µg/g) was measured in dugongs from the Pacific Ocean; the lowest average liver level (0.069 µg/g) was found in striped dolphins from the Atlantic Ocean. Average chromium skin levels in our samples (3.3 to 44.3 µg/g) were much higher than these reports (Figure 5).

Considering chromium levels by ocean, average published liver levels in dolphins, porpoises, and other marine mammal species from the Pacific Ocean range from 0.165 to 2.07 µg/g (Table 13) with the one skin study reporting a level of 0.14 µg/g (Yang et al., 2006). Our average levels for Pacific Ocean sperm whale skin (9.63 µg/g) were much higher, as were our average levels for specific Pacific Ocean regions. They ranged from 5.5 to 44 µg/g (Figure 5). Published average chromium levels in the livers of dolphins, porpoises, and other marine mammal species from the Atlantic Ocean ranged from 0.069 to 0.279 µg/g. Our average level for the Atlantic Ocean (4.48 µg/g) was much higher, as were our average levels for specific regions in the Atlantic, which ranged from 0.018 to 1.7 µg/g (Figure 5). We were unable to locate reports of chromium levels in dolphins, porpoises, and other marine mammals in the Indian Ocean or the Mediterranean Sea. The differences between our data and the published levels most likely reflect differences between liver and skin accumulation and/or different exposures to chromium in different regions. Some may also reflect species differences.

What Do These Chromium Levels Mean?

First and foremost, these data indicate that sperm whales are exposed to chromium and second that chromium levels are high. Third, the data show that chromium levels are more significant in the Pacific and Indian Oceans than in the Atlantic Ocean or the Mediterranean Sea, with particularly high levels in Kiribati, the Seychelles, and the Galapagos. Finally, the data indicate that chromium can reach and has reached even the remotest ocean locations (though we need more work to determine whether it is carried to these regions by air, by water, or just within the whales' tissues).

A study of chromium levels from 150 randomly chosen people who suffered accidental deaths (Shroeder et al., 1970) gives chromium levels in skin. This is unusual because skin levels are not often studied after metal exposure. The study also showed that skin, lung, muscle, ovary, and fat tissues have the highest chromium levels in the body, and that the levels in all of these body areas are similar. The average level for human skin was 0.31 +/- 0.099 µg/g, which is 28 times lower than the average level we found for sperm whale skin. These data suggest that the sperm whale levels are extremely high and that they may well reflect levels found inside the body.

If our assumption is correct that in sperm whales, lung levels are indeed equal to skin levels, then the sperm whale lung levels of chromium would be expected to reflect the sperm whale skin levels and be at the average level of 8.8 +/- 0.9 µg/g (with a range of 0.9 to 122.6 µg Cr/g). If such a level is correct, then sperm whales have chromium levels in their lungs that closely resemble chromium lung levels in chromium workers who are known to have died of lung cancer induced by chromium. Interestingly, skin level was measured in one worker with chromium-induced lung cancer and found to be much lower than the lung level. This difference is probably because the worker was poisoned by inhaling chromium rather than by handling it. If sperm whales also inhale chromium, then it is possible that their lung levels may be much higher and therefore of greater concern.

The fact that chromium can induce reproductive effects and that it may accumulate in testes and ovaries raises concerns about its effects on whale reproduction. The fact that level of chromium may be high in the lung tissue raises concerns about its effects on respiration and its possible role in the development of lung cancer. However, we need to investigate these concerns more fully before we can draw any firm conclusions. In recognition of these needs, our ongoing and future work are both aimed at understanding how skin chromium levels relate to chromium levels in internal organs. We are also continuing to work to understand how metals are causing toxicity in whales and humans and how much exposure is too much. Some toxicity data are presented and discussed in the Data Report VII: Cell Lines section on page 103 of the full report.

Where Is the Chromium Coming From?

Because the whales are migratory, we cannot tell exactly where the chromium is coming from. We believe that sperm whales are experiencing significant air and foodborne exposures. The major route of exposure to chromium is through the ingestion of contaminated food. Inhalation is another possible exposure route, but is rarely considered in the marine environment. The environment receives chromium from both natural and anthropogenic sources. The dominant natural source is continental dust. Anthropogenic sources include the combustion of fuels and ores; waste discharges from the electroplating, leather tanning, and textile industries; and the disposal of contaminated products (ATSDR, 2000a). The atmospheric half-life of chromium (VI) ranges from 16 hours to 5 days. Rural areas of the United States had atmospheric levels of chromium less than 10 ng/m3. Urban areas had levels of chromium ranging from 10 to 5,500 ng/m3. Remote areas were found to have chromium concentrations ranging from 0.005 to 2.6 ng/m3 (ATSDR, 2000a).

Chromium enters water sources from municipal and industrial wastewater discharges and atmospheric deposition. The dominant source of chromium from anthropogenic inputs comes from industrial (electroplating, leather tanning, and textile manufacturing) discharges. It is estimated that the annual inputs of anthropogenic chromium to water systems exceed atmospheric anthropogenic inputs. The average oceanic chromium concentration is 0.3 µg/L, ranging from 0.2 to 50 µg/L (Cary, 1982). Chromium (VI) has a much longer reduction half-life in water systems than in the atmosphere (4 to 140 days) (ATSDR, 2000a). Levels of chromium (VI) in the Pacific Ocean, Atlantic Ocean, Mediterranean Sea, and English Channel range from 2 to 4 nM (Connelly et al., 2006). The lowest levels of chromium in seawater appear to be in the Indian Ocean; however, this is an area with little research.

Pinpointing the sources of chromium more precisely would require additional data including levels in local water and air as well as in nonmigratory species. However, our data indicate that chromium is a major concern in the marine environment.

Mercury and Selenium

Mercury and Selenium in Marine Mammals: A Detoxification Mechanism

Because marine mammals detoxify mercury by binding it with selenium, we will present our data on these two elements together, even though selenium is not a metal. The principle to bear in mind is that as long as the amounts of selenium and mercury are equimolar, mercury should not be toxic to marine mammals. Figure 15 shows that in our studies there was a greater molar amount of selenium than mercury, so the mercury levels we are reporting here are not likely to be toxic to the whales themselves.

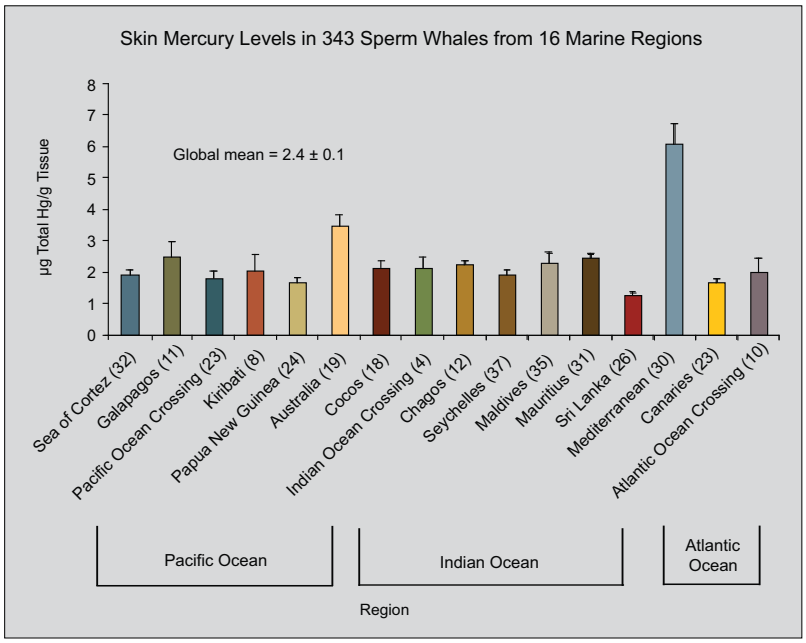


Figure 6. *The global distribution of mean mercury (Hg) levels in sperm whales grouped by sampling region. Specific regions are named for the nearest body of land or ocean region. Data are shown as µg total Hg/g tissue (ppm) +/- standard error. Half the detection level was used for samples with undetectable levels.*

What Are the Mercury and Selenium Baselines?

► **Mercury Levels**

We measured mercury (Hg) levels in 343 whales. Mercury was present in all but three whales. Detectable levels ranged from 0.1 to 16 ug Hg/g tissue with a global average level equal to 2.4 +/- 0.1 µg/g (ppm). Considered by ocean, the average Pacific Ocean mercury level was 2.22 +/- 0.13 µg/g; the average Indian Ocean mercury level was 2.04 +/- 0.1 µg/g; and the average Atlantic Ocean mercury level was 1.76 +/- 0.17 µg/g. Mercury concentrations were higher in some ocean regions than in others (Figure 6).

► Selenium Levels

We measured selenium (Se) levels in 342 sperm whale samples. Detectable levels of selenium were found in all samples. Levels ranged from 2.5 to 179 ug Se/g tissue with a global average level equal to 33.05 +/- 1.1 µg/g (ppm). Considered by ocean, the average Pacific Ocean selenium level was 40 +/- 2.0 µg/g; the average Indian Ocean selenium level, 29 +/- 1.6 µg/g; and the average Atlantic Ocean selenium level, 16 +/- 1.3 µg/g. Selenium concentrations were higher in some ocean regions than in others (Figure 7).

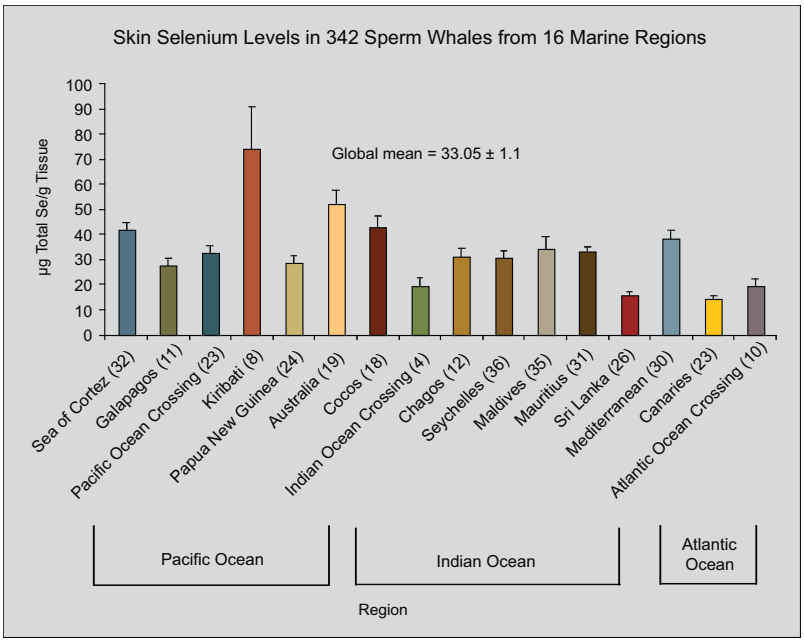


Figure 7. *The global distribution of mean selenium (Se) levels in sperm whales grouped by sampling region. Specific regions are named for the nearest body of land or ocean region. Data are shown as µg total Se/g tissue (ppm) +/- standard error. Half the detection level was used for samples with undetectable levels.*

What Do These Mercury Levels Mean?

First and foremost, these levels indicate that sperm whales are exposed to mercury. Second, they indicate that levels of mercury are high in the Mediterranean Sea. Third, the data indicate that mercury levels are more significant in the Mediterranean Sea than in the Pacific, Atlantic, or Indian Oceans. Finally, the data indicate that mercury can reach and has reached even the remotest ocean locations, though we need to conduct more work to determine whether it is carried to these regions by air, by water, or principally within the whales' tissues.

It is difficult to assess how high the internal body mercury levels actually are because the levels we measured were in skin and it is uncertain how skin levels reflect levels elsewhere in the body. Skin tissue levels are usually lower than those of other vital organs such as the liver, kidneys, and lungs; thus, the expectation is that the mercury levels inside the body will be much higher. This expectation is certainly true in the studies that measured both skin and liver in bottlenose and striped dolphins and in Dall's porpoise (Roditi-Elasar et al., 2003 and Yang et al., 2006). It remains to be determined for sperm whales. The key mercury levels will be in those organs that are likely to incur toxicity from exposure to mercury. It is difficult to assess whether the mercury levels we measured are toxic. Mercury does not serve any known normal function in mammalian physiology. Therefore, its presence in whales' bodies cannot be considered as positive. Mercury is at best neutral and lacking any effect, and at worst leads to some level of toxicity. The presence of selenium at equimolar levels suggests that the mercury that sperm whales are getting may not be toxic to them. However, more work is needed to confirm the mercury/selenium relationship based on skin levels before we can safely conclude that the mercury concentrations we found are not posing a toxic threat to sperm whales.

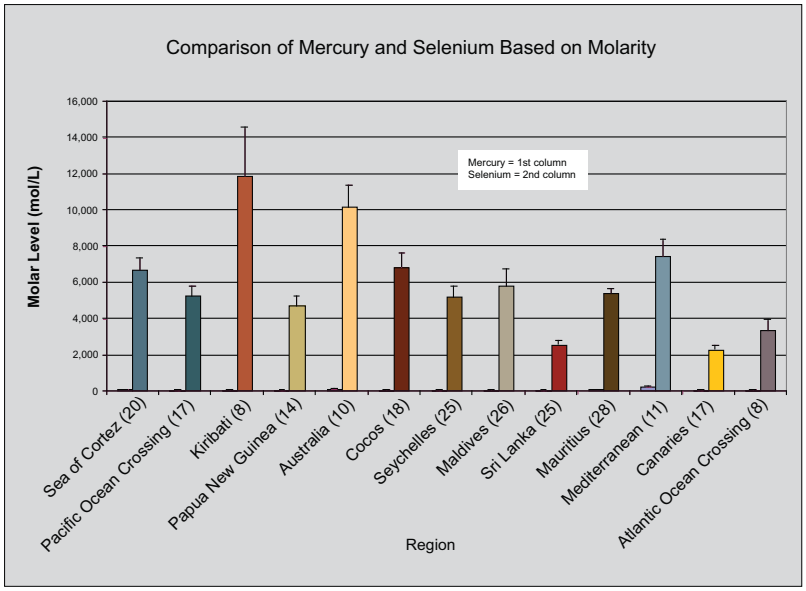


Figure 8. *The global distribution of mean molar ratio of mercury (Hg) and selenium (Se) levels in sperm whales grouped by sampling region. Specific regions are named for the nearest body of land or ocean region. Data are shown as molar concentrations of Hg and Se (ppm) +/- standard error. Half the detection level was used for samples with undetectable levels.*

Where Are the Mercury and Selenium Coming From?

► Mercury Levels

Because the whales are migratory, we cannot tell at this time exactly where the mercury is coming from. We believe the whales are experiencing significant air and foodborne exposures. The major route of exposure to mercury is through the ingestion of contaminated food, but inhalation is another possible exposure route, though rarely considered in the marine environment. Mercury intake through ingestion comes primarily from methylmercury, whereas inorganic and elemental mercury are more likely to be inhaled. Mercury accumulates primarily in the brain, liver, and kidneys and has a long biological half-life (>10 years, Hutton, 1987). Mercury is found naturally

in deposits of cinnabar (mercuric sulfide). However, deposits of cinnabar contribute an insignificant amount of available mercury to the biosphere. Cinnabar (HgS) is insoluble, very stable, and found at depths that allow little environmental release. For mercury to be released into the biosphere, deposits of cinnabar must be disturbed by natural or anthropogenic processes. Natural sources of mercury primarily release mercury in its elemental form through volatilization from soils and oceans, volcanic activity, forest fires, and biological emissions. Anthropogenic sources of mercury account for approximately 60% of global mercury released annually and include industrial applications, wastewater effluents, the combustion of fossil fuels, the degassing of landfills, and the application of sewage sludge for agriculture.

Lead

The Lead Baseline

We measured lead (Pb) levels in 337 sperm whales. Detectable lead levels were present in all but 24 whales (Figure 9). Detectable levels in sperm whale skin samples ranged from 0.1 to 129.6 ug Pb/g tissue with a global average level equal to 1.9 +/- 0.6 µg/g (ppm). Considered by ocean, the average Pacific Ocean lead level in our samples was 2.73 +/- 1.02 µg/g; the average in Indian Ocean samples was 0.94 +/- 0.11 µg/g; and the average in Atlantic Ocean in sperm whales was 1.2 +/- 0.34 µg/g. Lead concentrations from some ocean regions were higher than others (Figure 9).

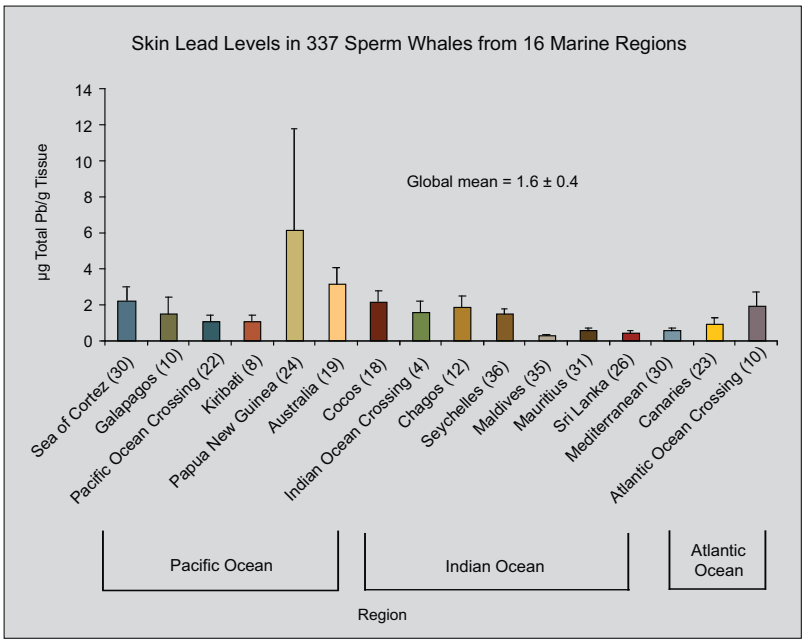


Figure 9. *Global distribution of mean lead (Pb) levels in sperm whales grouped by sampling region. Specific regions are named for the nearest land body or ocean region. Data are shown as µg total Pb/g tissue (ppm) +/- standard error. Half the detection level was used for samples with undetectable levels.*

Cadmium

The Cadmium Baseline

We measured cadmium (Cd) levels in 342 whales. It was nondetectable in 65 of these whales (Figure 10). Detectable levels ranged from 0.1 to 12.4 ug Cd/g tissue with a global average level equal to 0.3 +/- 0.04 µg/g (ppm). Considered by ocean, the average Pacific Ocean cadmium level was 0.29 +/- 0.02 µg/g; the average Indian Ocean cadmium level was 0.32 +/- 0.09 µg/g; and the average Atlantic Ocean cadmium level was 0.09 +/- 0.01 µg/g. Cadmium concentrations from some ocean regions were higher than others (Figure 10).

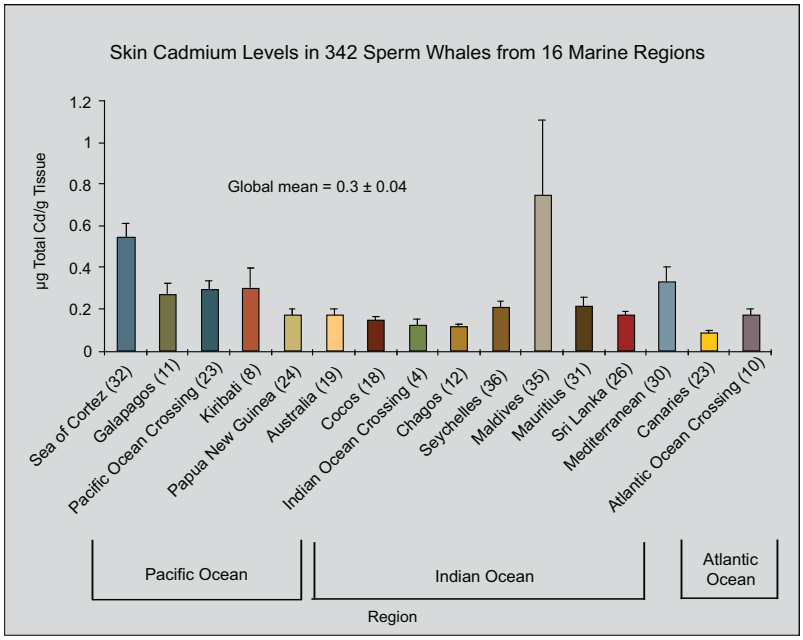


Figure 10. *Global distribution of mean cadmium (Cd) levels in sperm whales grouped by sampling region. Specific regions are named for the nearest body of land or ocean region. Data are shown as µg total Cd/g tissue (ppm) +/- standard error. Half the detection level was used for samples with undetectable levels.*

Data Report V: Emergent Toxic Metal Nanomaterials: Silver, Gold, and Titanium

What Are Nanoparticles?

Along with the metals mentioned earlier, silver, gold, and titanium are also toxic metals. We present them here in a separate nanoparticle section because we believe their threat to the marine environment will be greatly increased as they come into wide usage as nanomaterials. We could also present them in the section on toxic metals as they can be toxic and are not essential. It should be noted that although any metal may potentially be developed as a nanomaterial, the three we

are focused on are already in extensive commercial use and serve no known biological function. During the Voyage we also expected their levels to be low in the marine environment because the Voyage occurred prior to their release in commercial products. Thus, the baseline we have created with these three metals serves as a precommercial use baseline and will be a valuable tool for following the impact of nanomaterials on the marine environment. These baselines may be the only ones to have been developed prior to the commercial use of a new industrial material.

Nanotechnology is considered to be the next industrial revolution and is widely predicted to become a $1 trillion industry by 2018. The U.S. government is already investing more than $1 billion in nanotechnology development. Nanoparticles are currently in use in over 300 commercial products including sunscreen, stain-resistant clothing, tires, refrigerators, washing machines, and sports equipment. They are in clinical trials for drug delivery in diseases such as pancreatic cancer. The military is using nanomaterials in advanced electronics, munitions, propellants, fuels, nanocomposites, nano-controlled dielectrics, and nanoscale photonics. The world is at the beginning of the nanotechnology era.

Nanoparticles are defined as having at least one dimension less than 100 nm. They exist in the quantum scale, which means they don't follow the familiar laws of solids, liquids, or gases. Instead, they follow the laws of quantum mechanics, which is what gives them their unique value. They exhibit mechanical, magnetic, electronic, and color properties unachievable by the same chemicals at larger particle sizes. However, the same properties that make these particles such an exciting technology also make them daunting human health concerns. Simply put, it is unknown how these new properties will enhance, diminish, or otherwise alter the toxicity of the compounds that they are made from because the toxicity of nanoparticles is uncertain and relatively unexplored.

The likelihood of nanoparticles entering the environment is high given their extensive use in consumer products. Currently, there is no way to distinguish a nanoparticle from an ion in a biopsy. Simply put, a silver nanoparticle and a silver ion are measured the same way. As already noted, the Voyage of the *Odyssey* represented a rare opportunity to establish a baseline before significant exposures occurred. Accordingly, given the current use of silver, gold, and titanium as nanoparticles, our biopsies represent a baseline of these chemicals because our biopsies were collected before these nanoparticles came into widespread use.

The Health Effects of Nanomaterials

The toxicity of nanomaterials is unknown and poorly understood. They are presented by industry as having the same toxicity as their parent compounds on a larger scale, but because the nanoscale gives them new properties and activities, this claim is unlikely.

The Titanium Baseline

We measured titanium (Ti) levels in 298 whales. Titanium was present in all animals. Detectable levels ranged from 0.1 to 29.8 µg Ti/g tissue with a global average level equal to 4.5 +/- 0.25 µg/g (ppm). Considered by ocean, the average Pacific Ocean titanium level in sperm whale skin was 5.01 +/- 0.44 µg/g; the average in the Indian Ocean was 4.22 +/- 0.34 µg/g; and the average in the Atlantic Ocean was 2.34 +/- 0.27 µg/g. Titanium concentrations were higher in some ocean regions than in others (Figure 11).

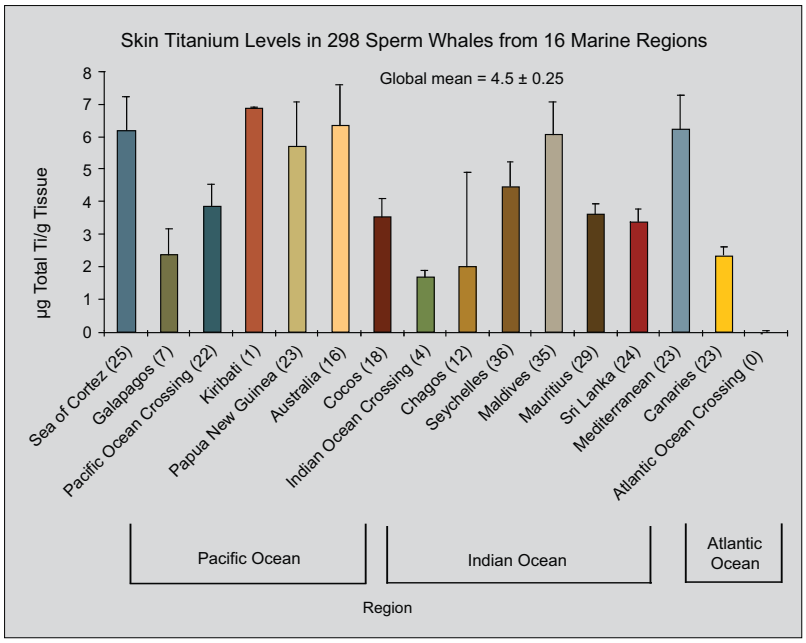


Figure 11. *Global distribution of mean titanium (Ti) levels in sperm whales grouped by sampling region. Specific regions are named for the nearest body of land or ocean region. Data are shown as µg total Ti/g tissue (ppm) +/- standard error. Half the detection level was used for samples with undetectable levels.*

What Is Next for Metal Nanoparticle Work?

1. Publish the results

To meet this goal, we are in the process of writing these data into manuscripts for publication. We have already submitted a manuscript on the silver data.

2. Develop toxicity data for the nanoparticles made from these metals

Nanotoxicology is a field in its infancy with only a few laboratories studying the potential toxic effects of these metals (our laboratory is one). Currently, the field urgently needs cell line–based studies to evaluate these materials. We have already conducted some initial silver nanoparticle toxicology studies in the sperm whale cell lines. We plan to expand these and also study gold and titanium. In manuscripts addressing these toxicology data, we will also be presenting the baseline data developed for these metals from samples provided by the Voyage of the *Odyssey*.

3. Monitor key sites again in a few years to determine whether the levels are changing
Now that nanomaterials are in widespread commercial use, in a few years we will need to revisit key sites and compare new levels to our baseline in order to determine whether exposures are increasing.

Data Report VI: Essential Metals That Become Toxic at Higher Doses

Several metals are required in trace amounts for good health. Their functions are usually as enzyme cofactors. We have determined baselines for some of these essential metals in sperm whales, including copper, iron, magnesium, manganese, nickel, and zinc. Because we are in the process of contextualizing these data, the information concerning how their levels compare to previous studies and what their sources are in the marine environment are not ready yet. However, because the baselines are complete, they are included here and will be contextualized in the near future.

The Copper Baseline

We measured copper (Cu) levels in 342 whales. Copper was present in all whales. Detectable levels in our sperm whale samples ranged from 0.2 to 1,855.5 ug Cμ/g tissue with a global average level equal to 14.9 +/- 5.5 μg/g (ppm). Considered by ocean, the average Pacific Ocean copper level was 10.6 +/- 1.3 μg/g; the average in the Indian Ocean was 20.7 +/- 12.9 μg/g; and the average in the Atlantic Ocean was 14.1 +/- 8.8 μg/g.

Copper concentrations were higher in some ocean regions than in others (Figure 12).

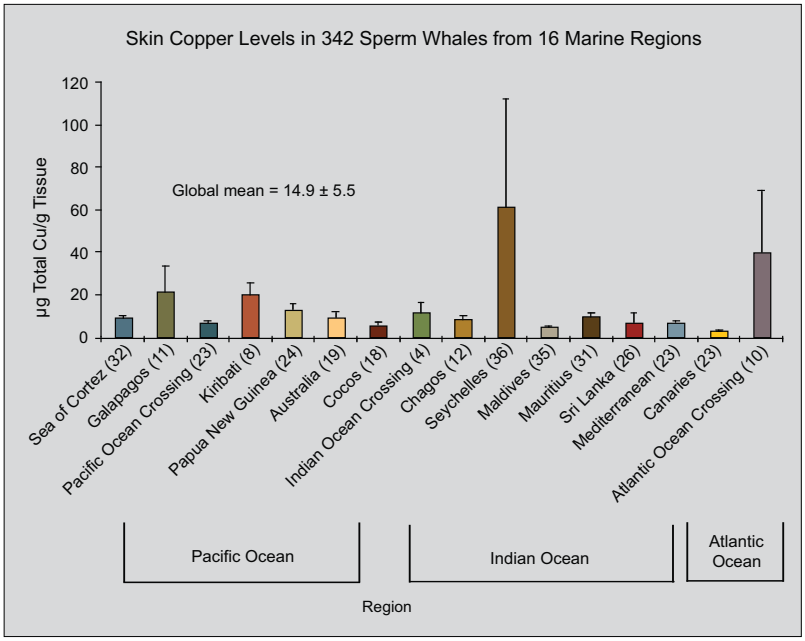


Figure 12. *Global distribution of mean copper (Cu) levels in sperm whales grouped by sampling region. Specific regions are named for the nearest body of land or ocean region. Data are shown as μg total Cu/g tissue (ppm) +/- standard error. Half the detection level was used for samples with undetectable levels.*

The Magnesium Baseline

We measured magnesium (Mg) levels in 298 sperm whale samples. Magnesium was present in all whales. Detectable levels ranged from 38 to 2,021 μg Mg/g tissue with a global average level equal to 548.6 +/- 21.1 μg/g (ppm). Considered by ocean, the average Pacific Ocean magnesium level in sperm whale skin was 704 +/- 35 μg/g; the average in the Indian Ocean was 465 +/- 28.4 μg/g; and the average in the Atlantic Ocean was 283 +/- 15.3 μg/g.

Magnesium concentrations were higher in some ocean regions than in others (Figure 13). The highest average magnesium level was found in sperm whales sampled in the Sea of Cortez in the Pacific Ocean (957 +/- 69 μg/g). The lowest average magnesium level was found in whales off the coast of the Canary Islands in the Atlantic Ocean (283 +/- 15.3 μg/g).

Considering magnesium levels by individual whales, the highest magnesium level (2,021 μg/g) was found in a whale from the Sea of Cortez in the Pacific Ocean. The lowest magnesium level (38 μg/g) was found in a whale from Cocos in the Indian Ocean.

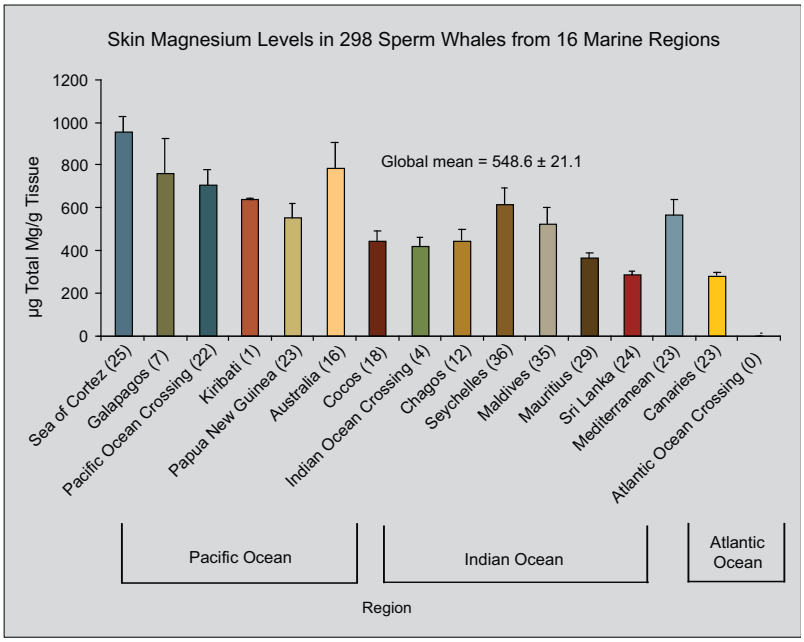


Figure 13. *Global distribution of mean magnesium (Mg) levels in sperm whales grouped by sampling region. Specific regions are named for the nearest body of land or ocean region. Data are shown as μg total Mg/g tissue (ppm) +/- standard error. Half the detection level was used for samples with undetectable levels.*

Data Report VII: Cell Lines

A Brief Overview of Some Challenges in Marine Toxicology That Can Be Helped with Cell Lines

Dr. Wise (on the right) obtaining the biopsies from Gen Johnson, Odyssey *crew member, near the end of the Voyage. The cooler contains three biopsies that were turned into three cell lines. The* Odyssey *is in the background.*

One of the fundamental shortcomings in marine toxicology studies, particularly of marine mammals, is a lack of understanding of how metals and chemical compounds affect marine species and how much is too much. Human toxicology and risk assessment rely on epidemiological studies of exposed human populations, controlled exposures of laboratory animals, and controlled exposures of cell cultures. Because rodent models have proven inaccurate for predicting human response, the demand for and amount of work in human cell lines has increased. Marine toxicology studies are hampered by the fact that good epidemiology studies of exposed populations are difficult to do because exposure levels are uncertain and the results cannot tell us the animals' exposure or travel histories. Such studies are also hampered by the fact that few of them correlate effects in rodents with effects in marine species. Finally, most are hampered by a lack of marine cell lines.

The second prong in our approach is to work with cell lines. During the Voyage we used a few of the biopsies to create the first sperm whale cell lines. Shown above, Dr. Wise obtains the biopsies from *Odyssey* crew member Gen Johnson near the end of the Voyage.

Using these cell lines we are beginning to determine (1) the toxic effects of these metals and chemical compounds at cellular and molecular levels and (2) how well toxic outcomes and responses in cell lines from marine species mimic responses in humans and rodents. In the future we believe that the data from the cell lines will help us determine (1) a model for how much exposure is too much, (2) a ranking system to define the chemicals of most concern in terms of their toxic effects, (3) a risk assessment paradigm for marine species, and (4) possible adaptations in marine species that may be adoptable to improve human health.

What Is a Cell Line and Why Do We Want One?

A cell line is a renewable population of cells grown in culture from the cells of a species of interest. Such a cell line contains all of the genetic information (DNA) of a species be it whale, human, or plant. Cell lines can be experimentally controlled and manipulated in the laboratory to study toxicology, genetics, and other important health issues. Cell lines and tissue slices are the only means available to generate sperm whale-specific information in a controlled toxicological experiment because bringing the whales into the laboratory and exposing them directly is both illegal and impractical. Of these two methods – cell lines and tissue slices – only the cell lines are renewable. Thus, having a cell line allows us to obtain species-specific information in a controlled laboratory environment. We can expose the cells to various levels of chemicals and observe the outcomes. For whales and many other marine mammals, cell lines are the best laboratory approach to studying the effects of chemicals, becoming an extremely powerful approach when combined with a marine mammal population study such as the Voyage of the *Odyssey*.

How Do We Create a Cell Line?

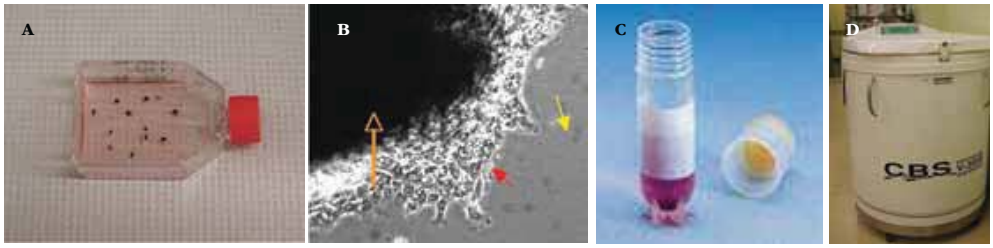


Figure 14. *The process of making a sperm whale cell line from the initial minced tissue pieces to the freezer. (A) These are some of the actual sperm whale samples about to be placed in the incubator. The dark chunks are the tissue pieces. The bottom of this flask is about the size of a credit card. The cells attach to the bottom and grow out of the tissue pieces. (B) Using high magnification through a microscope, the picture shows cells growing out of a piece of sperm whale tissue. The red arrow points to the growing cells. The orange arrow points to the tissue piece. The yellow arrow points to a clear area, which is the bottom of the flask. (C) A vial into which cells are put for freezing. Each vial holds at least 1 million cells. (D) The nitrogen freezer that stores the cells. The freezer can hold 25,000 vials of cells.*

Once the biopsy is in the laboratory and able to be handled in a clean and sterile environment, we process the tissue and mince it into small pieces and place them inside a tissue culture flask. The flask is specially treated so that cells will attach to the bottom of it. A special growth medium, with the appearance of a light red juice, is placed on top of the flask, and the flask is placed in an incubator. After several days, the cells begin to grow out of the tissue and onto the bottom of the flask. The cells continue to grow until they fill the flask, at which point they need to be either moved into more or bigger flasks or placed in a tube and frozen for later use. Cells continue to grow for several months and can be recovered from being cryogenically frozen in liquid nitrogen. Alas, they do have a finite life span that differs for each cell line, cell type, and species. Our laboratory (the Wise Laboratory) is pioneering genetic ways to make these cells immortal with minimal alterations in their normal phenotype. Figure 14 shows the stages of cell line creation from the initial pieces in a flask, to cells growing out of a tissue, to the freezer.

What Toxic Endpoints Are We Studying and Why?

Cell lines are limited by the fact that we can study only cellular and molecular effects. We cannot study organ failure or organ malfunction. But we can study the cellular and molecular effects that underlie organ failure and malfunction. We have chosen to start by looking at two major endpoints: cytotoxicity, which measures cell death, and genotoxicity, which measures damage to DNA. Measuring cell death will tell us what amount of a chemical kills cells. It also gives us a context for our DNA damage studies so that we do not work at doses so high that all cells are killed. Damage to DNA is an important measure of toxicity because it is known to lead to cancer, affect reproduction, and cause developmental problems, and other health effects. DNA damage is commonly used as a regulatory endpoint by the U.S. Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and Occupational Safety and Health Administration (OSHA).

What Are We Finding?

Because of the high chromium levels we found in Sperm Whales we studied the effects of chromium on cells grown in culture first. We are also testing silver nanoparticles because of the possible imminent threat they pose to human and aquatic health.

Is the Chromium Damaging the Whales' DNA?

The data cause us to ask whether the whales are exposed to doses that are breaking their chromosomes. The quick answer is that we do not know yet as we need more information about exposure route. We do know the following:

Sperm whale chromium levels are very high.

The range of the measured chromium levels is much higher than has ever been reported before for wildlife. The average global chromium level in sperm whale skin is 28 times higher than the average chromium skin level in humans who do not work directly with chromium.

The chromium levels in the whales from Kiribati almost perfectly match the levels seen in the lung tissue of human workers who died of chromium-induced lung cancer after more than 10 years of occupational exposure. Most of the chromium in the bodies of the workers got there through inhalation. The full report explores this further, concluding that it is not an unreasonable possibility that sperm whales may be inhaling significant amounts of chromium.

Data Report VIII: Genetics and Genetic Fingerprinting

One of our goals is to complete a genetic fingerprint for every sample.
These data will allow us to understand where the whales come from as well as to identify instances in which we have sampled a particular whale more than once. We extracted DNA from 932 samples and ran a multiplex PCR assay to identify gender. In all, 906 samples were genetically identified by gender: 221 males and 685 females. Only 26 samples (2.8%) failed to be identified, a gratifyingly low failure rate for this technique. Additional efforts could be pursued for these samples (e.g., more robust and expensive enzymes could be used) should the need arise. In the meantime, we have partnered with Dr. Scott Baker at the University of Oregon to conduct the genetic fingerprinting, and that work is in progress.

Data Report IX: Database

The Voyage of the *Odyssey* Database

While collecting data on sperm whales during the Voyage, the crew of the *Odyssey* also built the Voyage of the *Odyssey* database using three software programs. Rainbow Click is a program designed to track whales by isolating the echolocation "clicks" that sperm whales make from noise and, giving the helmsperson a relative bearing to the whale. Logger 2000 is a data logging program that acts as an interface to a database program. In particular, Logger was able to collect data directly from the GPS on the bridge; display a real-time map of the ship's track and any other data we collected such as estimated range and bearing to cetacean sightings, biopsy locations, acoustic detections, etc.; run a software tape recorder for making hard disk recordings; link comments by scientists and crew on those recordings, and display data from the Rainbow Click sperm whale detection program. Microsoft ACCESS (2000) is a standard database program that stores these data. Using these software packages, the crew and staff of Ocean Alliance created 21 Microsoft Access database files (essentially one for each region and subregion). Collectively these make up the first version of the Voyage of the *Odyssey* database.

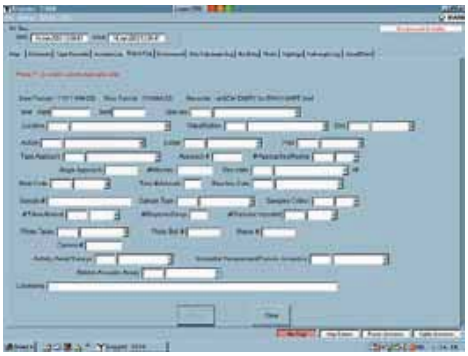


Figure 15. *Data entry screen for the Voyage of the* Odyssey *database. Each tab at the top indicates a different entry form for each data point. By the end of the voyage, the crew had collected over two terabytes of raw data.*

Each regional database contains large tables of information that hold all of the data. The specific tables had categories with fixed "scroll-down" options. The categories included the acoustic log, the biopsy log, the biopsy master log, comments, environmental data, GPS data, miscellaneous subsample log, photos, reaction-of-whale log, recordings, sightings, subsample log, and visual effort. Figure 15 shows an illustration of one of these tables and a typical level of detail it contains.

The Data Context

Data Contextualization I: A Global Pollution Baseline

What Is the Global Baseline?

We considered four marine areas: the Pacific Ocean, the Indian Ocean, the Mediterranean Sea, and the Atlantic Ocean. Whales from these regions were tested for the presence of *toxic metals* (arsenic, aluminum, barium, cadmium, chromium, mercury, lead, strontium, tin, and titanium), *essential metals* (copper, iron, magnesium, manganese, nickel, selenium, and zinc), and *metals related to nanoparticles in current commercial use* (gold, silver, and titanium). Figures 16 through 19 show the global baseline levels for these contaminants. Perspectives on where many of these contaminants originate from are discussed in the full report by individual metal.

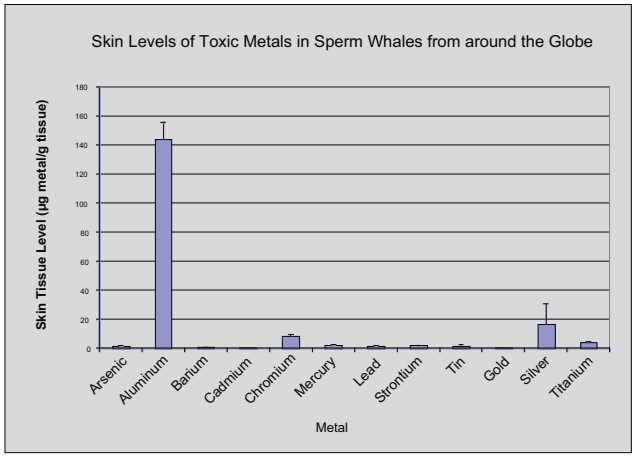


Figure 16. Levels of toxic metals in sperm whales from around the world. All data are presented in µg total metal/g tissue +/- standard error. For whales with undetectable levels, half the detection level was used in the analysis.

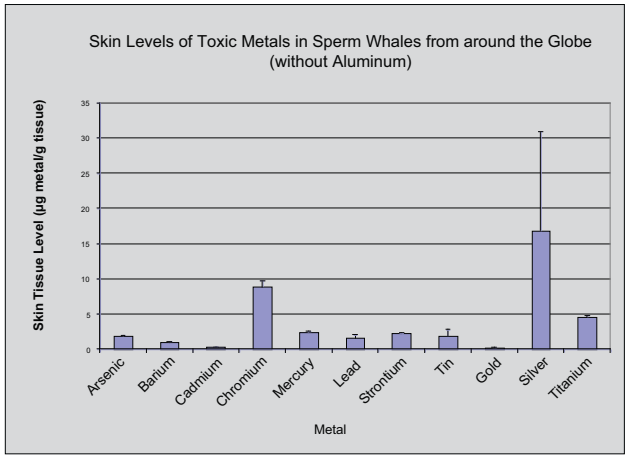


Figure 17. Levels of toxic metals in sperm whales from around the world (excluding aluminum). This figure shows the levels of toxic metals in sperm whales from around the world. All data are presented in µg total metal/g tissue +/- standard error. For whales with undetectable levels, half the detection level was used in the analysis. Aluminum was excluded to allow for a smaller scale that would allow better visualization of the levels of the other metals.

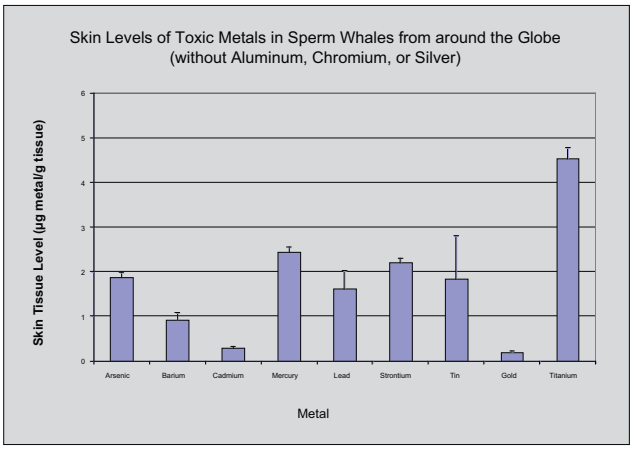


Figure 18. Levels of toxic metals in sperm whales from around the world (excluding aluminum, chromium, and silver). This figure shows the levels of toxic metals in sperm whales from around the world. All data are presented in µg total metal/g tissue +/- standard error. For whales with undetectable levels, half the detection level was used in the analysis. Aluminum, chromium, and silver were excluded to allow for a smaller scale that would allow better visualization of the levels of the other metals.

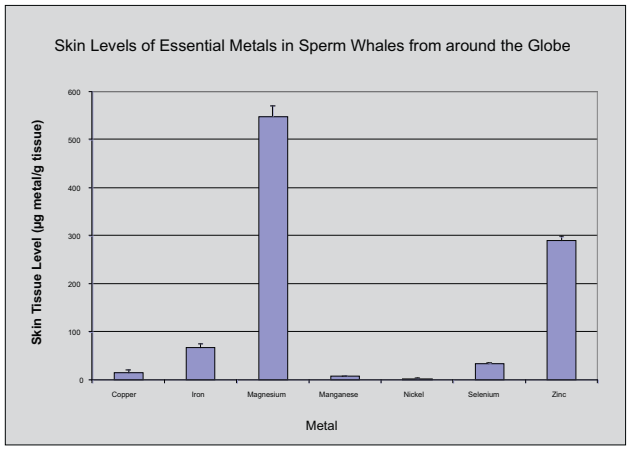


Figure 19. Levels of essential metals in sperm whales from around the world. All data are presented in µg total metal/g tissue +/- standard error. For whales with undetectable levels, half the detection level was used in the analysis.

Could These High Aluminum and Chromium Levels Be an Artifact?

We believe that these high aluminum and chromium levels are real and not the result of sample contamination during collection (e.g., by a biopsy dart) or during sample handling. To ensure that there was no contamination of the sample by the biopsy dart, the stainless steel biopsy tips were rinsed extensively in ethanol and cleaned between each use. However, we base our final conclusion on a number of observations. First, there is the broad distribution of levels in the samples. If the darts/tools were contaminated with aluminum or chromium, one would expect that all samples would be high within, a narrow range. In fact, the ranges are large and span 3 orders of magnitude. On the other hand, if only a few darts were contaminated, then one would expect the high end of the range to be similar in every region, and it is not. There was a 13-fold difference in range for chromium with Sri Lanka having the lowest value maximum chromium level (9.3 µg/g) and the Seychelles having the highest value for its maximum (122.6 µg/g). For aluminum there was also a large difference in range with Kiribati at 23.1 µg/g (only one sample) and the Galapagos at 95.3 µg/g having the lowest maximum aluminum levels, and the Mediterranean (1,870 µg/g) having the highest. This range is 81-fold if we compare levels to the Kiribati levels or 20-fold if we use the level from the Galapagos.

Second, as discussed earlier, a reasonable exposure scenario can explain these chromium levels. The whales could be expected to reach these high levels by some means, perhaps through inhalation of sufficient amounts of chromium. If this were merely artifactual contamination of some sort, it would be more difficult to conceive of a way for the whales to actually reach such high levels. Thus, although this exposure scenario on its own proves nothing, when considered with the distribution levels and the absence of chromium leaching from the biopsy tips (see next paragraph), it is consistent with a conclusion that these levels are real and exposure is occurring. We are currently considering exposure scenarios for aluminum.

Finally, to confirm that no chromium was leaching from the biopsy tips, we tested whale tissue provided by Alaskan Inupiat hunters during their annual fall hunt of bowhead whales (*Balaena mysticetus*). We sampled this tissue using two means to procure tissue: with a biopsy dart from the Voyage and a ceramic knife. The measured chromium levels were all low, and there was no difference between samples obtained by the two methods, indicating that the darts did not contaminate the tissue. In addition, we attempted to leach chromium from the dart with a 2% HNO3 solution and found the leachate to be nondetectable for chromium. We will soon be performing the same tests with aluminum.

Data Contextualization II: A Baseline for the Ocean Regions around the Globe

What Is the Baseline for the Ocean Regions?

We considered four marine areas: the Pacific Ocean, the Indian Ocean, the Mediterranean Sea, and the Atlantic Ocean. Whales from these regions were tested for the presence of *toxic metals* (arsenic, aluminum, barium, cadmium, chromium, mercury, lead, strontium, tin, and titanium), *essential metals* (copper, iron, magnesium, manganese, nickel, selenium, and zinc), and *metals related to nanoparticles in current commercial use* (gold, silver, and titanium). In the Pacific Ocean we also identified a baseline for three organochlorine compounds (PCB, DDT, and HCB). Baselines and perspectives on where many of these contaminants may be originating from are discussed in the full report.

Data Contextualization III: A Finer Look — Baselines for the Pacific Ocean Regions

What Is the Baseline for the Pacific Ocean Regions?

We considered five sites in the Pacific Ocean: the Sea of Cortez, the Galapagos, the Pacific Ocean Crossing, Kiribati, and Papua New Guinea. From these regions, whales were tested for the presence of toxic metals (arsenic, aluminum, barium, cadmium, chromium, mercury, lead, strontium, tin, and titanium), essential metals (copper, iron, magnesium, manganese, nickel, selenium, and zinc), metals related to nanoparticles of interest (gold, silver, and titanium), and organochlorines (PCB, DDT, and HCB). Previous sections provide context about many of these contaminants, based on the Pacific Ocean as a whole (see page 112 of the full report). Generally, specific data on marine mammals, air, and water, are not readily available for the regions where we worked. We are in the process of trying to find any data that will help contextualize the contaminants based on specific Pacific Ocean regions, but that information is not yet ready for presentation here.

Data Contextualization IV: A Finer Look: Baselines for the Indian Ocean Regions

What Is the Baseline for the Indian Ocean Regions?

We considered eight sites in the Indian Ocean: Australia, Chagos, Cocos, Indian Ocean Crossing, Maldives, Mauritius, the Seychelles, and Sri Lanka. From these regions, whales were tested for the presence of toxic metals (arsenic, aluminum, barium, cadmium, chromium, mercury, lead, strontium, tin, and titanium), essential metals (copper, iron, magnesium, manganese, nickel, selenium, and zinc), and the metals related to nanoparticles of interest (gold, silver, and titanium). Of course, any metal could potentially be developed as a nanomaterial, but the three we are focused on are already in extensive commercial use and serve no known biological function. The full report provides context about many of these contaminants, based on the Indian Ocean as a whole. Generally, specific data on marine mammals, as well as levels in air and water, are not

readily available for the specific regions in which we worked. We are still searching for data that will help contextualize the contaminants based on specific Indian Ocean regions, but that effort is incomplete and consequently there are no data to present.

The Most Polluted Region in the Indian Ocean

To provide some context in regard to the relative pollution of the sperm whales we sampled in each Indian Ocean region, we ranked the relative concentration of each pollutant as low, medium, or high. These levels were determined based on the global average for each metal. We defined as low those average levels that were equal to the global average minus 2 standard errors for that metal. In other words, we subtracted twice the standard error from the global average and set that level as low. We defined as high those average levels that were equal to the global average plus 2 standard errors for that metal. In other words, we added twice the standard error to the global average and set that level as high. A medium level was then defined as between those values.

The full report shows maps of the relative levels for the toxic metals. The high values are shown as red dots; the low values, as yellow dots; and the medium values, as orange dots. We then assigned an arbitrary score to each color. Low values were given a score of 0; medium values, a score of 1; and high values, a score of 2. Based on this approach, the Maldives and Australia appear to be the most polluted regions, and the Idian Ocean Crossing and Cocos the least, leaving Mauritius, Chagos, and Sri Lanka comparatively in the middle for pollution of the sperm whales at the sites we studied (See full report). We did not include the essential metals in this presentation because we are uncertain about whether their levels are beneficial or deleterious to the whales. When we do include them, although the scores are higher, the pattern is the same.
Of course, these data are specific for the metals we considered and may not reflect the relative amounts of the pollutants that we did not consider. Nevertheless, the data provide an interesting picture of relative pollutant levels in the Indian Ocean.

Data Contextualization V: A Finer Look – Baselines for the Mediterranean Sea Regions

What Is the Baseline for the Mediterranean Sea Regions?

Although we collected samples from whales from multiple sites in the Mediterranean Sea, we analyzed them as a group. Thus, we did not create separate baselines for individual locations within the Mediterranean Sea. This approach was consistent with our analysis for the Pacific, Atlantic, and Indian Oceans, although the centers of the regions we sampled within those areas were separated by much greater distances. The data from the Mediterranean Sea are presented in the full report on page 114.

Data Contextualization VI: A Finer Look – Baselines for the Atlantic Ocean Regions

What Is the Baseline for the Atlantic Ocean Regions?

We collected samples from sperm whales in three sites in the Atlantic Ocean: the Canary Islands, our Atlantic Ocean Crossing, and the Bahamas. The majority of the samples from the Bahamas, however, were from sloughed skin rather than from biopsies obtained with a dart. The sloughed skin samples were not included in the initial analysis studies because of inherent differences between the two types of samples. Because only a few metals were considered in the Atlantic Ocean Crossing, the bulk of the Atlantic Ocean region analysis is just from sperm whales in the waters around the Canary Islands area. Remarkably, this area tended to have the lowest levels of pollutants in the world. Earlier sections of the full report provide context about many of these contaminants, based on the Atlantic Ocean as a whole (see page 115 of the full report). We have found very few specific data on marine mammals. Furthermore air and water levels of those contaminets are not readily available for the specific regions in which we worked. We are looking for specific Atlantic Ocean regions data that can help contextualize the contaminants, but our information is incomplete and consequently it is not included.

Biopsy samples are inventoried and stored in a variety of ways: at -80 degrees celsius; in a solution within individual vials; and embedded in wax trays.

Part 4: The Future

Ocean Alliance now holds a unique, enormously valuable sample set that for the first time allows for a rigorous assessment of the impact of toxic chemicals on whales from around the world. Until their historic sample collection adventure, no rigorous studies of toxic impacts on these majestic creatures, high on the food chain and hence so susceptible to toxic impact, was possible. NRDC has been following this work with great interest over the last few years and believes that the data set from this voyage has great potential to impact environmental policy both here in the U.S. and, perhaps more importantly, abroad. We strongly encourage you to support their efforts so that the sample set can be fully analyzed and mined for information so crucial for now and the future.

– Linda E. Greer, Ph.D., Director, Health Program, Natural Resources Defense Council

Implications of the Data: What Does All of This Mean?

Working up the data, contextualizing it, and writing it up as scientific papers is still underway. Nevertheless, some important discoveries that can be expected to alter fundamentally the way the world thinks about marine pollution are already apparent. The first Voyage of the *Odyssey* provides clear evidence that pollutants have reached the deep oceans of the entire world; the extent of the accumulation of contaminants in apex predators makes it an indisputable fact that humans are polluting the oceans. Aluminum, chromium, lead, silver, and titanium are all metals that are found in high concentrations and/or appear to be increasing in ocean life. The Voyage has already achieved a number of important goals and breakthroughs as follows:

1. Established a baseline of ocean contaminants

As evidenced by the many contaminant baseline concentrations presented in Part 3 of this report, we achieved our fundamental goal many times over. We can now talk and think about many pollutants in a global context, something not possible before the Voyage. This context is critically important because it allows us to see in objective terms that even animals living in the most remote ocean regions are heavily contaminated and that pollution is now a global problem. Voyage of the *Odyssey* results encourage us to begin thinking more about global movements of pollutants by such natural forces as wind and ocean currents. Scientists have long questioned whether pollutants were moving across the globe at rates and concentrations that would constitute any kind of significant threat to ocean life or, by extension, to human life. But the baseline concentrations provided in this report now show that this is indeed the case. The presence of high levels of pollutants in remote locations near areas of land without major industry such as Kiribati and the Galapagos demonstrate that contaminants are everywhere whether they are carried there by wind, water, or animals. We now have proof that humanity is polluting all oceans. In the future, the baselines provided by the first Voyage of the *Odyssey* will tell us whether things are getting better or worse and in applicable cases whether remediation efforts are working.

2. Conducted the first and only large study of marine contaminants in free-ranging animals

The Voyage of the *Odyssey* transforms the way we will think about contaminant studies. The study encompassed hundreds of apparently healthy animals of a single species that is found across the globe. It is the first of its kind. Even if we consider studies of dead animals, it is the largest single marine contaminant study. Previous studies have relied on small numbers of animals, usually a handful, from just one species. Moreover, these animals were usually found stranded and dead, though in a few cases, such as subsistence-hunted bowhead whales, the specimen animals were presumed healthy when they were killed. In previous whale studies the animals came to the researchers: the researchers did not go to the animals. The Voyage of the *Odyssey* proves that a large dataset from multiple regions can be developed and, more important, provides critical insights into a complex problem. Scientists can now use these data to provide context and guidance for their own smaller or more local studies and may now seek with greater confidence of possible success, new ways to perform larger and more ambitious studies.

3. Showed that hundreds of healthy sperm whales, from even the remotest ocean regions, have been exposed to pollutants, thus demonstrating that marine pollution is both a global and a local problem

With the dataset from the Voyage we now have the statistical power to show marine baselines with unprecedented authority. Critics of previous studies by others have pointed out that the number of animals considered was so low that the studies probably did not reflect the whole population of that species, and disputed that any definite conclusion could be drawn regarding a large pollution problem. In addition, they argued that because the studied animals were sick, the levels were quite probably elevated as a result of the underlying disease that caused the animals to strand and die in the first place. Finally, they argued that if there were any validity to the levels found, they probably reflected an unusually high and localized exposure to some rare pollution event such as an oil spill or a sunken ship.

The Voyage of the *Odyssey* overcomes all of these criticisms. Hundreds of animals were considered, not just a handful. Because all of the animals were alive and exhibiting normal healthy behaviors, disease is not likely to be a factor, nor is it likely to be responsible for the elevated levels found. Eighteen broad regions from around the globe were studied so this is not merely a local phenomenon. Moreover, the vast majority of sperm whales are not found in coastal waters so this is not just a near shore problem. Finally, because this work was done on a single species, critics cannot argue that the observed differences come from studying several different species. Because of the dataset we have provided, it is no longer possible to argue that marine pollution is not a major global health concern.

4. Compiled the first extensive pollutant data for the Indian Ocean

Very few studies have considered contaminants in the Indian Ocean. This data gap seems remarkable given the size of the Indian Ocean and the numbers of peoples who depend on seafood from it. The Voyage of the *Odyssey* has now provided substantial insights into pollution levels in the Indian Ocean, which will help officials and scientists in that area better understand the challenges they face in protecting and conserving this important area.

5. Identified aluminum and chromium as global pollutants

The high aluminum and chromium levels we discovered are surprising and provocative. Aluminum and chromium have only rarely been considered in marine studies as they were not thought to be major concerns. Now, with data showing chromium levels previously seen only in workers, with long occupational exposure, the discussion changes and we have to consider aluminum and chromium as important global health concerns.

6. Brought attention to and started a significant discussion on the importance of air pollution in the marine environment

One discussion we have already started concerns the possibility that whales are exposed to chromium via inhalation. This argument stems from the fact that chromium is poorly absorbed by eating food or via direct absorption through the skin. Thus, it would be logical that in order to have the high levels we observed, whales have to have been inhaling substantial quantities. In starting this discussion, we have learned that few have considered the impact of air pollution on marine mammals, the focus having almost entirely been on diet and water exposure. The Voyage data will fundamentally change the way scientists perceive the marine environment, by forcing them to consider air quality as an important health concern to marine life.

7. Created the first sperm whale cell lines

The creation of the world's first sperm whale cell lines will allow us to investigate the toxic effects of metal and chemical pollutants we found in the biopsy samples we obtained. We will also be able to compare the effects of pollutants on sperm whale cell cultures to their effects on human cell cultures, thereby gaining important insights into both whale and human health. Finally, using these cell lines, we can create a number of important cellular and molecular tools that can be used to gain a better understanding of sperm whale biology.

8. Established baselines for nanoparticle-related metals

Nanotechnology is an unstoppable wave of the future that has raised concerns globally about its strong potential to cause serious environmental problems. Because of the Voyage of the *Odyssey*, we are now armed with key baseline data showing levels of three of the major nanomaterials (silver, gold, and titanium) before they came into widespread usage. As that usage grows, our results will help assess the effects of the widespread use of nanoparticles on the marine environment, and can be expected to be of key value in helping to minimize negative effects.

9. Established baselines for organochlorines

Although the levels of organochlorines in the oceans are low, the Pacific baseline for these chemicals tells us that they appear to be decreasing. This observation is important because it tells us whether remediation methods such as banning a chemical can work – an important consideration when considering how to control other pollutants with very high levels, such as chromium.

10. Are establishing a baseline for polybrominated flame retardants

PBDEs are a ubiquitous contaminant of emerging concern. Once we complete the work of establishing the baseline for them, we will have provided a key insight into the extent of their reach into the oceans and how urgent a problem they constitute there.

There are of course many more important but less consequential achievements from the Voyage that we will not attempt to discuss further here. Examples are the data showing that chromium can damage whale DNA and the determination that CYP1A1 is not a sufficient biomarker for assessing contaminant exposure levels in sperm whales. As we continue to analyze our samples, we are confident that many more discoveries will come to light.

Next Steps for the Toxicology Program

The biopsies, cell lines, and data from the Voyage of the *Odyssey* have provided important baseline information on contaminant concentrations in the oceans. Our future plans, short-, medium-, and long-term, are ambitious, but we believe that with the necessary support they can significantly add to humanity's understanding of how pollutants affect marine species and will lead to important changes in the management and conservation of the seas.

Short-Term

1. Publish our existing data

Publishing scientific data in peer-reviewed literature is a slow, careful, and deliberate process. We have been devoting substantial time to analyzing and contextualizing data. As of April, 2009, our scientific team has produced 14 peer reviewed journal articles, 4 more are in press, 2 have been submitted for publication, and 15 are in preparation. We have published 22 abstracts and presented 12 posters, 19 technical lectures, and 4 technical reports. Eighteen posters were presented at the 18th Biennial Conference of the Society for Marine Mammalogy, October 2009, Quebec, Canada (see page 164 of the full report).

2. Conduct additional analyses

In 2006 we received a grant from the National Fish and Wildlife Foundation to measure PDBE concentrations in whales from several regions, principally the Pacific. We will be seeking additional funds to analyze our biopsy samples from the other regions of the world for PBDEs.

We also plan to begin studying the levels of drugs that reach the sea via the waste stream. Pharmaceuticals such as antibiotics, birth control pills, and other medications are becoming a concern for marine species. We will be seeking funding to test our archive of sperm whale biopsies to see if they show significant accumulations of any of these drugs or their metabolites.

3. Conduct additional toxicity tests on sperm whale cell lines

We intend to test the effects of a number of chemicals on cell survival and DNA damage. We are seeking funding to conduct these tests on our sperm whale cell lines. We also hope to continue without delay our examination of the effects of nanoparticles on marine mammal cells grown in culture.

4. Develop more cell lines including other species and cell types

We have recently completed construction of a comprehensive cell line laboratory onboard the *Odyssey*. Using this platform, we plan to develop more marine mammal cell lines. We will also attempt to develop cell lines from corals and mollusks, neither of which has been achieved before. We will be seeking funding to help develop these programs.

5. Determine a baseline for pollutants in the Gulf of Mexico and on the Eastern seaboard of the United States

We are planning to collect and study sperm whale biopsies from the Eastern seaboard and the Gulf of Mexico as they are not well represented in our sample collection. We have submitted several grants to achieve this and hope to start the studies in the spring of 2010.

6. Compare pollutant levels in skin with levels in internal organs

We are currently seeking tissue samples from dead, stranded marine mammals. We will be seeking funding to collect these tissues and analyze them for levels in both internal organs and skin. The internal organs of greatest interest include brain, liver, kidneys, lungs, testes, ovaries, heart, and spleen. We have recently received a grant to analyze samples for 22 metals from bowhead, skin, liver, lung and reproductive organs.

7. Complete Phase 4 of the database plan and integrate the laboratory and the *Odyssey*

We are in the process of planning ways to link the cell culture work with the rest of the Voyage database. We will then try to create real-time linkages with existing data collection systems carried by the *Odyssey*. This work will result in the first fully integrated, land-based and oceangoing laboratory that can carry out studies at both molecular and cellular levels, and also pursue field work on wild populations. While we will be seeking funding to build these systems, much work has been done. In June 2009 Ocean Alliance received a gold medal at the Computerworld honors program for their innovative use of data technology for the benefit of society.

Medium-Term

1. Develop tests for other endpoints in marine mammal cell lines

We plan to use these cell lines to develop tests that will determine more about how contaminants disrupt the endocrine system and how they affect marine mammal brain and immune-system cells.

2. Determine where the high exposures are occurring

We plan a follow-up voyage to sites where the data we have already obtained lie at the extremes of normal values, either because they are exceptionally high or exceptionally low. We will revisit sites with very high levels of chromium (e.g., Kiribati and the Seychelles). For comparison we will also visit sites with low levels (e.g, Sri Lanka). In addition, we will visit areas to which the whales may be migrating and from which metals such as chromium that are accumulating in them may be coming (e.g., Australia and Papua New Guinea). During this trip, in addition to sampling more sperm whales, we will sample

(a) other marine mammals in the area, (b) sessile species (e.g., clams, mollusks and seaweeds), (c) local air and seawater, and (d) prey species that are occur in sperm whale food chains. We will be able to monitor all of the metals from one sample which will provide insight into metal nanoparticle exposure levels. This will help us to track the paths taken by contaminants through food chains.

3. Develop better risk assessment models

We plan to master an understanding of current state-of-the-art human risk assessment as a step toward developing better risk assessment techniques for marine species. We expect to develop the cell line data as a key component of this approach. One of the tools we will use for this work is our long-standing right whale population study in Argentina, which will enable us to look at contaminant levels in known individual whales for their potential effects on behavior and reproduction.

4. Develop a priority chemical intervention list

Using the Voyage of the *Odyssey* data, the cell line toxicity work, and improved risk assessment models, we plan to develop a priority list to guide risk managers, government agencies, and conservation groups to the chemicals and metals that pose the greatest hazard in the marine environment.

5. Develop an educational and research laboratory in our new paint factory building

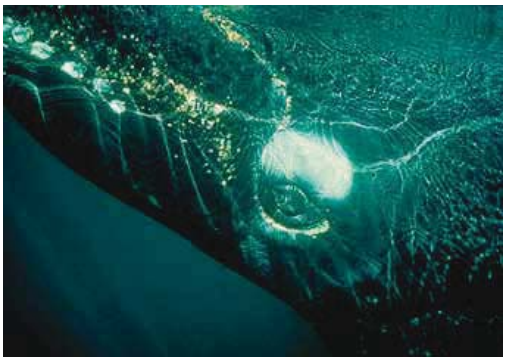
In 2008 Ocean Alliance purchased a historic paint factory in Gloucester, Massachusetts. We plan to convert these buildings into our new headquarters, which will include facilities for our our toxicology program. The buildings will also serve as an educational and outreach hub, allowing people to participate in our ongoing research work.

Long-Term

Our long-term goal is to establish pollutant monitoring sites in key areas, which will enable us to identify when and where exposures are reaching dangerous concentrations and at what rate.

Core Voyage Leadership, Scientists, and Crew

Roger Payne – *Ocean Alliance Founder and President*
Iain Kerr - *CEO / Voyage of the* Odyssey – *Expedition Leader*
John Wise – *Ocean Alliance Toxicology Program Director*
Celine Godard-Codding – *Voyage of the* Odyssey *Chief Scientist*
Genevieve Johnson – Odyssey *Education Director and Marine Coordinator*
Chris Johnson – *Voyage of the* Odyssey *Multimedia Producer/Videographer*
Josh Jones – *Marine Coordinator/First Mate*
Rebecca Clark – *Science Manager, Voyage of the* Odyssey
Kim Marshall – *Senior Director/Biologist*
Robert Wallace – Odyssey *Chief Captain/Engineer*
Rodrigo Olsen – Odyssey *Captain*



Voyage Publications

- **Journal Articles: 16**
- **Published Abstracts: 22**
- **Publications Submitted or In Preparation: 20**
- **Posters: 12**
- **Posters Presented at the Society of Marine Mammalogy 18th Biennial Conference on the Biology of Marine Mammals, October 2009, Quebec, Canada: 18**
- **Presentation/Reports: 23**

For the list of resources please see the full report. For additional reading sources please visit our website at www.oceanalliance.org or contact Iain Kerr at iaink@oceanalliance.org.

Thank You

We are greatly indebted to the individuals, foundations, organizations, and corporate sponsors that contributed with unstinting generosity and steadfast support to the success of the Voyage of the *Odyssey.*

It was they who made The Voyage of the *Odyssey* possible and their names can be found in the full report. By investing in such an ambitious research expedition they assured its success, and for that we extend our deepest thanks.

It is only with your continued support can the Voyages of the *Odyssey* continue.

Oceans Matter

WWW.OCEANALLIANCE.ORG

Please visit our website to find out more about the ongoing research and activity of the Ocean Alliance. We still have much to do. The oceans need your help.

"The environmental crisis we face provides this generation with the most singular opportunity for greatness ever offered to any generation in any civilization, ancient or modern. If we hesitate we will write our names in infamy. If we seize it, we can take our place among the stars."
– Roger Payne, PhD

"To all those that made this possible. We are eternally grateful."
– Iain Kerr

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