An investigation into a possible relationship between metals and death loss in dolphins

by

Wynne W. Landgraf

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INTRODUCTION

Much of the earth is covered with salt water. Since the oceans are extensive, they are important to the ecological health of the planet, and are a possible monitor of the general environment. One of the few indicators of oceanic health is the condition of its inhabitants; any noticeable disruption of their normal equilibrium needs to be studied and evaluated.

In the spring of 1987, Atlantic bottlenose dolphins (Tursiops truncatus) began stranding on the New Jersey and Virginia shores, with an estimated final loss of 740 animals. This phenomenon continued for 11 months; dolphins appeared on shores from South Carolina to Florida. These dolphins were part of a mid-Atlantic stock living just off-shore and seasonally migrating up and down the east coast of the United States. Because of the unprecedented nature of the stranding, an investigation was begun to determine the cause of these unusual dolphin deaths.

The dead animals were of varying sex and age, ranging from calves to mature adults. In general, they exhibited emaciation, ulcerations around the mouth, on the gingiva (Figure 1), and on the skin surface, where sloughing occurred. Some had barnacles attached to their skin, which is a sign of lethargy. A few affected dolphins were live-captured and reported to be "non-robust", a term indicating lethargy, inadequate blubber accumulation, and general ill-health (Saari et al., 1987). Several dolphins died during capture (Figure 2), and they showed the same lesions as those which had washed up ashore.

Necropsies performed on 97 dolphins (both dead animals and those that were live-caught and subsequently died) showed a variety of microscopic changes (Geraci, 1989, Saari et al., 1987). Sixty-seven had liver involvement, ranging from fatty degeneration, fibrosis, hyperplasia, and periportal degeneration to cirrhosis and necrosis (Figures 3 and 4). Fifty-two had ulcer-like skin and mouth lesions, apparently caused by bacteria-congested thrombi in the blood vessels. This finding was "one manifestation of systemic bacterial invasion which seems to have been the ultimate cause of death of many of the dolphins" (Geraci, 1989). The skin of a dolphin has less collateral circulation than the skin of many land mammals, and thrombi of major vessels in any area lead to ischemia, tissue damage, and eventual ulceration (Saari et al., 1987). Many of the labial and gingival lesions contained protozoa, thought to be secondary invaders (Saari et al., 1987).

Several dolphins had hemorrhages and vascular degeneration with bacteria-filled thrombi in vessels of the brain. Deep muscle hemorrhages were also found. The lymph nodes from 18 of 62 dolphins showed lymphadenitis, and 38 of 62 animals had lymphoid depletion of follicles in the intestine, spleen, and lymph nodes (Figure 5). These follicles were characterized by hyaline centers and a noticeable lack of lymphocytes (Geraci, 1989). The dolphins also evidenced a probable endotoxemia from the breakdown of dead bacteria and subsequent release of toxic cell metabolites. These toxins caused hemorrhages which compromised the integrity of the vascular system. There was a marked

coagulopathy and bacteria-containing thrombi in arteries, veins, and lymphatics (Saari et al., 1987).

Bacteriologic cultures performed on 48 dolphins indicated invasion by 21 bacterial species in 10 different tissues (liver, spleen, lung, lymph node, blood, urine, blubber, abdominal fluid, kidney, and brain). Fifty-two percent of the total isolates were Vibrio sp. (the most prevalent isolate was V. parahaemolyticus), but Edwardsiella sp., Alteromonas sp., Pseudomonas putrefaciens, Enterobacter cloacae, Acinetobacter lwoffi, Streptococcus sp., Staphylococcus sp., Proteus sp., Escherichia coli, and Morganella morganii were also isolated (Geraci, 1989).

Livers from 26 affected dolphins were analyzed for marine toxins by mouse bioassay and high performance liquid chromatographic confirmation. Eight of 26 livers were positive for brevetoxin, a lipid soluble polyether toxin produced by an unarmored dinoflagellate, Ptychodiscus brevis. This organism is responsible for red tide, and secretes a powerful neurotoxin that acts at the voltage-dependent sodium channels on excitable membranes. No other biotoxins were isolated (Geraci, 1989).

Figure 1. Gingival ulceration in the mouth of a dolphin that washed up ashore

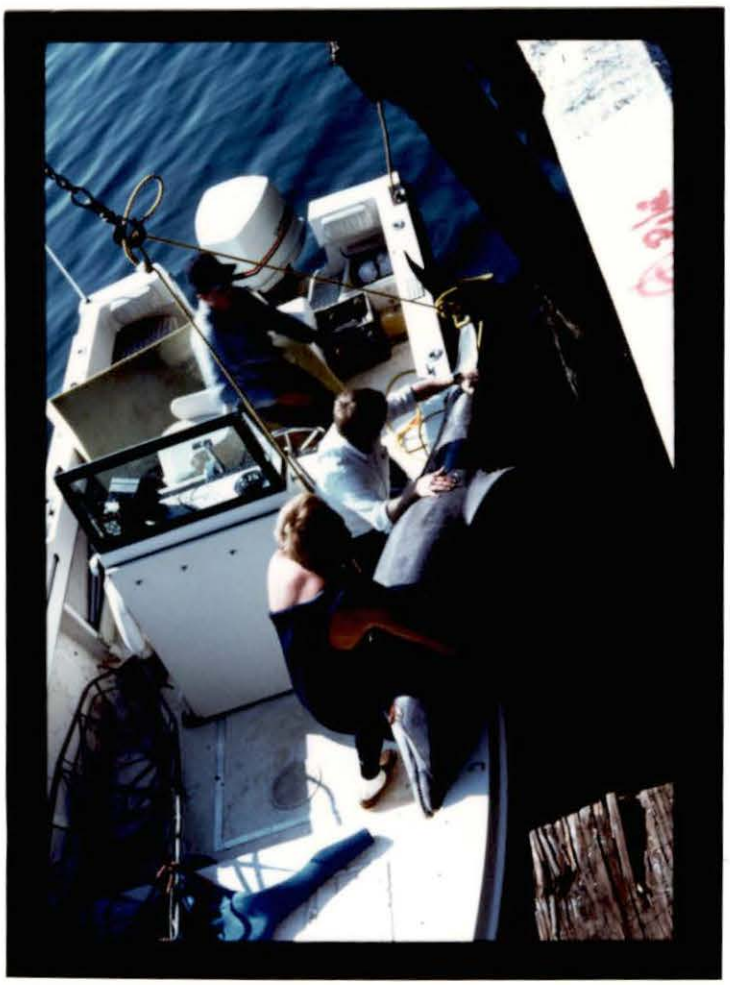

Figure 2. Live-captured dolphin off the shore of Virginia. Dolphin subsequently died from ill-health and the stress of being captured

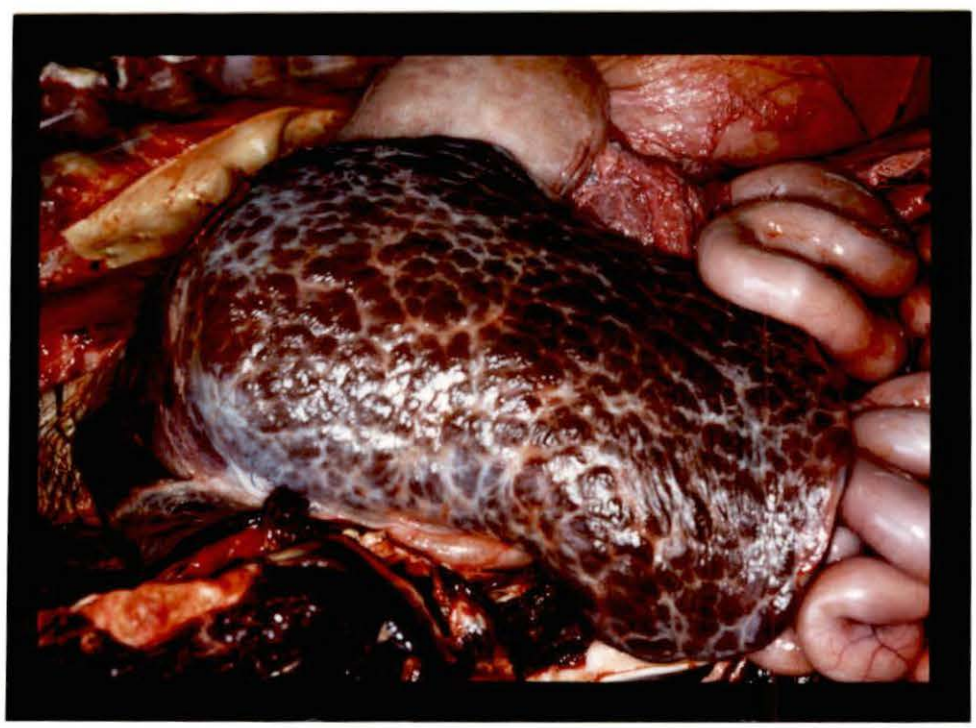

Figure 3. Hepatic cirrhosis in a liver of a dolphin that stranded on the Virginia shore. Note lobular appearance of surface

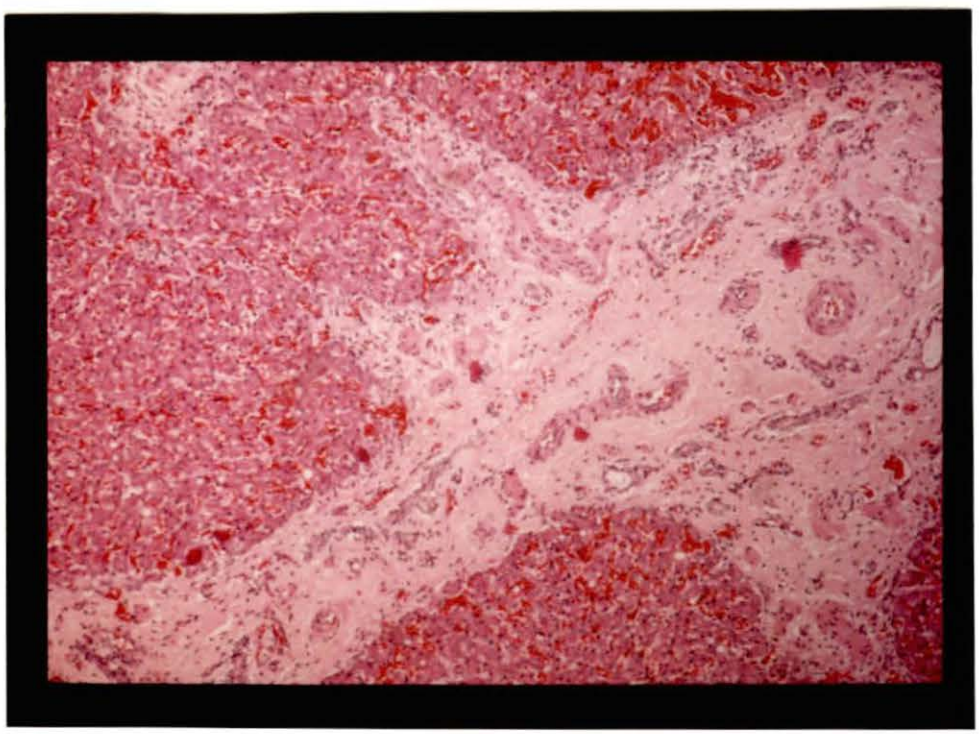

Figure 4. Liver with periportal fibrosis (whitish area indicates normal tissue replacement by fibrous tissue). Hematoxylin-eosin stain (250x)

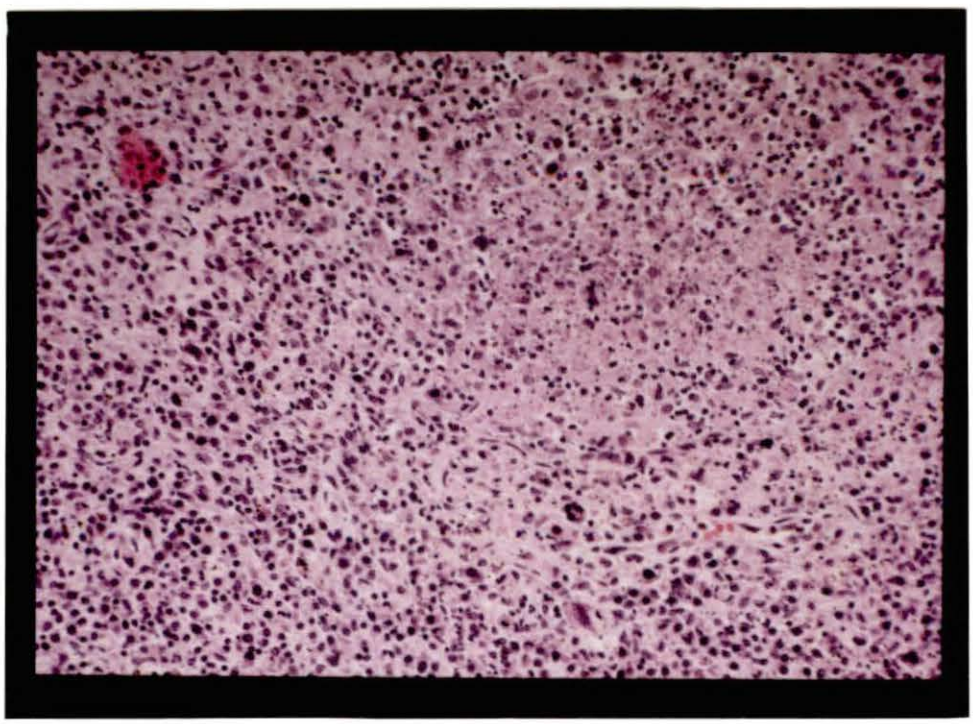

Figure 5. Lymph node with depletion. Entire area shows loss of lymphocytes. Hematoxylin-eosin stain (250x)

HYPOTHESIS

It has been hypothesized that the dolphins succumbed to a syndrome beginning with sublethal brevetoxin ingestion through the food chain and ending with death due to invasion by bacterial species normally found in the dolphin environment (Geraci, 1989). Since the literature supports connections between certain metals and the functioning of the immune system, this is an investigation into the possibility that the dolphins were victims of a change in normal elemental concentrations that could favor increased susceptibility to brevetoxin and bacterial invasion.

LITERATURE

Cetacean Metal Concentrations

Tissue metal concentrations have been determined in a variety of cetaceans that were stranded, drowned, ice-entrapped, or live-captured. Data are primarily for the striped or blue-white dolphin (Stenella coeruleoalba) and the harbor popoise (Phocoena phocoena), with a few values for Risso dolphin (Grampus griseus), Gill's or Pacific bottlenose dolphin (Tursiops gilli), long snouted dolphin (Stenella longirostris), white dotted dolphin (Stenella attenuata), white beaked dolphin (Lagenorhynchus albirostris), and a finless black porpoise (Neophocaena phocaenoides) fetus. Most studies included levels of lead (Pb), mercury (Hg), cadmium (Cd), and selenium (Se); a few determined concentrations of zinc (Zn), copper (Cu), iron (Fe), nickel (Ni), and manganese (Mn). Literature values and data are tabulated in the Appendix.

Copper levels in liver, kidney, and muscle of the white beaked dolphin, striped dolphin and the harbor porpoise are similar, with kidney values ranging from 1.2 to 6.05 ug/g for 81 animals. Muscle values among genera are also close, ranging from 1.1 to 4.4 ug/g for 90 dolphins. Liver Cu values vary slightly more, ranging from 0.97 to 40.3 ug/g for 114 cetaceans; most values fell between 2.6 and 15.2 ug/g. No explanation was given for the very low value of 0.97 ug/g in a white beaked dolphin, but the high value of 40.3 ug/g may be explained by the fact that this animal was a fetus, and Cu concentrations are known to decrease with age (Muir et al., 1988).

Concentrations of Zn reported for striped dolphins, harbor porpoises, and white beaked dolphins follow the same trend as the Cu; muscle and kidney values are close among genera, with a larger range for liver values. There are few differences among genera for kidney (8.3 to 41.2 ug/g) and muscle (6.86 to 24.5 ug/g) levels for 104 and 153 animals respectively, but Zn values for liver ranged from 12 to 109 ug/g for 148 cetaceans. Zinc is an inducer of metallothionein (MT), which binds and detoxifies the Cd accumulated in striped dolphins' primary diet of squid. The elevated Zn may be a compensatory increase to induce sufficient MT for enhanced Cd binding (Honda et al., 1983).

Values for Pb in white beaked dolphins, striped dolphins, and harbor porpoises in kidney (60 animals), liver (94 animals), and muscle (96 animals) were consistent among genera with the exception of the harbor porpoise. These cetaceans contained at least 10 times the Pb in the liver and muscle (but not the kidney) of the white beaked and striped dolphins. Striped and white beaked dolphins live off-shore in deep water, while many harbor porpoises spend their lives near shore in areas more likely to be polluted. Although soft tissue is not the storage tissue of choice for Pb, Muir et al. (1988) proposed that the higher value in muscle of the harbor porpoises (4.7 ug/g) may indicate long-term exposure, while the high liver level (5.3 ug/g) reflects short-term or continuous exposure.

Cadmium concentrations in white beaked dolphins, striped dolphins and harbor porpoises also reflect the animals' diets. Concentrations for white beaked dolphins (26 animals) and harbor porpoises (25 animals)

ranged from <0.05 to 2.3 ug/g in liver, 0.077 to 9.5 ug/g in kidney, and 0.002 to 0.07 ug/g in muscle. Striped dolphins, however, have liver Cd levels ranging from <0.005 to 11.1 ug/g. According to Honda et al. (1983), this elevated Cd indicates continuous exposure through a squid diet. Kidney levels are seven times higher (0.06 to 69.9 ug/g) in striped dolphins than in other genera that do not feed on squid. This accumulation of Cd in the kidney is due to the excretion of the Cd-MT complex (Honda et al., 1982).

Literature Hg concentrations are reported higher in mature adult tissues than in fetuses (Muir et al., 1988, Itano et al., 1984c), reflecting domain and diet (Muir et al., 1988, Itano et al., 1984a, Zonfrillo et al., 1987, Viale, 1978). Some harbor porpoises live in more polluted areas and accumulate Hg in the liver at 15 to 100 ug/g, while the Risso dolphin and long snouted dolphin live deeper and farther from pollution and have lower liver Hg (1.16 to 12 ug/g) (Itano et al., 1984b).

Values for Hg and Se in Risso dolphin, white beaked dolphin, striped dolphin, harbor porpoise, blue-white dolphin, white dotted dolphin, finless black porpoise, and Gill's bottlenose dolphin reaffirm the positive correlation between these two metals found in the literature (Koeman et al., 1973). A pattern is also seen in liver, kidney, and muscle values for mature, immature, and fetal cetaceans. In adults, liver, kidney, and muscle Hg levels are 3 to 5 times the Se levels, while liver, muscle, and kidney Hg and Se levels are approximately equal in immature and fetal cetaceans. Most of this work has been done in striped

dolphins, but the same pattern occurs in white beaked and blue-white dolphins.

Role of Metals in Immunocompetency

Metals and metalloids have been implicated in alteration of the functioning of the immune system. This alteration may take the form of a suppression of the humoral system, suppression of cellular immunity, a disruption of both forms of immunity, or an enhancement of immunocompetency.

Among the heavy metals, Cd is one of the most thoroughly studied inhibitors of the immune system. Koeller (1973) demonstrated that 300 ug/g Cd in the drinking water of rabbits caused a 4-fold decrease in antibody (ab) titers to pseudorabies virus; Pribyl and Treagan (1977) showed that Cd caused lowered interferon synthesis in cell culture, which increased susceptibility to viruses. Both Krzystyniak et al. (1987) and Graham et al. (1978) exposed mice to Cd by aerosol and found fewer ab-producing cells because of Cd toxicity to splenic lymphocytes. Similar results occurred using cells in vitro (Lawrence, 1981). Cadmium also caused a marked increase in susceptibility to several bacterial endotoxins in the rat (Cook et al., 1975) and an increase in susceptibility in mice to encephalomyocarditis (EMC) virus (Gainer, 1977). Cadmium also impaired phagocytic activity by several proposed mechanisms. Waters et al. (1975) theorized that Cd reduced acid phosphatase activity, thereby impairing phagocytosis. Levy et al. (1986) concluded that decreased phagocytosis in mouse macrophages was due to

decreased internalization of particles. This loss could be explained by Cd interference with the lymphokine that signals for internalization. Increased susceptibility to Salmonella sp. was produced by injection of 10 ug CdO/mouse, resulting in 63% greater mortality than the controls (Hatch et al., 1985). Finally, Mills and Dalgarno (1972) produced lowered plasma and liver Zn levels in lambs exposed to 12.3 ug/g Cd in utero. This was of concern because Zn is essential to immunocompetence.

Immunosuppression by Ni has been extensively studied. A 500 ug/m^3 aerosol of Ni increased susceptibility of mice to Streptococcus pyogenes (Adkins et al., 1979). Nickel also interfered with macrophages by reducing interferon synthesis and ab production against EMC virus, causing 30% more mortality in nickel-fed mice than in controls (Gainer, 1977). Waters et al. (1975) found that Ni also reduced acid phosphatase activity by 50%, and Pribyl and Treagan (1977) illustrated a 5-fold loss in viral protection from Ni interference in interferon synthesis. Mice given 0.98 ug/g Ni by aerosol had decreases in ab producing cells in the spleen, potentially decreasing bacterial and viral protection (Graham et al., 1978). Work with salmon and carp demonstrated that Ni in water suppressed immune clearance of phage particles (O'Neill, 1981).

Lead has been shown to be immunosuppressive. Five mg of Pb increased susceptibility of rats, mice, baboons, and chicks to several bacterial endotoxins by 100,000-fold (Cook et al., 1975). Lead acetate in drinking water (0.01M) increased murine susceptibility 70% to EMC virus by reducing both ab and interferon synthesis (Gainer, 1977). Lead

at 2500 ug/g lowered serum ab to pseudorabies (PRV) 10-fold in rabbits by interfering with phagocytosis and ab binding (Koeller, 1973).

Immunocompetence has also been affected by inorganic Hg compounds. Toyama and Kolmer (1918) employed mercuric chloride intravenously in rabbits to show a loss of anti-human hemolysin production. Mercuric oxide (0.10 ug) injected in mice increased their susceptibility to Salmonella sp. by 30% (Hatch et al., 1985). Ten ug/g mercuric chloride in drinking water of rabbits caused a 3-fold decrease in serum ab to PRV (Koeller, 1973). Lawrence (1981) found that mercuric chloride not only inhibited ab production, but also the development of the plaque-forming cell response. Gainer (1977) found significantly increased susceptibility to EMC virus with 0.44 - 44.0 ug/g mercuric chloride in drinking water of mice. This author theorized that the effect of Hg was due to binding of Hg with the sulfhydryl groups on the macrophage, causing death.

Metalloids also have been implicated in loss of immunocompetence. Toyama and Kolmer (1918) studied the effect of arsphenamine on rabbits. They concluded that large doses of this arsenical (0.006 g/kg) reduced resistance to typhoid bacilli by reducing both ab and complement production. In contrast, they discovered that small doses of the same compound stimulated ab and complement production. Thus, arsenic (As) may be an element that both promotes and depresses immunocompetence, depending on the dosage. Later work with As supported the data of Toyama and Kolmer. In 1975, Nielsen et al. demonstrated that As is an essential element for health. Rats receiving only 30 ng/g As (as sodium arsenite

and sodium arsenate) had rough hair coats, low growth rates and black, swollen spleens with unusual architecture. Hatch et al. (1985) showed that As was immunosuppressive by injecting 10 ug of sodium arsenite into mice and observing a 73% increase in susceptibility to Salmonella sp.. In other trials in mice, Gainer and Pry (1972) used sodium arsenate, sodium arsenite, p-arsanilic acid as trioxide and 4-hydroxy-3-nitro-benzarsonic acid in drinking water in combination with PRV, EMC, St. Louis encephalitis, and Western equine encephalitis viruses. These authors demonstrated a 23-60% increase in deaths of treated mice over controls with all compounds and all viruses except Western equine encephalitis. Using this data, Gainer and Pry (1972) proposed that As decreased interferon production and therefore increased viral infectivity.

Waters et al. (1975) found a 50% reduction in macrophage phagocytic activity with 250 ug/g Mn in rabbits; Hahon and Booth (1984) concluded that Mn in vitro depressed formation of interferon in response to infection by influenza virus. Dietary Mn (6000 ug/g) in lambs decreased liver Zn by 14% and liver Fe by 34%; Fe deficiency increased bacterial susceptibility (Watson et al., 1973). Increased Mn also inhibited ab-producing cells both in vitro (Lawrence, 1981) and in vivo (Srisuchart et al., 1987). Srisuchart et al. (1987) theorized that suppression may be due either to inhibition of protein synthesis by Mn, or Mn interference with rapid calcium uptake necessary for viable plasma cells.

Work with tin (Sn) compounds showed their adverse effects on the immune system. In 1979, Seinen and Pennicks fed 150 ug/g dialkyl and

tributyl tin compounds to mice. They discovered that organic Sn compounds were reversibly cytotoxic to lymphoid tissues by inhibiting division of thymic lymphocytes, thereby depressing cell-mediated immunity and T-cell mitogen response. Sensitivity to E. coli endotoxins was increased 25-fold by Sn, and the authors stated that Sn acted by interfering with the energy metabolism of thymic lymphocytes through the tri-carboxylic-acid cycle. Sn also has been implicated in the inhibition of in vitro ab production (Lawrence, 1981).

Limited work has been done with other metals and metalloids. Vanadium (V) at 10 ug/g reduced phagocytosis 90% by reducing acid phosphatase specific activity (Waters et al., 1975). Copper suppressed the immune response in C. carpio (O'Neill, 1981). Cobalt inhibited ab production in vitro (Lawrence, 1981), and Se caused leukopenia in cynomolgus monkeys (Loew et al., 1975). Chromium (Cr) (330 ug/g) reduced phagocytosis by rabbit alveolar macrophages (Waters et al., 1975), depressed interferon production in cell monolayers infected with influenza virus (Hahon and Booth, 1984), and inhibited ab production in vitro (Lawrence, 1981). Finally, thallium and scandium both have been shown to increase susceptibility to bacterial endotoxins (Cook et al., 1975).

Certain metals are necessary to a well-functioning immune system, although high levels of these elements can damage immunocompetence. Zinc deficiencies in cattle caused severe immunodeficiencies because the thymus did not develop. Low Zn in rats caused atrophy of lymphoid tissues and a loss of ab production. Zinc functions as an essential

element in the metalloenzymes involved in protein and ribonucleic acid synthesis (Fernandes et al., 1979). However, Zn toxicosis interferes with macrophage mobility (Fernandes et al., 1979) and decreases ab production in vitro (Lawrence, 1981).

Calcium (Ca) is also essential to the immune system. It is required for T-cell stimulation (Srisuchart et al., 1987), but excess dietary Ca will cause a deficiency of Zn and Fe by interfering with absorption where intake of these elements is marginal (Davis, 1959). Iron is a necessary element for a functioning immune system. Rats fed a diet deficient in Fe (15 ug/g) were more susceptible to infection by Salmonella typhimurium (Baggs and Miller, 1973). Baggs and Miller (1973) therefore concluded that low Fe caused a drop in production of myeloperoxidase, an enzyme which functions in lysosomal bacteriocide by increasing bacterial susceptibility.

MATERIALS AND METHODS

Tissue Collection

Whenever stranded dolphins were discovered on the beach, the Stranding Center in the area was notified, a truck was dispatched, and the entire carcasses were retrieved. Dolphins were necropsied immediately upon arrival at the Center by personnel from the United States Department of Agriculture, the University of Guelph, and the Smithsonian. The skull and a tooth were collected from each animal by a member of the Smithsonian team for aging calculations. Dolphins were measured and sexed and an estimated age was assigned; specimens were collected for bacteriology, virology, histopathology, bioassays, and toxicology. The specimens for toxicology were bagged, frozen immediately upon collection, and delivered on dry ice to the Pathobiology Laboratory of the National Veterinary Services Laboratories (NVSL) for analyses.

Sample Preparation

In general, samples were received frozen at the laboratory and kept frozen (0° C) until sample preparation. All tissue samples were ground in toto in a blender. One gram of blended liver, kidney, or muscle was weighed into a 15 ml teflon screw cap vial[1]. Five ml of high purity

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[1]Thomas Scientific, Swedesboro, New Jersey.

nitric acid[2] were added, and the vials were placed in a muffle oven at 60°C for overnight digestion. After cooling, the digestate was poured into a 2N HCl-rinsed 100 ml volumetric flask. Scandium at 5 ug/ml was added to each flask to function as an internal standard, and the volume was adjusted to 100 ml with Super Q water[3].

National Board of Standards Standard Reference Materials[4] (SRM) bovine liver 1577a, oyster tissue 1566, and a normal bovine liver with added Pb (25 ug/g) and Hg (10 ug/g) were run with each batch of ten samples. SRM 1577a has guaranteed values for Ca (120±7 ug/g), Mn (9.9±0.9 ug/g), Cu (158±7 ug/g), Zn (123±8 ug/g) and Fe (194±20 ug/g); SRM 1566 contains a guaranteed amount of As (13.4±1.9 ug/g), Cd (3.5 ± 0.4 ug/g), and V (2.8 ug/g). The control sample for Hg and Pb was prepared similarly to the samples; SRM materials are lyophilized and only 0.25 gram quantities were used.

For Se analyses, one gram of blended liver, kidney, or muscle, one gram of SRM 1577a (with a guaranteed Se value of 0.71±0.07 ug/g), and standards containing 0.01, 0.1 and 0.2 ug/ul Se were heated overnight at 90°C with 10 ml of concentrated nitric acid and 4 grams of magnesium nitrate. The dried samples and standards were placed in a muffle oven preheated to 500°C and ashed for 30 minutes. After cooling, samples and

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[2]J. T. Baker, Baker Instra-Analyzed, Phillipsburg, New Jersey.

[3]Millipore, Bedford, Massachusetts.

[4]National Board of Standards, Washington, D.C..

standards were redissolved in 8 ml concentrated hydrochloric acid on a 90°C hot plate for 15 minutes. Ten ml of 20% urea in water were added directly to the hot samples and standards and allowed to cool. Four ml of 1,2-diamino-4-nitrobenzene[5], 5 ml Super Q water and 5 ml toluene were added to each sample and standard and then shaken. After separation of the aqueous and toluene phases, the toluene layer was removed for analysis by gas chromatograph.

Standard Conditions

Quantitation of all elements except Se was conducted on a Model 6500 Inductively Coupled Argon Plasma Emission Spectrometer[6] (ICP) equipped with a Czerny-Turner 409 mm focal length monochrometer with holographic grating (UV, 2880 lines/mm and visible, 1440 lines/mm). The ICP was also equipped with a U-5000 Ultrasonic Nebulizer[7]. The optics were purged with argon; argon was also used as the plasma, auxiliary, and nebulizer gas. The plasma gas flow rate was 16 standard liters per minute (SLPM), nebulizer flow rate was 1.0 SLPM, and the auxiliary flow rate was 0.8 SLPM. Samples were introduced into the system by a peristaltic pump and the ultrasonic nebulizer at a rate of 3.0 ml/minute. Standard emission lines used were:

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[5]Sigma Diagnostics, St. Louis, Missouri.

[6]Perkin Elmer, Norwalk, Connecticut.

[7]CETAC Technologies, Inc., Omaha, Nebraska.

Copper	324.754 nm	Cobalt	228.610 nm
Cadmium	214.438 nm	Calcium	317.933 nm
Iron	259.940 nm	Tin	189.989 nm
Chromium	267.716 nm	Zinc	213.856 nm
Lead	220.353 nm	Manganese	257.610 nm
Mercury	194.227 nm	Arsenic	189.043 nm
Vanadium	292.402 nm	Nickel	231.604 nm

Selenium values were obtained by gas-liquid chromatographic analysis (GLC) on a Hewlett-Packard 5840 gas chromatograph[8] equipped with a packed column[9] (OV17, QF1) and an electron capture detector. The ICP was not used for Se analyses because of spectral interferences and a lack of sensitivity.

All standards were prepared directly from 1000 ug/ul stock standards with the exception of the Hg, which was prepared from a 1000 ug/ul stock standard and digested in a manner similar to the samples. Standards for ICP were diluted to a final volume in high purity nitric acid (5%) after the addition of 5 ug/ul scandium with the exception of Hg, which was diluted in Super Q water. The standards for Se analysis were diluted from stock to 100 ug/g in Super Q water and further diluted daily. Standard concentrations were as follows:

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[8]Hewlett-Packard, Avondale, Pennsylvania.

[9]Supelco, Bellefonte, Pennsylvania.

Calcium	5.0 ug/ul	Iron	2.0 ug/ul
Zinc	1.0 ug/ul	Manganese	0.1 ug/ul
Cobalt	0.1 ug/ul	Cadmium	0.1 ug/ul
Tin	0.1 ug/ul	Chromium	0.13 ug/ul
Lead	0.5 ug/ul	Nickel	1.0 ug/ul
Mercury	5.0 ug/ul	Vanadium	0.1 ug/ul
Arsenic	0.2 ug/ul	Copper	2.0 ug/ul
Selenium	0.02 ug/ul, 0.2 ug/ul, 0.4 ug/ul		

A blank of 5% high purity nitric acid was analyzed with each batch of 10 samples. Samples, standards, and blanks were all analyzed in duplicate on the ICP, singularly by GLC.

Operation and Principles of ICP

The ICP operating unit consists of a quartz torch, an optical system, a radiofrequency (RF) generator, and a computer-aided data analysis package (Figure 6). The operating principle of the ICP states that a radiofrequency of 1250 watts produced by the generator moves through a coil surrounding the torch. The RF generates an oscillating magnetic field which accelerates the electrons in the argon passing through the center of the torch. These electrons encounter resistance, and heating and ionization of the argon occurs. A plasma of 10,000° K is formed; this plasma is hollow, with a center temperature of 7000° K. The injected sample stream moves through the center of the plasma, where the temperature is high enough to result in atomization and free ion formation. These free ions exist in an inert environment and radiate energy at a wavelength specific for each element. The ion emission is received by the optic system (Figure 7), and data are relayed to the

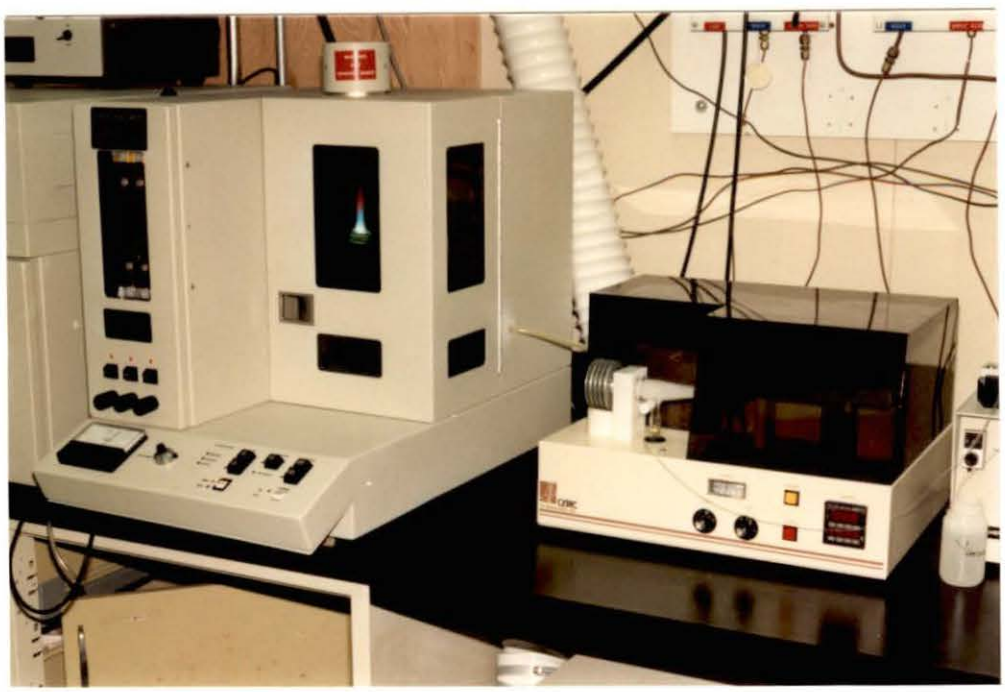


Figure 6. The Inductively Coupled Argon Plasma Emission Spectrograph (ICP) with ultrasonic nebulizer. A yttrium standard being aspirated into the torch demonstrates the different temperature zones (white - 10,000° K, blue - 7,000° K, and red - gases)

Figure 7. Schematic showing the operation of the ICP Optical System

computer, which optimizes by selection of the most intense of five points on either side of the specific wavelength (Fassel and Kniseley, 1974a).

The ICP may be operated in either of two modes, sequential or graphics. The sequential mode utilizes the scandium standard to eliminate drift, and the elements chosen by the operator are analyzed in sequence according to their wavelength. The graphics mode analyzes individual elements and does not use the internal scandium standard. This mode allows the operator to visualize elemental spectra, calibrate the wavelengths, and set background limits (Figure 8). This mode is especially useful when only one elemental analysis is required, when dilution of a sample is necessary for analysis, when there are obvious background interferences in the sequential mode, or when it is necessary to verify the existence of an unexpected element in a sample. Alternate or secondary wavelengths may be used to verify results. Both modes were used for elemental analyses.

Sample Analysis

All readings in the sequential mode were done in duplicate, the results averaged, and standard deviations and coefficients of variation were calculated.

All individual elements were quantitated against known standards. Except for Se, single standards were run for instrument calibration because standard curves from the ICP are linear over a concentration range of 4 - 5 orders of magnitude (Fassel and Kniseley, 1974b). Three standards were run for each group of Se samples to insure GLC linearity;

comparison of samples, SRM and standards was done by measuring and graphing peak heights.

Calculations and Statistics

Calculations of metals concentrations from the ICP for kidney, liver, muscle, spike, and SRM were performed according to the following formula:

reading from computer (both sequential and graphics) x 100 (dilution) x added dilution if necessary ÷ weight in grams

Calculations of Se concentrations from the GLC for kidney, liver, muscle, and SRM were performed according to the following formula:

$$\frac{\text{peak height sample}}{\text{peak height standard}} \times \frac{\text{ng standard}}{\text{ul sample}} \times \frac{5\text{ ml}}{\text{weight}} \times \text{added dilution if needed}$$

Statistically, the means for the affected dolphins versus the NVSL normal means were compared by the F-test from the Analysis of Variance for a Completely Randomized Design. An overall weighted mean was calculated from the literature values and compared to the affected mean by a one sample t-test.

Correlations between metal concentrations in liver for both affected and NVSL normal dolphins were performed using the following formula:

$$\epsilon(y_1 - \bar{y}_1)\ (y_2 - \bar{y}_2) \div (n - 1)\ s_1 s_2$$

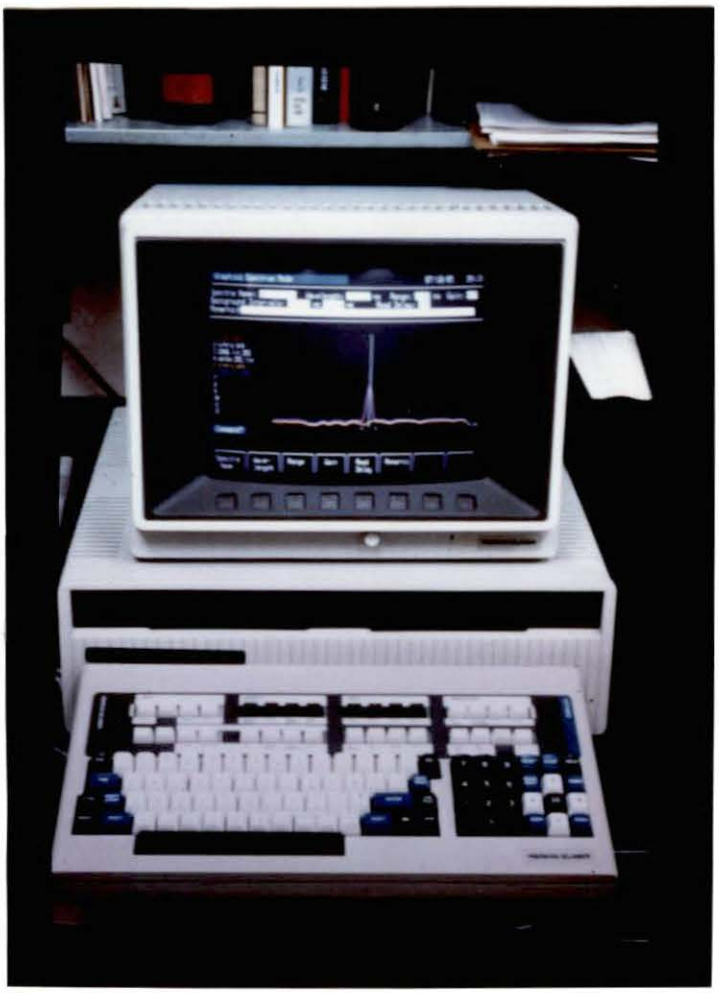


Figure 8. Computer in graphics mode: calcium standard (pink), and nitric acid blank (yellow). Visualization of the spectrum allows the operator to determine interferences with the sample

RESULTS

Analyses of the metals data indicate some differences between the literature values, NVSL normals (dolphin tissues analyzed at the National Veterinary Services Laboratories that were not part of the die-off), and the values for the affected dolphins (those animals from the East coast die-off). Only significant differences will be discussed (for complete data, see Appendix).

In liver tissue, the means for literature values and affected dolphin values differ significantly for 7 of 15 metals. The affected dolphin means for Zn and Fe were significantly higher ($p \leq 0.0001$) than the literature means. Manganese and Ni affected means were also larger than literature values, but the p values ($p \leq 0.05$) indicated a lower probability of significance than the p value for Fe and Zn ($p \leq 0.0001$). Affected means were significantly lower than literature means for Cd ($p = 0$), mature dolphin Hg ($p = 0$), and mature dolphin Se ($p < 0.00001$). See Table 1 and Figure 9 for a statistical summary of evaluation.

Kidney concentrations for eight elements (Table 2 and Figure 10) differed significantly between literature and affected dolphin means. Affected means were higher than literature means for Cu ($p = 0.00001$) and immature dolphin Hg ($p = 0.0042$). Affected values were lower than literature values for Zn ($p = 0.002$), Fe ($p = 0.0001$), Mn ($p = 0.0014$), Cd ($p = 0$), Pb ($p = 0.0011$), and immature Se means ($p = 0$).

Comparison of muscle tissue means for literature and affected dolphins (see Table 3 and Figure 11) showed the largest number of

differences. Affected dolphin means were significantly higher than literature means for Zn and immature dolphin Hg, but significantly lower than literature means for Fe, Cd, Mn, Pb, immature and mature dolphin Hg and Se.

Analysis of data from the NVSL normals (liver was the only tissue available) showed that only the affected mean was higher than the normal dolphin mean for Hg ($p = 0.0043$) (Table 4 and Figure 12). Mercury values in this case for affected dolphins include both mature and immature animals since ages were not indicated for the NVSL normals.

Correlations between metals of NVSL normals and affected dolphin livers were also calculated. Twelve significant correlations were found between metals in affected livers only (Table 6), and five correlations were significant in the NVSL normal livers (Table 5). Only one correlation, mercury-selenium was seen in both affected and NVSL normal tissues (Table 7).

Figure 9. Significant differences ($p < 0.05$) between Literature and Affected means for liver (individual p values listed on each graph)

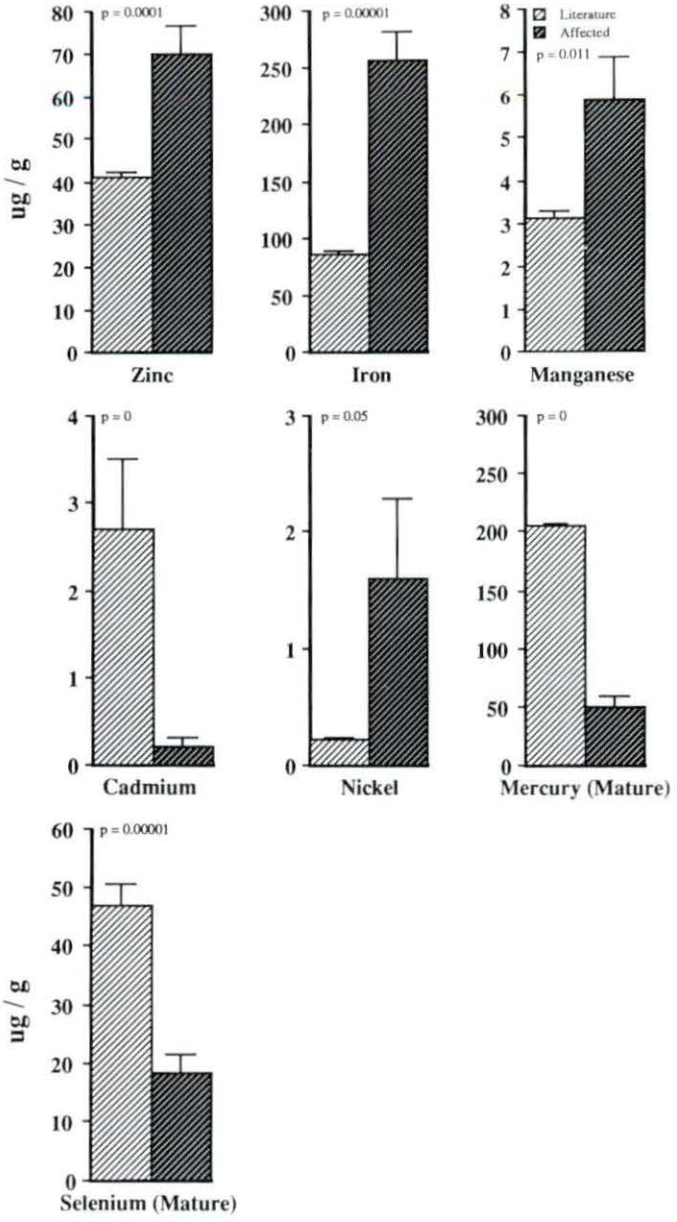

ug / g
p = 0.0001
Zinc
p = 0.00001
Iron
Literature
Affected
p = 0.011
Manganese
p = 0
Cadmium
p = 0.05
Nickel
p = 0
Mercury (Mature)
p = 0.00001
Selenium (Mature)

Figure 10. Significant differences ($p < 0.05$) between Literature and Affected means for kidney (individual p values listed on each graph)

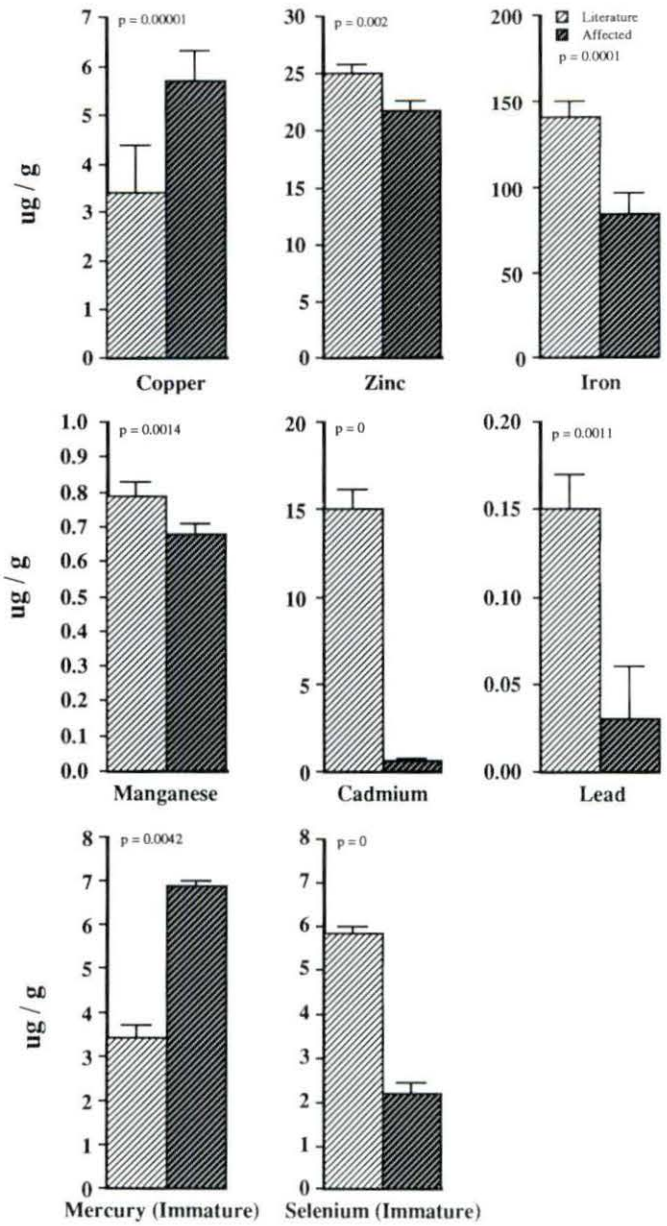
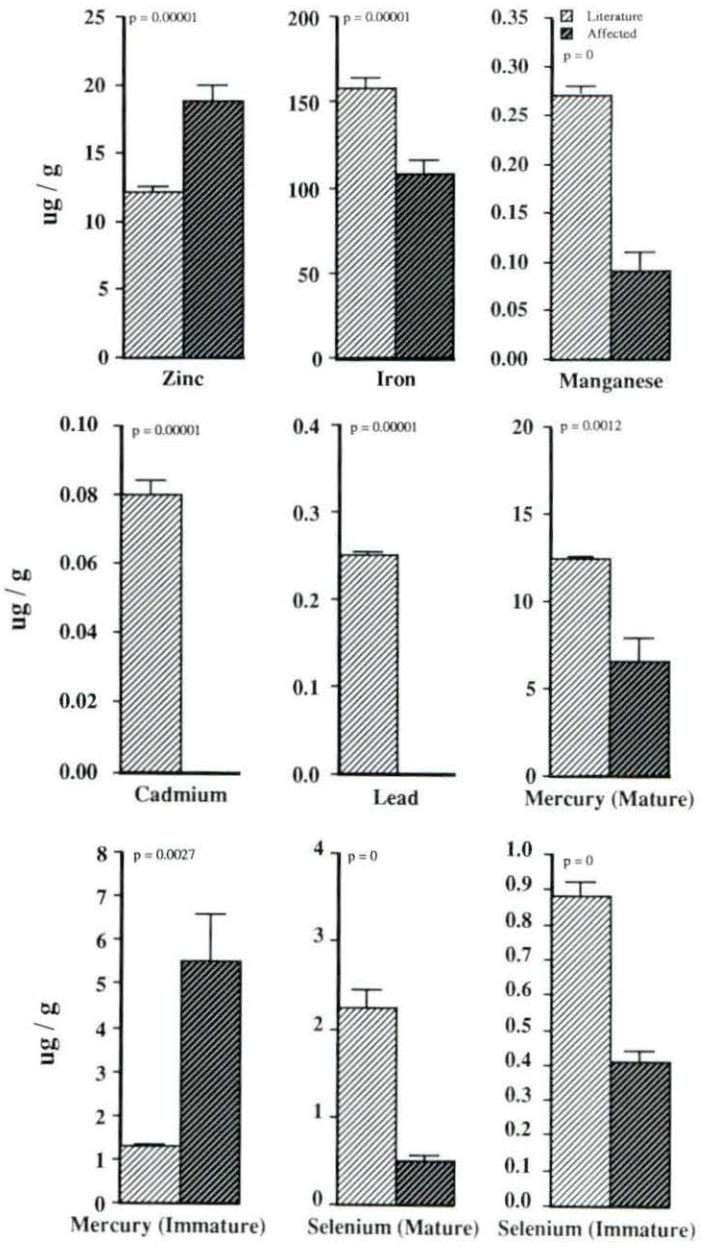

ug / g
p = 0.00001
Copper
p = 0.002
Zinc
Literature
Affected
p = 0.0001
Iron
p = 0.0014
Manganese
p = 0
Cadmium
p = 0.0011
Lead
p = 0.0042
Mercury (Immature)
p = 0
Selenium (Immature)

Figure 11. Significant differences ($p < 0.05$) between Literature and Affected means for muscle (individual p values listed on each graph)


Literature
Affected
ug / g
p = 0.00001
Zinc
p = 0.00001
Iron
p = 0
Manganese
p = 0.00001
Cadmium
p = 0.00001
Lead
p = 0.0012
Mercury (Mature)
p = 0.0027
Mercury (Immature)
p = 0
Selenium (Mature)
p = 0
Selenium (Immature)

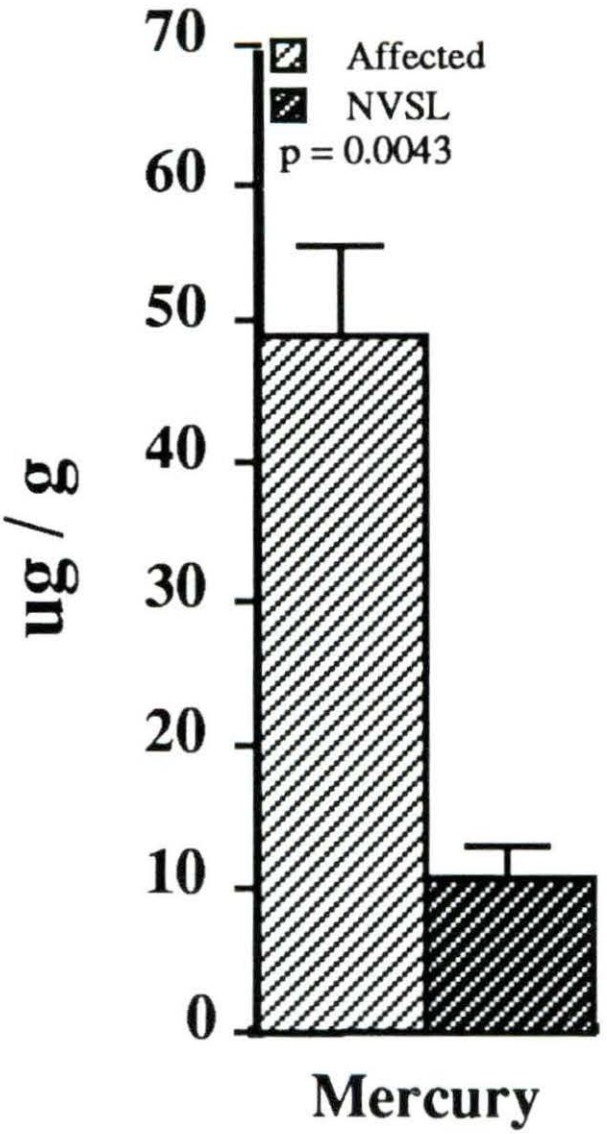


Figure 12. Significant difference between NVSL Normal and Affected mean for liver (p value listed on graph)

Table 1. Literature values vs. affected values: means, standard errors and p values for comparisons of significant difference in liver tissue

	MEAN	STANDARD ERROR	p VALUE
Zinc			
Affected	70	6.4	0.0001
Literature	41	1.3	
Iron			
Affected	256	2.5	0.00001
Literature	86	2.3	
Manganese			
Affected	5.9	1.0	0.011
Literature	3.1	0.17	
Cadmium			
Affected	0.21	0.10	0
Literature	2.7	0.80	
Nickel			
Affected	1.6	0.70	0.05
Literature	0.22	0.14	
Mercury MATURE			
Affected	49	10.8	0
Literature	205	0.08	
Selenium MATURE			
Affected	18.2	3.2	0.00001
Literature	47	3.8	

All means expressed as ug/g.

Table 2. Literature values vs. affected values: means, standard errors and p values for comparisons of significant difference in kidney tissue

	MEAN	STANDARD ERROR	p VALUE
Copper			
Affected	5.7	0.62	0.00001
Literature	3.4	0.84	
Zinc			
Affected	21.7	0.96	0.002
Literature	25	0.83	
Iron			
Affected	84	11.9	0.0001
Literature	141	9.7	
Manganese			
Affected	0.68	0.03	0.0014
Literature	0.79	0.04	
Cadmium			
Affected	0.58	0.16	0
Literature	15	1.2	
Lead			
Affected	0.03	0.03	0.0011
Literature	0.15	0.02	
Mercury			
IMMATURE			
Affected	6.9	0.92	0.0042
Literature	3.4	0.27	
Selenium			
IMMATURE			
Affected	2.16	0.23	0
Literature	5.8	0.18	

All means expressed as ug/g.

Table 3. Literature values vs. affected values: means, standard errors and p values for comparisons of significant difference in muscle tissue

	MEAN	STANDARD ERROR	p VALUE
Zinc			
Affected	18.8	1.1	0.00001
Literature	12.2	0.32	
Iron			
Affected	108	7.9	0.00001
Literature	157	5.5	
Manganese			
Affected	0.09	0.02	0
Literature	0.27	0.01	
Cadmium			
Affected	0	0	0.00001
Literature	0.08	0.004	
Lead			
Affected	0	0	0.00001
Literature	0.25	0.005	
Mercury MATURE			
Affected	6.5	1.4	0.0012
Literature	12.4	0.47	
Mercury IMMATURE			
Affected	5.5	1.1	0.0027
Literature	1.3	0.04	
Selenium MATURE			
Affected	0.49	0.07	0
Literature	2.24	0.21	
Selenium IMMATURE			
Affected	0.41	0.03	0
Literature	0.88	0.04	

All means expressed as ug/g.

Table 4. Affected value vs. NVSL normal value: means, standard error and p value for comparison of significant difference in liver tissue

	MEAN	STANDARD ERROR	p VALUE
Mercury			
Affected	49	6.5	0.0043
NVSL Normal	11	2.0	

All means expressed as ug/g.

Table 5. Correlations between metal values for NVSL normal livers

Normal Liver ($|r| \geq .51$)

Cu	Zn	Fe	Mn	Sn	Cd	Pb	Ni	Cr	Hg	Ca	As	Se
Cu	-.16	0.08	-.23	-.04	-.26	0.32	-.21	-.23	-.41	0.24	-.27	-.24
Zn		0.02	0.46	0.39	-.33	0.08	-.18	-.10	-.03	-.43	-.09	-.18
Fe	Fe		-.38	-.46	-.27	0.04	-.10	-.22	-.29	0.02	-.13	-.17
Mn	Mn	Mn		0.55*	-.36	-.12	0.20	0.39	-.07	-.58*	0.04	-.16
Sn	Sn	Sn	Sn		0.02	0.00	-.29	0.03	0.03	-.04	-.34	-.16
Cd	Cd	Cd	Cd	Cd		-.01	-.13	-.17	0.66*	0.36	0.35	0.80*
Pb	Pb	Pb	Pb	Pb	Pb		-.12	-.13	-.29	-.21	-.18	-.14
Ni	Ni	Ni	Ni	Ni	Ni	Ni		0.92*	-.12	-.21	-.10	-.07
Cr	Cr	Cr	Cr	Cr	Cr	Cr	Cr		-.13	-.17	-.17	-.17
Hg	Hg	Hg	Hg	Hg	Hg	Hg	Hg	Hg		-.02	0.36	0.68*
Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca		0.09	-.06
As	As	As	As	As	As	As	As	As	As	As		0.50
Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	

* Indicates significant correlation ($p \leq 0.05$)

Table 6. Correlations between metal values for affected dolphin livers

Affected Liver ($|r| \geq .35$)

Cu	Zn	Fe	Mn	Sn	Cd	Pb	Ni	Cr	V	Hg	Ca	As	Se
Cu	0.10	0.23	0.02	0.03	-.16	0.46*	-.14	0.54*	-.09	-.08	-.14	-.07	0.08
Zn		-.13	0.02	-.18	-.04	-.10	0.06	0.03	-.26	-.33	0.29	-.14	-.56*
Fe	Fe		-.04	-.11	-.07	0.02	-.25	0.05	0.00	0.60*	-.13	0.10	0.51*
Mn	Mn	Mn		0.19	0.05	0.06	0.02	0.36*	0.11	-.01	0.04	0.61*	-.00
Sn	Sn	Sn	Sn		-.10	0.31	0.50*	0.14	0.10	0.10	-.21	0.40*	0.16
Cd	Cd	Cd	Cd	Cd		0.36*	-.15	-.02	0.01	-.04	0.62*	0.18	-.00
Pb	Pb	Pb	Pb	Pb	Pb		0.02	0.44*	-.09	0.12	0.06	0.30	0.24
Ni	Ni	Ni	Ni	Ni	Ni	Ni		-.08	-.10	-.21	-.03	-.02	-.26
Cr	Cr	Cr	Cr	Cr	Cr	Cr	Cr		-.04	-.03	-.10	0.23	-.08
V	V	V	V	V	V	V	V	V		0.13	0.02	-.12	0.20
Hg	Hg	Hg	Hg	Hg	Hg	Hg	Hg	Hg	Hg		-.16	0.20	0.71*
Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca		0.16	-.22
As	As	As	As	As	As	As	As	As	As	As	As		0.13
Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	

* Indicates significant correlation ($p \leq 0.05$)

Table 7. Significant correlations between metals for affected dolphin and NVSL normal livers

Affected dolphin liver correlations only, $\|r\| \geq 0.35$	
copper - lead	+
cadmium - lead	+
tin - nickel	+
calcium - cadmium	+
zinc - selenium	-
iron - selenium	+
copper - chromium	+
manganese - chromium	+
lead - chromium	+
mercury - iron	+
manganese - arsenic	+
tin - arsenic	+

Normal dolphin liver correlations only, $\|r\| \geq 0.51$	
manganese - tin	+
mercury - cadmium	+
cadmium - selenium	+
calcium - manganese	-
chromium-nickel	+

Significant correlation common to both normal NVSL and affected dolphins	
mercury - selenium	+

DISCUSSION

Introduction

The results of analyses of NVSL normal and affected dolphin tissues in comparison with the literature normal concentrations show some definite differences, as do the correlations between metal values for affected and NVSL normal livers. Histopathology results are compatible with a possible immunosuppression process because of lymphoid depletion of follicles in the spleen and intestine, accompanied by a lack of lymphocytes (Saari et al., 1987-1988).

Analysis of Liver Data

Comparisons between NVSL normals, literature values and affected dolphin liver values show that the metals and metalloids considered most immunosuppressive from the literature (Cd, Pb, Ni, As, Mn, Hg, and Sn) are in lower concentrations in the affected animals than in the literature dolphins. The affected Cd mean is 10 times lower than the literature mean. This higher level in the literature may be explained by the inclusion of striped dolphins and harbor porpoises in the overall literature mean. Striped dolphins accumulate Cd from their diet of squid (Honda et al., 1983) and harbor porpoises live very close to shore, placing them in areas of potential pollution (Viale, 1978). Examination of the literature values excluding striped dolphins and harbor porpoises shows the mean for Cd is 0.67 ug/g, a figure much closer to the affected mean of 0.21 ug/g, but still higher. In addition, the NVSL normal mean

of 0.80 ug/g is also higher than the affected mean. Since the affected mean is smaller than both the NVSL normal and literature means, Cd levels in the affected dolphins are probably not involved in immunosuppression.

Differences between Pb, Sn, and As means in the affected, NVSL normals, and literature dolphins are insignificant. Statistical evaluation shows no differences between affected, NVSL normal, and literature means for Pb. One set of literature concentrations is quite high for Pb as compared to the other literature dolphins, which can also be explained by the proximity of habitats to potential pollution (Viale, 1978). In fact, the affected mean for Pb is slightly lower than both the normal and literature means. Literature values are unavailable for Sn or As; affected means for these metals are insignificantly different from the NVSL normal means.

A significant difference occurs between affected and literature means for Mn ($p = 0.011$), but there are no differences between NVSL normal and affected means. The same is true for Ni means. The affected Hg mean is much lower (49 ug/g) than the literature mean (205 ug/g) for mature animals, which may be attributed to values from Japanese striped dolphins (Itano et al., 1984). When these are excluded, the affected mean is significantly higher than both literature and NVSL normal means. This may be an important difference, but the high Hg content in apparently healthy striped dolphins indicates that dolphins can carry a heavy Hg load when it is balanced by Se (Koeman et al., 1973). In the case of both the striped dolphins and the affected animals, the Se is elevated to protect against the higher Hg concentrations.

Affected animals show significantly higher Zn and Fe levels than what is described in the literature. Deficiency of either of these two elements usually lowers immunocompentency (Fernandes et al., 1979, Baggs and Miller, 1973). Comparison of the affected Zn mean with the NVSL normal mean shows no significant difference; the affected Fe mean is significantly higher than the normal mean. This difference may be explained by the inclusion of three very high Fe values in the normal mean. Dropping these three outliers, the mean calculates to 356 ug/g, insignificantly different from the affected mean.

Other metals that may cause immune system changes are Cu, Cr, V, and Ca. There are no differences between the affected and literature mean for Cu (literature values are unavailable for Cr, Ca, and V), or NVSL normal means and the affected means for Cr, V, or Ca.

Correlations between metals in the liver of both NVSL normals and affected dolphins were examined (Tables 5, 6 and 7). A significant correlation common to both groups was seen for Hg and Se, as noted in the literature (Koeman et al., 1973). Some of the correlations in NVSL normals may be attributed to known mineral interrelationships found in land mammals. Mercury competes with Cd for binding sites on MT (Magos et al., 1974), and Se reduces the toxicity of Cd (Parizek et al., 1974). There is no known interrelationship between Ca and Mn or Cr and Ni.

Several correlations found only in affected animal livers may also be explained in the same way; Pb reduces Cu utilization, Ca lowers Cd absorption, and Zn is antagonistic to Se metabolism (Puls, 1988). However, nine correlations noted in the affected dolphin livers have not

yet had biological interrelationships identified. Positive correlations between Sn and Ni and Sn and As are noted. Since the biological significance of Sn is unknown, these correlations have not been explained (Puls, 1988). Iron is positively correlated with both Hg and Se, which agrees with the known mercury-selenium interrelationship (Koeman et al., 1973). Chromium is positively correlated with Pb, Mn, and Cu; the literature only notes Cr interrelationship with V and Zn (Puls, 1988). Cadmium-lead and manganese-arsenic are two more positive correlations found in affected dolphins without known biological significance (Nordberg et al., 1979).

There is no evidence of an alteration in immunocompetence or toxicosis in affected dolphins from evaluation or comparisons of liver metal concentrations.

Analysis of Kidney Data

Analysis of the data for kidney metal concentrations shows several differences between literature and affected dolphin means (no tissue was available for NVSL normals). No comparison will be made in this section between literature and affected means for Sn, Cr, V, Ca, and As since literature values for these metals were unavailable. Levels for the above metals found in the affected dolphins are included for information only.

The well-researched immunosuppressive metals (Cd, Pb, Ni, Hg, and Mn) follow the trend set in the liver of being generally found in lower

concentrations in the affected dolphins than in the literature population.

The affected Cd mean for kidney is 30 times lower than the literature mean ($p = 0$). As in the liver, the explanation lies with the diet of the striped dolphin and the function of the kidney in Cd excretion (Honda et al., 1982). The Pb mean is also significantly lower for affected dolphins than literature normals ($p = 0.0011$). Higher Pb levels were found in all the literature populations, indicating more Pb exposure to literature animals than to affected dolphins (Muir et al., 1988).

The affected mean for Mn was significantly lower than the literature mean ($p = 0.0014$), but the means are numerically very close (difference of 0.11 ug/g). No explanation can be given for this statistical importance.

Significant differences were not found between literature and affected means for Ni and mature Hg and mature Se levels. There is, however, a significant difference between the literature and affected means for Hg ($p = 0.0042$) and Se ($p = 0$) in immature dolphins. Affected immature dolphins contained almost twice as much Hg as did the literature animals, and the Hg mean was three times higher than the Se mean. This ratio was reversed for literature means, where the selenium-mercury ratio was 1.5:1. Although the main tissues of accumulation are liver, brain, and kidney, kidney is the route of Hg excretion (Berlin, 1979). In the case of affected immature dolphins, it appears that they are actively excreting Hg. However, if a loss of immunocompetence due to Hg was a

major factor leading to the dolphin die-off, both the mature and immature animals would have had high Hg profiles. Mercury in the affected adult animals was lower than in literature dolphins, and adequate protective Se was available (Koeman et al., 1973). This would indicate that there may be a Hg accumulation problem in immature animals, but it probably was not responsible for the die-off.

Affected means for Cu, Zn, and Fe are all significantly different from literature means. For both Fe and Zn, the affected mean is lower. In the case of Zn, the difference may be due to a number of literature values coming from striped dolphins (64/119 animals). These dolphins accumulate Cd from their diet, and high Zn helps to induce sufficient MT to complex and remove the Cd (Nordberg et al., 1979). Without striped dolphin values, the affected mean is actually higher than the literature mean (21 ug/g to 18.6 ug/g). This comparison indicates that there is probably no Zn deficiency in the affected animals.

The difference between literature and affected means for Fe is also significant ($p = 0.0001$). The affected mean is half as large as the literature mean, which might predispose a loss of immunocompetence in affected animals due to lowered myeloperoxidase and lead to increased bacterial susceptibility (Baggs and Miller, 1973). Geraci (1989) established that the affected dolphins succumbed to normal bacterial flora. However, Fe ranges given for other mammals such as sheep and cattle indicate that the values found in affected dolphins could be considered normal (Puls, 1988). Whether extrapolation between land and

oceanic mammal metal values may be made is unknown. In this case, the low Fe may or may not be significant.

The difference between the affected Cu mean and the literature Cu mean is considered significant statistically ($p = 0.00001$), but the actual difference between the means is very small (5.7 ug/g to 3.4 ug/g). The affected mean still lies within the normal range for most land mammals. Given the small difference and the consistency of affected values with other mammals (Puls, 1988), there is probably insufficient extra Cu in affected dolphins to cause a dramatic sequella such as this massive die-off.

The only significant findings for kidney are the differences in Cu, Fe, and immature Hg means. In light of the similarity between Fe and Cu values for land mammals, and the lack of Hg in adult dolphins, the significance of these values is unknown.

Analysis of Muscle Data

The highest number of significant differences between the affected means and the literature means is seen in muscle tissues. Statistical differences occurred for Zn, Fe, Mn, Cd, Pb, Hg (mature and immature animals), and Se (mature and immature animals). No NVSL normal values are given because of lack of tissue. As with the kidney and liver, no literature concentrations were available for Sn, Ca, As, V, and Cr, so affected values are given for information only.

The same trend seen in kidney and liver is also found in muscle; those metals considered to be more immunosuppressive are in lower

concentrations in the affected dolphins than in the literature populations.

Lead, Mn, Cd, Hg (mature dolphins), and Se (both adult and immature animals) means were all significantly lower in affected animals than in literature dolphins. Therefore, none of these metals accumulated to cause a loss of immunocompetence in the affected dolphins. Both Cu and Ni means for affected animals were insignificantly different from literature means and were probably not involved in the die-off.

Iron concentrations in affected dolphins were statistically lower than in literature animals, indicating that an Fe toxicity did not occur. The fact that the Fe mean was lower in affected animals might suggest a loss of immunocompetence (Baggs and Miller, 1973). However, a comparison to land mammal Fe ranges indicates that the affected animals had no Fe shortage in muscle (Puls, 1988). Further comparison between literature and affected means for liver shows no Fe deficiency, so the significance of lower Fe mean here is unknown.

Immature affected dolphins did have a higher Hg concentration than what was found in the literature. Muscle tissue will also accumulate Hg (Itano et al., 1984b). However, as was discussed concerning the high kidney Hg, any elevated Hg in the immature animals is probably not the root of the problem.

It does not appear that any of the metals analyzed in this study was sufficiently elevated or deficient to affect the immunocompetence of the dolphins or to cause the unusual die-off seen in 1987-88.

SUMMARY

Over 700 bottlenose dolphins stranded on the east coast of the United States during an 11 month period in 1987 and 1988. Lesions present in the affected dolphins suggest that this die-off may have resulted from a loss of immunocompetence; the cause of this loss may have been a change in tissue metals concentrations. Analyses of affected dolphin tissues for 15 metals were performed. Results were compared to literature values as well as to values from similar analyses conducted on tissues from dolphins uninvolved in the die-off. Although there were many significant differences between literature and affected values, no support for a loss of immunocompetency due to the concentrations of metals was noted.

APPENDIX

Table 1. Individual Metal Concentrations[a] for Affected Dolphins

Animal Number	Copper	Zinc	Iron	Manganese	Tin	Cadmium
CWP 273						
Liver	14	47	495	5.4	0.40	0.20
Kidney	5.5	17	100	0.67	NDA	NDA
Muscle	0.88	18	130	0.18	NDA	NDA
WAM 269						
Liver	7.4	100	465	2.9	1.4	NDA
Kidney	6.1	19	57	0.91	0.23	0.46
CWP 272						
Liver	15	97	120	17	NDA	NDA
Kidney	17	32	67	0.64	NDA	NDA
Muscle	0.79	11	46	0.15	NDA	NDA
CWP 271						
Liver	2.1	85	85	5.4	NDA	2.4
Kidney	7.8	22	100	0.70	NDA	NDA
Muscle	NDA	19	120	NDA	0.16	NDA
VB 87 005						
Liver	17	40	290	12	4.1	0.45
Kidney	3.9	16	57	0.81	NDA	2.2
Muscle	0.80	15	99	NDA	NDA	NDA
VB 87 012B						
Liver	7.2	29	235	11	1.7	0.16
Kidney	4.4	8.7	54	0.18	0.72	0.18
Muscle	0.92	17	96	0.13	NDA	NDA
VB 87 014						
Liver	6.6	25	235	10	1.7	0.26
Kidney	6.0	15	69	0.68	NDA	0.60
VB 87 004						
Liver	6.7	25	255	9.7	2.1	0.23
Muscle	0.92	27	140	0.15	NDA	NDA

[a]All values expressed as ug/g wet weight. NDA Copper, zinc, manganese, tin, cadmium, lead, cobalt, chromium, vanadium, arsenic - <0.10 ug/g, NDA nickel - <0.50 ug/g, NDA mercury - <2.0 ug/g

Table 1. (continued)

Animal Number	Lead	Nickel	Cobalt	Chromium	Vanadium	Mercury
CWP 273						
Liver	NDA	NDA	NDA	0.30	NDA	INSUFFICIENT
Kidney	NDA	3.9	NDA	0.18	NDA	16
Muscle	NDA	NDA	NDA	0.38	NDA	12
WAM 269						
Liver	NDA	NDA	NDA	0.69	NDA	77
Kidney	NDA	NDA	NDA	0.18	NDA	6.6
CWP 272						
Liver	NDA	NDA	NDA	1.4	NDA	INSUFFICIENT
Kidney	0.87	19	NDA	0.20	NDA	NDA
Muscle	NDA	NDA	NDA	0.21	NDA	4.8
CWP 271						
Liver	0.83	NDA	NDA	0.38	NDA	INSUFFICIENT
Kidney	NDA	NDA	NDA	1.2	NDA	10
Muscle	NDA	NDA	NDA	0.14	NDA	12
VB 87 005						
Liver	1.9	NDA	NDA	1.2	NDA	87
Kidney	NDA	NDA	NDA	0.23	NDA	16
Muscle	NDA	NDA	NDA	0.21	NDA	8.8
VB 87 012B						
Liver	NDA	NDA	NDA	0.60	NDA	19
Kidney	NDA	21	NDA	0.22	NDA	17
Muscle	NDA	NDA	NDA	NDA	NDA	7.5
VB 87 014						
Liver	NDA	NDA	NDA	0.45	0.43	61
Kidney	NDA	NDA	NDA	1.1	NDA	9.2
VB 87 004						
Liver	NDA	3.4	NDA	0.44	NDA	39
Muscle	NDA	NDA	NDA	0.15	NDA	8.0

Table 1. (continued)

Animal Number	Arsenic	Selenium	Calcium
CWP 273			
Liver	INSUFFICIENT	36	60
Kidney	NDA	0.41	150
Muscle	0.22	0.39	35
WAM 269			
Liver	0.69	6.7	77
Kidney	NDA	0.99	93
CWP 272			
Liver	INSUFFICIENT	INSUFFICIENT	88
Kidney	NDA	1.49	58
Muscle	NDA	0.39	49
CWP 271			
Liver	0.33	INSUFFICIENT	215
Kidney	NDA	3.04	99
Muscle	NDA	0.43	41
VB 87 005			
Liver	0.91	39	57
Kidney	0.19	6.52	210
Muscle	NDA	1.09	38
VB 87 012B			
Liver	0.57	13	66
Kidney	NDA	3.42	59
Muscle	0.60	0.61	32
VB 87 014			
Liver	NDA	24	77
Kidney	NDA	3.79	150
VB 87 004			
Liver	0.69	14	71
Muscle	NDA	0.39	35

Table 1. (continued)

Animal Number	Copper	Zinc	Iron	Manganese	Tin	Cadmium
VB 87 009						
Liver	4.0	89	285	30	1.4	0.27
Kidney	2.4	27	39	0.80	NDA	0.19
WAM 209						
Liver	6.8	57	220	5.0	NDA	NDA
Muscle	5.0	17	125	0.12	0.20	NDA
WAM 253						
Liver	6.0	32	725	2.8	NDA	0.28
Kidney	4.9	23	340	0.83	NDA	NDA
Muscle	2.8	33	190	0.24	NDA	NDA
WAM 258						
Liver	9.9	68	265	5.2	NDA	0.42
Kidney	3.2	20	84	0.59	NDA	2.2
Muscle	0.76	15	120	NDA	NDA	NDA
WAM 264						
Liver	6.8	42	330	4.3	0.36	0.18
Kidney	4.6	18	65	0.66	0.18	0.64
Muscle	0.98	20	105	0.11	NDA	NDA
S 88 Tt 10						
Liver	20	76	165	3.7	0.49	NDA
Kidney	6.4	26	48	0.74	NDA	0.35
S 88 Tt 32						
Liver	28	80	330	4.7	0.88	0.20
Kidney	5.8	21	97	0.50	NDA	2.1
Muscle	0.94	23	105	0.10	0.25	NDA
S 88 Tt 01						
Liver	11	69	190	3.2	0.37	NDA
Muscle	8.2	24	120	0.84	0.30	0.22

Table 1. (continued)

Animal Number	Lead	Nickel	Cobalt	Chromium	Vanadium	Mercury
VB 87 009						
Liver	NDA	5.3	NDA	0.65	NDA	41
Kidney	NDA	NDA	NDA	0.42	NDA	7.1
WAM 209						
Liver	NDA	NDA	NDA	0.31	NDA	7.5
Muscle	NDA	NDA	NDA	0.33	NDA	NDA
WAM 253						
Liver	NDA	NDA	NDA	0.35	NDA	165
Kidney	NDA	NDA	NDA	0.16	NDA	17
Muscle	NDA	NDA	NDA	1.9	NDA	5.8
WAM 258						
Liver	NDA	NDA	NDA	0.55	NDA	28
Kidney	NDA	NDA	NDA	0.15	NDA	6.5
Muscle	NDA	NDA	NDA	0.17	NDA	9.4
WAM 264						
Liver	NDA	NDA	NDA	0.49	0.14	27
Kidney	NDA	NDA	NDA	0.24	NDA	15
Muscle	NDA	NDA	NDA	0.35	NDA	5.1
S 88 Tt 10						
Liver	NDA	NDA	NDA	0.25	NDA	INSUFFICIENT
Kidney	NDA	NDA	NDA	0.23	NDA	12
S 88 Tt 32						
Liver	0.98	NDA	NDA	1.7	NDA	19
Kidney	NDA	NDA	NDA	0.12	NDA	16
Muscle	NDA	NDA	NDA	0.26	0.10	6.1
S 88 Tt 01						
Liver	0.92	11	NDA	0.47	NDA	13
Kidney	NDA	NDA	NDA	0.44	NDA	8.1

Table 1. (continued)

Animal Number	Arsenic	Selenium	Calcium
VB 87 009			
Liver	0.89	5.73	81
Kidney	NDA	2.13	190
WAM 209			
Liver	0.47	1.67	145
Muscle	0.10	0.56	40
WAM 253			
Liver	NDA	33.62	57
Kidney	NDA	3.94	150
Muscle	NDA	0.56	55
WAM 258			
Liver	0.20	9.92	105
Kidney	0.24	4.05	130
Muscle	0.19	0.52	41
WAM 264			
Liver	NDA	12	92
Kidney	0.60	3.65	150
Muscle	NDA	0.39	49
S 88 Tt 10			
Liver	NDA	6.89	74
Kidney	0.29	1.18	100
S 88 Tt 32			
Liver	NDA	2.00	57
Kidney	0.15	2.06	115
Muscle	NDA	0.54	57
S 88 Tt 01			
Liver	NDA	4.4	58
Kidney	NDA	2.81	74

Table 1. (continued)

Animal Number	Copper	Zinc	Iron	Manganese	Tin	Cadmium
S 88 Tt 27						
Liver	15	53	370	2.3	0.85	0.34
Kidney	4.7	21	210	0.64	NDA	1.1
Muscle	0.63	14	110	NDA	NDA	NDA
S 88 Tt 33						
Liver	5.2	125	240	2.6	0.41	NDA
Kidney	7.2	27	85	0.66	NDA	0.18
Muscle	NDA	13	36	0.20	NDA	NDA
S 88 Tt 34						
Liver	11	81	340	3.7	0.55	NDA
Kidney	3.7	30	73	0.65	NDA	0.26
Muscle	9.0	22	56	NDA	NDA	NDA
S 88 Tt 07						
Liver	3.8	67	85	2.4	5.7	NDA
Kidney	6.0	24	64	0.94	NDA	0.13
S 88 Tt 48						
Liver	9.4	87	145	4.4	0.37	NDA
Kidney	14	23	47	0.96	0.36	NDA
Muscle	7.0	20	66	0.16	NDA	NDA
S 88 Tt 57						
Liver	12	68	180	3.6	0.34	0.25
Kidney	4.1	25	66	0.61	NDA	0.82
Muscle	0.98	15	115	NDA	NDA	NDA
S 88 Tt 49						
Liver	5.0	100	280	5.4	NDA	NDA
Kidney	3.7	21	51	0.57	NDA	NDA
Muscle	1.2	19	94	NDA	NDA	NDA
S 88 Tt 11						
Liver	2.8	22	250	1.4	NDA	0.28
Kidney	2.5	15	125	0.49	NDA	2.3

Table 1. (continued)

Animal Number	Lead	Nickel	Cobalt	Chromium	Vanadium	Mercury
S 88 Tt 27						
Liver	0.85	NDA	NDA	0.22	NDA	48
Kidney	NDA	NDA	NDA	0.20	0.10	6.8
Muscle	NDA	NDA	NDA	0.15	NDA	NDA
S 88 Tt 33						
Liver	NDA	7.3	NDA	0.60	NDA	7.8
Kidney	NDA	NDA	NDA	0.23	NDA	4.8
Muscle	NDA	NDA	NDA	0.35	NDA	5.3
S 88 Tt 34						
Liver	NDA	NDA	NDA	0.33	NDA	28
Kidney	NDA	NDA	NDA	0.15	NDA	8.6
Muscle	NDA	NDA	NDA	0.51	NDA	15
S 88 Tt 07						
Liver	NDA	16	0.19	0.34	NDA	NDA
Kidney	NDA	NDA	NDA	0.22	NDA	4.2
S 88 Tt 48						
Liver	NDA	NDA	NDA	0.29	NDA	21
Kidney	NDA	1.4	NDA	0.64	NDA	2.5
Muscle	NDA	2.0	NDA	0.51	NDA	5.6
S 88 Tt 57						
Liver	NDA	7.9	NDA	0.39	NDA	21
Kidney	NDA	0.82	NDA	0.27	NDA	6.3
Muscle	NDA	NDA	NDA	0.67	NDA	NDA
S 88 Tt 49						
Liver	NDA	NDA	NDA	0.46	NDA	7.0
Kidney	NDA	NDA	NDA	0.19	NDA	4.5
Muscle	NDA	1.9	NDA	0.21	NDA	8.3
S 88 Tt 11						
Liver	NDA	NDA	NDA	0.83	NDA	41
Kidney	NDA	NDA	NDA	0.24	NDA	15

Table 1. (continued)

Animal Number	Arsenic	Selenium	Calcium
S 88 Tt 27			
Liver	NDA	20	76
Kidney	NDA	4.01	185
Muscle	NDA	0.44	36
S 88 Tt 33			
Liver	NDA	1.78	140
Kidney	0.63	1.81	85
Muscle	NDA	0.31	61
S 88 Tt 34			
Liver	NDA	8.7	59
Kidney	0.22	2.66	99
Muscle	NDA	0.26	56
S 88 Tt 07			
Liver	NDA	0.41	59
Kidney	NDA	1.67	89
S 88 Tt 48			
Liver	NDA	0.49	61
Kidney	0.16	1.27	76
Muscle	NDA	0.39	37
S 88 Tt 57			
Liver	NDA	5.5	76
Kidney	NDA	3.54	160
Muscle	NDA	0.26	29
S 88 Tt 49			
Liver	NDA	0.96	39
Kidney	NDA	1.96	110
Muscle	NDA	0.34	37
S 88 Tt 11			
Liver	NDA	17	50
Kidney	NDA	5.53	220

Table 1. (continued)

Animal Number	Copper	Zinc	Iron	Manganese	Tin	Cadmium
S 88 Tt 44						
Liver	9.4	58	150	3.3	0.20	0.10
Kidney	4.5	26	46	0.69	NDA	0.44
Muscle	9.8	30	165	NDA	NDA	NDA
S 88 Tt 51						
Liver	4.0	155	92	4.3	0.53	0.21
Kidney	4.3	21	62	0.73	NDA	NDA
Muscle	4.0	21	91	0.25	NDA	NDA
S 88 Tt 50						
Liver	2.4	100	85	4.0	NDA	NDA
Kidney	4.8	22	28	0.70	NDA	0.52
Muscle	3.2	13	75	0.10	NDA	NDA
S 88 Tt 04						
Liver	15	170	430	4.4	0.46	0.19
Muscle	1.9	16	170	NDA	NDA	NDA
S 88 Tt 55						
Liver	5.1	46	315	3.3	NDA	0.71
Muscle	0.79	16	110	NDA	0.31	NDA
S 88 Tt 19						
Liver	3.3	25	135	2.3	0.47	NDA
Kidney	4.4	18	62	0.65	NDA	0.59
Muscle	1.6	20	120	0.10	0.39	NDA
S 88 Tt 39						
Liver	3.9	52	135	2.3	1.1	NDA
Kidney	3.4	24	62	0.65	NDA	0.59

Table 1. (continued)

Animal Number	Lead	Nickel	Cobalt	Chromium	Vanadium	Mercury
S 88 Tt 44						
Liver	NDA	NDA	NDA	0.69	NDA	11
Kidney	NDA	NDA	NDA	0.32	NDA	7.1
Muscle	NDA	1.5	NDA	0.33	0.10	5.5
S 88 Tt 51						
Liver	NDA	NDA	NDA	0.26	NDA	7.8
Kidney	NDA	NDA	NDA	0.41	NDA	7.0
Muscle	NDA	2.2	NDA	1.8	NDA	NDA
S 88 Tt 50						
Liver	NDA	NDA	NDA	0.29	NDA	6.8
Kidney	NDA	NDA	NDA	0.20	NDA	4.4
Muscle	NDA	1.6	NDA	0.42	NDA	4.0
S 88 Tt 04						
Liver	NDA	NDA	NDA	0.43	NDA	53
Muscle	NDA	NDA	NDA	0.42	NDA	9.5
S 88 Tt 55						
Liver	NDA	NDA	NDA	0.29	NDA	25
Muscle	NDA	NDA	NDA	0.19	NDA	NDA
S 88 Tt 19						
Liver	NDA	NDA	NDA	0.14	NDA	110
Kidney	NDA	0.74	NDA	0.64	NDA	28
Muscle	NDA	NDA	NDA	0.22	NDA	5.9
S 88 Tt 39						
Liver	NDA	NDA	NDA	0.25	NDA	33
Kidney	NDA	0.74	NDA	0.49	NDA	8.7

Table 1. (continued)

Animal Number	Arsenic	Selenium	Calcium
S 88 Tt 44			
Liver	NDA	2.09	39
Kidney	NDA	2.00	110
Muscle	NDA	0.34	37
S 88 Tt 51			
Liver	NDA	0.59	55
Kidney	0.72	1.83	83
Muscle	0.25	0.38	53
S 88 Tt 50			
Liver	NDA	2.27	74
Kidney	NDA	2.47	76
Muscle	0.60	0.31	41
S 88 Tt 04			
Liver	NDA	12	130
Muscle	NDA	0.48	49
S 88 Tt 55			
Liver	NDA	11	59
Muscle	NDA	0.32	105
S 88 Tt 19			
Liver	NDA	39	71
Kidney	NDA	15.7	255
Muscle	NDA	0.64	50
S 88 Tt 39			
Liver	NDA	6.00	62
Kidney	0.78	3.51	170

Table 2. Individual Metal Concentrations[a] for Normal NVSL Dolphins

Animal Number	Copper	Zinc	Iron	Manganese	Tin	Cadmium
MH 82 227 Liver	27	42	3000	1.7	INSUFFICIENT	
MH 78 08 Liver	6.4	65	2000	1.9	NDA	0.21
MH 75 16 Liver	13	68	1900	1.8	0.31	0.20
MH 79 179 Liver	39	30	265	NDA	NDA	NDA
MH 83 216 Liver	7.6	41	235	2.5	0.28	5.6
MH 87 460 Liver	8.5	59	625	5.1	NDA	0.20
MH 87 250 Liver	23	54	430	2.1	0.30	1.2
MH 87 462 Liver	13	145	475	5.5	1.4	NDA
MH 87 479 Liver	7.8	25	120	0.92	1.3	4.0
MH 87 468 Liver	24	36	230	5.2	1.1	NDA
MH 87 455 Liver	6.2	60	440	5.1	1.2	NDA
MH 87 454 Liver	14	72	135	6.9	0.95	0.19
MH 87 453 Liver	31	75	485	7.0	1.7	0.41

[a]All values expressed as ug/g wet weight. NDA copper, zinc, manganese, tin, cadmium, lead, cobalt, chromium, vanadium, arsenic - <0.10 ug/g, NDA nickel - < 0.50 ug/g, NDA mercury - < 2.0 ug/g

Table 2. (continued)

Animal Number	Lead	Nickel	Cobalt	Chromium	Vanadium	Mercury
MH 82 227 Liver	----------INSUFFICIENT TISSUE FOR ANALYSES----------					
MH 78 08 Liver	NDA	NDA	NDA	0.20	NDA	24
MH 75 16 Liver	1.0	NDA	NDA	0.28	NDA	INSUFFICIENT
MH 79 179 Liver	NDA	NDA	NDA	NDA	NDA	5.5
MH 83 216 Liver	NDA	NDA	NDA	NDA	NDA	29
MH 87 460 Liver	NDA	NDA	NDA	0.30	NDA	11
MH 87 250 Liver	2.0	NDA	NDA	NDA	NDA	7.8
MH 87 462 Liver	NDA	NDA	NDA	0.37	NDA	13
MH 87 479 Liver	NDA	NDA	NDA	0.64	NDA	17
MH 87 468 Liver	NDA	NDA	NDA	0.54	NDA	14
MH 87 455 Liver	NDA	NDA	NDA	0.91	NDA	6.5
MH 87 454 Liver	NDA	NDA	NDA	0.42	NDA	9.2
MH 87 453 Liver	1.0	NDA	NDA	1.0	NDA	8.4

Table 2. (continued)

Animal Number	Arsenic	Selenium	Calcium
MH 82 227 Liver	INSUFFICIENT	INSUFFICIENT	110
MH 78 08 Liver	NDA	6.98	44
MH 75 16 Liver	INSUFFICIENT	INSUFFICIENT	81
MH 79 179 Liver	NDA	INSUFFICIENT	130
MH 83 216 Liver	0.22	120	64
MH 87 460 Liver	0.35	3.67	100
MH 87 250 Liver	NDA	1.60	42
MH 87 462 Liver	NDA	1.36	56
MH 87 479 Liver	NDA	5.32	200
MH 87 468 Liver	NDA	2.92	75
MH 87 455 Liver	NDA	2.89	54
MH 87 454 Liver	NDA	2.94	60
MH 87 453 Liver	NDA	1.29	60

Table 2. (continued)

Animal Number	Copper	Zinc	Iron	Manganese	Tin	Cadmium
MH 82 472 Liver	7.6	40	430	5.5	NDA	NDA
MH 87 456 Liver	5.1	62	400	6.4	1.2	NDA

Table 2. (continued)

Animal Number	Lead	Nickel	Cobalt	Chromium	Vanadium	Mercury
MH 87 472 Liver	NDA	41	NDA	3.2	NDA	7.4
MH 87 456 Liver	NDA	NDA	NDA	0.21	NDA	8.4

Table 2. (continued)

Animal Number	Arsenic	Selenium	Calcium
MH 87 472			
Liver	NDA	2.96	42
MH 87 456			
Liver	NDA	1.42	15

Table 3. Published Metals Concentrations[a] in Dolphins

Element	Tissue	Values (# of animals)	Species	Reference	Location
Copper	Liver	0.97 - 8.9 (24) $\bar{x}$ - 5.62	White beaked dolphin	Muir et al., 1988	New-foundland
	Kidney	2.2 - 5.9 (23) $\bar{x}$ - 3.4			
	Muscle	1.1 - 4.4 (25) $\bar{x}$ - 2.58			
	Liver	3.57 - 15.2 (57) $\bar{x}$ - 8.09	Striped dolphin	Honda et al., 1983	Japan
	Kidney	1.46 - 6.05 (30) $\bar{x}$ - 3.13			
	Muscle	1.28 - 3.37 (59) $\bar{x}$ - 2.04			
	Liver	8.03 - 40.3 (3) $\bar{x}$ - 19.2	Striped dolphin	Honda et al., 1982	Japan
	Kidney	1.2 - 3.19 (3) $\bar{x}$ - 2.52			
	Muscle	0.81 - 2.16 (3) $\bar{x}$ - 1.78			
	Liver	2.65 - 12.8 (23) $\bar{x}$ - 7.25	Harbor porpoise	Falconer et al., 1983	Scotland east coast
	Kidney	2.64 - 4.77 (23) $\bar{x}$ - 3.71			
	Liver	4.0 - 15 (3) $\bar{x}$ - 8.33	Harbor porpoise	Wagemann & Muir, 1984	Baltic Sea
	Kidney	3.1 - 3.2 (2) $\bar{x}$ - 3.2			
	Muscle	1.8 - 2.7 (3) $\bar{x}$ - 2.2			
	Liver	2.6 - 8.3 (4) $\bar{x}$ - 4.5	Harbor porpoise	Wagemann & Muir, 1984	Denmark

[a]All values expressed as ug/g wet weight.

Table 3. (continued)

Element	Tissue	Values (# of animals)	Species	Reference	Location
Zinc	Liver	12 - 37.7 (27) $\bar{x}$ - 27.6	White beaked dolphin	Muir et al., 1988	New-foundland
	Kidney	8.3 x 24.4 (25) $\bar{x}$ - 18.5			
	Muscle	10 - 24.5 (27) $\bar{x}$ - 14.6			
	Liver	26.5 - 109 (57) $\bar{x}$ - 44.5	Striped dolphin	Honda et al., 1983	Japan
	Kidney	22.8 - 41.2 (30) $\bar{x}$ - 30.1			
	Muscle	6.86 - 20.4 (59) $\bar{x}$ - 11.4			
	Liver	26 - 35.5 (3) $\bar{x}$ - 32.3	Striped dolphin	Honda et al., 1982	Japan
	Kidney	16.7 - 27.7 (3) $\bar{x}$ - 22.8			
	Muscle	12.2 - 23.9 (3) $\bar{x}$ - 16.8			
	Liver Kidney Muscle	43.7 ± 14.2 (31) 26.4 ± 16.2 (31) 11.4 ± 2.44 (57)	Striped dolphin	Honda & Tatsukawa, 1984	Japan
	Liver Kidney	18.4 - 67.6 (23) 19.5 - 33.1 (23)	Harbor porpoise	Falconer et al., 1983	Scotland east coast
	Liver	34 - 50 (3) $\bar{x}$ - 44.3	Harbor porpoise	Wagemann & Muir, 1984	Baltic Sea
	Kidney	20 - 24.4 (2) $\bar{x}$ - 22.2			
	Muscle	12.4 - 21 (3) $\bar{x}$ - 15.9			
	Liver	34 - 50 (4) $\bar{x}$ - 59	Harbor porpoise	Wagemann & Muir, 1984	Denmark
	Kidney	18 - 21 (4) $\bar{x}$ - 15			

Table 3. (continued)

Element	Tissue	Values (# of animals)	Species	Reference	Location
Lead	Liver	0.15 - 0.34 (27) $\bar{x}$ - 0.14	White beaked dolphin	Muir et al., 1988	New-foundland
	Kidney	0.002 - 0.47 (25) $\bar{x}$ - 13			
	Muscle	0.008 - 0.22 (27) $\bar{x}$ - 0.02			
	Liver	0.03 - 0.64 (57) $\bar{x}$ - 0.22	Striped dolphin	Honda et al., 1983	Japan
	Kidney	0.01 - 0.71 (30) $\bar{x}$ - 0.17			
	Muscle	0.04 - 0.26 (59) $\bar{x}$ - 0.18			
	Liver	0.02 - 0.12 (3) $\bar{x}$ - 0.07	Striped dolphin	Honda et al., 1982	Japan
	Kidney	0.03 - 0.16 (3) $\bar{x}$ - 0.12			
	Muscle	0.06 - 0.08 (3) $\bar{x}$ - 0.07			
	Liver	0.17 - 0.43 (3) $\bar{x}$ - 0.32	Harbor porpoise	Wagemann & Muir, 1984	Baltic Sea
	Kidney	0.15 - 0.17 (2) $\bar{x}$ - 0.16			
	Muscle	0.03 - 0.07 (3) $\bar{x}$ - 0.05			
	Liver	1.9 - 5.3 (4) $\bar{x}$ - 3.5	Harbor porpoise	Wagemann & Muir, 1984	Denmark
	Muscle	1.6 - 4.7 (4) $\bar{x}$ - 3.3			

Table 3. (continued)

Element	Tissue	Values (# of animals)	Species	Reference	Location
Cadmium	Liver	0.6 - 2.3 (26) $\bar{x}$ - 0.67	White beaked dolphin	Muir et al., 1988	New-foundland
	Kidney	0.55 - 9.5 (25) $\bar{x}$ - 2.97			
	Muscle	0.006 - 0.07 (26) $\bar{x}$ - 0.02			
	Liver	0.04 - 11.1 (57) $\bar{x}$ - 6.26	Striped dolphin	Honda et al., 1983	Japan
	Kidney	0.06 - 69.9 (30) $\bar{x}$ - 24.8			
	Muscle	0.01 - 0.25 (59) $\bar{x}$ - 0.10			
	Liver	<0.005 - 7.03 (3) $\bar{x}$ - 3.05	Striped dolphin	Honda et al., 1982	Japan
	Kidney	<0.0005 - 63.7 (3) $\bar{x}$ - 0.13			
	Muscle	0.005 - 0.12 (3) $\bar{x}$ - 0.006			
	Liver	<0.05 ± 2.41 (31)	Striped dolphin	Honda & Tatsukawa, 1984	Japan
	Kidney	26.4 ± 16.2 (31)			
	Muscle	0.1 ± 0.06 (59)			
	Liver	<0.05 - 0.94 (23) $\bar{x}$ - 0.08	Harbor porpoise	Falconer et al., 1983	Scotland east coast
	Kidney	0.17 - 7.42 (23)			
	Liver	0.019 - 0.025 (3) $\bar{x}$ - 0.08	Harbor porpoise	Wagemann & Muir, 1984	Baltic Sea
	Kidney	0.077 - 0.95 (2) $\bar{x}$ - 0.51			
	Muscle	0.002 - 0.006 (3) $\bar{x}$ - 0.03			

Table 3. (continued)

Element	Tissue	Values (# of animals)	Species	Reference	Location
Mercury	Liver	1.16 (1)	Risso	Zonfrillo	Scotland
	Kidney	0.55 (1)	dolphin	et al., 1987	
	Liver	0.13 - 1.6 (27)	White	Muir	New-
		$\bar{x}$ - 0.83	beaked	et al., 1988	foundland
	Kidney	0 - 0.43 (25)	dolphin		
		$\bar{x}$ - 0.23			
	Muscle	0.19 - 0.72 (26)			
		$\bar{x}$ - 0.45			
	MATURE				
	Liver	205 ± 102 (15)	Striped	Itano	Japan
	Kidney	14.7 ± 6.6	dolphin	et al., 1984b	
	Muscle	15.2 ± 8 (26)			
	IMMATURE				
	Liver	5.81 ± 2.76 (6)			
	Kidney	3.38 ± 1.3 (5)			
	Muscle	1.27 ± 0.22 (6)			
	FETUS				
	Liver	1.76 ± 0.69 (16)	Striped	Itano	Japan
	Kidney	0.60 ± 0.16 (15)	dolphin	et al., 1984c	
	Muscle	0.90 ± 0.31 (16)			
	SUCKLING				
	Liver	1.72 ± 0.02 (3)			
	Kidney	0.89 ± 0.13 (4)			
	Muscle	0.52 ± 0.06 (4)			

Table 3. (continued)

Element	Tissue	Values (# of animals)	Species	Reference	Location
Mercury	Liver	204 ± 93 (4)			
	Kidney	17.7 ± 7.3 (4)			
	Muscle	11.1 ± 3.1 (4)			
	Muscle	2.05 - 22.2 (5) $\bar{x}$ - 12.3 ± 7.9	Striped dolphin	Itano et al., 1985	Japan
	Liver	1.7 - 485 (45) $\bar{x}$ - 205	Striped dolphin	Honda et al., 1983	Japan
	Kidney	0.91 - 17.6 (20) $\bar{x}$ - 8.71			
	Muscle	0.46 - 15.7 (51) $\bar{x}$ - 7.02			
	Liver Muscle	0.55 - 91.3 (20) 0.21 - 2.58 (41) $\bar{x}$ - 0.99	Harbor porpoise	Gaskin et al., 1972	Atlantic Ocean Canada
	Liver	6 - 13 (2) x - 9.5 ± 4.94	Long snouted dolphin	Gaskin et al., 1974	Lesser Antilles
	Kidney	2.28 - 2.68 (2) $\bar{x}$ - 2.48 ± 0.28			
	Muscle	0.87 - 1.33 (2) $\bar{x}$ - 1.1 ± 0.33			
	Liver	0.82 - 30.7 (87) $\bar{x}$ - 13.33 ± 14.3	Harbor porpoise	Gaskin et al., 1979	Atlantic Ocean Canada
	Kidney	0.68 - 4.71 (60) $\bar{x}$ - 1.88 ± 1.02			
	Muscle	0.25 - 1.69 (142) $\bar{x}$ - 0.95 ± 0.37			
	ADULT Muscle	2.28 - 9.43 (10) $\bar{x}$ - 5 ± 2.39	Blue white dolphin	Arima & Nagakura, 1979	Japan
	FETUS Muscle	0.95 (1)			
	ADULT Muscle	1.66 (1)	White dotted dolphin	Arima & Nagakura, 1979	Japan

Table 3. (continued)

Element	Tissue	Values (# of animals)	Species	Reference	Location
	FETUS Muscle	0.61 (1)			
	FETUS Muscle	0.16 (1)	Finless black porpoise	Arima & Nagakura, 1979	Japan
	Muscle	4.57 (1)	Gill's bottlenose dolphin	Arima & Nagakura, 1979	Japan
	Liver Kidney	0.28 - 15.9 (23) 0.23 - 2.82 (23)	Harbor porpoise	Falconer et al., 1983	Scotland
	Liver Muscle	0.7 - 28 (3) $\bar{x}$ - 10.4 0.15 - 3.3 (3) $\bar{x}$ - 1.46	Harbor porpoise	Wagemann & Muir, 1984	Baltic Sea
	Liver Muscle	1.5 - 69 (4) $\bar{x}$ - 22 0.8 - 3.2 (4) $\bar{x}$ - 1.9	Harbor porpoise	Wagemann & Muir, 1984	Denmark
Selenium	Liver Kidney Muscle	1 - 3.4 (27) $\bar{x}$ - 2.25 0 - 4.4 (25) $\bar{x}$ - 1.28 0.33 - 1.3 (26) $\bar{x}$ - 0.53	White beaked dolphin	Muir et al., 1988	New-foundland
	ADULT Liver Kidney Muscle	48 ± 28.7 (15) 5.6 ± 2.2 (14) 2.8 ± 2.2 (26)	Striped dolphin	Itano et al., 1984b	Japan
	IMMATURE Liver Kidney Muscle	1.97 ± 1.03 (6) 5.82 ± 0.94 (5) 0.88 ± 0.25 (6)			

Table 3. (continued)

Element	Tissue	Values (# of animals)	Species	Reference	Location
	FETUS				
	Liver	2.03 ± 1.03 (16)	Striped	Itano	Japan
	Kidney	0.67 ± 0.28 (15)	dolphin	et al., 1984b	
	Muscle	0.16 ± 0.05 (16)			
	SUCKLING				
	Liver	1.07 ± 0.46 (3)			
	Kidney	1.83 ± 0.33 (4)			
	Muscle	0.54 ± 0.15 (4)			
	ADULT				
	Liver	41.3 ± 13.8 (4)			
	Kidney	5.2 ± 2.5 (4)			
	Muscle	1.4 ± 0.7 (4)			
	Muscle	0.467 - 4.99 (5) $\bar{x}$ - 2.28 ± 1.7	Striped dolphin	Honda et al., 1983	Japan
	ADULT				
	Muscle	0.51 - 2.48 (10) $\bar{x}$ - 1.12 ± 0.68	Blue white dolphin	Arima & Nagakura, 1979	Japan
	FETUS				
	Muscle	0.22 (1)	Blue white dolphin	Arima & Nagakura, 1979	Japan
	ADULT				
	Muscle	0.68 (1)	White dotted dolphin	Arima & Nagakura, 1979	Japan
	FETUS				
	Muscle	0.21 (1)	Finless black porpoise	Arima & Nagakura, 1979	Japan
	Muscle	1.30 (1)	Gill's bottlenose dolphin	Arima & Nagakura, 1979	Japan

Table 3. (continued)

Element	Tissue	Values (# of animals)	Species	Reference	Location
Nickel	Liver	0.05 - 0.49 (57) $\bar{x}$ - 0.22	Striped dolphin	Honda et al., 1983	Japan
	Kidney	0.07 - 0.63 (30) $\bar{x}$ - 0.22			
	Muscle	0.04 - 0.25 (59) $\bar{x}$ - 0.14			
	Liver	0.18 - 0.19 (3) $\bar{x}$ - 0.19	Striped dolphin	Honda et al., 1982	Japan
	Kidney	0.07 - 0.18 (3) $\bar{x}$ - 0.13			
	Muscle	0.02 - 0.16 (3) $\bar{x}$ - 0.09			
Manganese	Liver	1.3 - 6.71 (57) $\bar{x}$ - 3.18	Striped dolphin	Honda et al., 1983	Japan
	Kidney	0.45 - 1.32 (30) $\bar{x}$ - 0.82			
	Muscle	0.15 - 0.46 (59) $\bar{x}$ - 0.27			
	Liver	0.94 - 3.06 (3) $\bar{x}$ - 2.17	Striped dolphin	Honda et al., 1982	Japan
	Kidney	0.45 - 0.56 (3) $\bar{x}$ - 0.50			
	Muscle	0.25 - 0.32 (3) $\bar{x}$ - 0.27			
Iron	Liver Kidney	55.8 - 95.5 (59) 39.6 - 267 (30) $\bar{x}$ - 143	Striped dolphin	Honda et al., 1983	Japan
	Muscle	47 - 222 (59) $\bar{x}$ - 159			
	Liver	104 - 448 (3) $\bar{x}$ - 304	Striped dolphin	Honda et al., 1982	Japan
	Kidney	87.9 - 169 (3) $\bar{x}$ - 129			
	Muscle	39.5 - 192 (3) $\bar{x}$ - 138			

Table 4. Means, Standard Deviations and Ranges for Affected, NVSL Normal and Literature Dolphins[a]

	Affected Dolphins	NVSL Normal Dolphins	Literature Dolphins
Copper			
Liver			
$\bar{x}$ (# of animals)	8.9 (31)	15.6 (15)	7.6 (114)
SD	± 5.9	± 10.6	± 2.8
Range	2.4 - 28	6.2 - 39	0.97 - 40.3
Kidney			
$\bar{x}$ (# of animals)	5.7 (27)	No tissue	3.4 (81)
SD	± 3.2		± 9.0
Range	2.4 - 17		1.2 - 6.5
Muscle			
$\bar{x}$ (# of animals)	2.4 (23)	No tissue	2.2 (90)
SD	± 2.8		± 0.61
Range	< 0.1 - 9.8		0.81 - 4.4
Zinc			
Liver			
$\bar{x}$ (# of animals)	70 (31)	58 (15)	41 (148)
SD	± 35.6	± 28.6	± 15.6
Range	22 - 155	25 - 145	12.0 - 109
Kidney			
$\bar{x}$ (# of animals)	21.7 (27)	No tissue	25 (118)
SD	± 5		± 9.0
Range	8.7 - 32		8.3 - 50
Muscle			
$\bar{x}$ (# of animals)	18.9 (23)	No tissue	12.2 (96)
SD	± 5.4		± 3.1
Range	11 - 33		6.86 - 24.5
Iron			
Liver			
$\bar{x}$ (# of animals)	256 (31)	744 (15)	86 (62)
SD	± 141	± 848	± 18.5
Range	67 - 725	120 - 3000	55.8 - 448

[a]All values in above chart express as ug/g wet weight.

Table 4. (continued)

	Affected Dolphins	NVSL Normal Dolphins	Literature Dolphins
Kidney			
$\bar{x}$ (# of animals)	84 (27)	No tissue	141 (33)
SD	± 62		± 55.2
Range	28 - 340		39.6 - 267
Muscle			
$\bar{x}$ (# of animals)	108 (23)	No tissue	157 (62)
SD	± 38		± 43.6
Range	36 - 190		39.5 - 222
Manganese			
Liver			
$\bar{x}$ (# of animals)	5.9 (31)	3.8 (15)	3.1 (60)
SD	± 5.6	± 2.4	± 1.3
Range	1.4 - 30	0.92 - 7	0.94 6.71
Kidney			
$\bar{x}$ (# of animals)	0.68 (27)	No tissue	0.79 (33)
SD	± 0.16		± 0.21
Range	0.18 - 0.96		0.45 - 1.32
Muscle			
$\bar{x}$ (# of animals)	0.09 (23)	No tissue	0.27 (62)
SD	± 0.09		± 0.08
Range	< 0.1 - 0.25		0.15 - 0.46
Tin			
Liver			
$\bar{x}$ (# of animals)	0.83 (31)	0.65 (14)	No values
SD	± 1.25	± 0.63	
Range	< 0.1 - 5.7	< 0.1 - 1.7	
Kidney			
$\bar{x}$ (# of animals)	0.07 (27)	No tissue	No values
SD	± 0.16		
Range	< 0.1 - 0.72		
Muscle			
$\bar{x}$ (# of animals)	0.06 (23)	No tissue	No values
SD	± 0.12		
Range	< 0.1 - 0.39		

Table 4. (continued)

	Affected Dolphins	NVSL Normal Dolphins	Literature Dolphins
Cadmium			
Liver			
$\bar{x}$ (# of animals)	0.21 (31)	0.80 (14)	2.7 (143)
SD	± 0.54	± 1.7	± 9.6
Range	< 0.1 - 2.4	< 0.1 - 5.6	<0.005 - 11.1
Kidney			
$\bar{x}$ (# of animals)	0.58 (27)	No tissue	15.1 (114)
SD	± 0.82		± 12.6
Range	< 0.1 - 2.3		< 0.005 - 69.9
Muscle			
$\bar{x}$ (# of animals)	< 0.1 (23)	No tissue	0.08 (150)
SD	0		± 0.05
Range	None		0.002 - 0.25
Lead			
Liver			
$\bar{x}$ (# of animals)	0.18 (31)	0.27 (14)	0.34 (94)
SD	± 0.44	± 0.59	± 15.6
Range	< 0.1 - 1.9	< 0.1 - 2.0	0.02 - 5.3
Kidney			
$\bar{x}$ (# of animals)	0.03 (27)	No tissue	0.15 (60)
SD	± 0.17		± 0.15
Range	< 0.1 - 0.87		0.002 - 0.71
Muscle			
$\bar{x}$ (# of animals)	< 0.1 (23)	No tissue	0.25 (96)
SD	0		± 0.05
Range	None		0.008 - 4.7
Nickel			
Liver			
$\bar{x}$ (# of animals)	1.6 (31)	2.7 (14)	0.22 (60)
SD	± 3.9	± 10.5	± 0.11
Range	< 0.5 - 16	< 0.5 - 41	0.05 - 0.49
Kidney			
$\bar{x}$ (# of animals)	1.8 (27)	No tissue	0.21 (33)
SD	± 5.3		± 0.14
Range	< 0.5 - 21		0.07 - 0.63

Table 4. (continued)

	Affected Dolphins	NVSL Normal Dolphins	Literature Dolphins
Muscle			
$\bar{x}$ (# of animals)	0.40 (23)	No tissue	0.14 (62)
SD	± 0.79		± 0.05
Range	< 0.5 - 2.0		0.02 - 0.25
Mercury, MATURE			
Liver			
$\bar{x}$ (# of animals)	49 (15)	11 (15)	205 (181)
SD	± 42	± 7.9	± 1.02
Range	< 0.1 - 165	< 0.1 - 290	
Kidney			
$\bar{x}$ (# of animals)	13 (12)	No tissue	15 (156)
SD	± 6.3		± 6.5
Range	6.8 - 28		
Muscle			
$\bar{x}$ (# of animals)	6.5 (12)	No tissue	12.4 (220)
SD	± 4.8		± 7.0
Range	< 0.1 - 15		
Mercury, IMMATURE			
$\bar{x}$(# of animals)	16 (16)	No tissue	5.8 (25)
SD	± 22.4		± 2.8
Range	< 0.1 - 77		
Kidney			
$\bar{x}$ (# of animals)	6.9 (15)	No tissue	3.4 (24)
SD	± 3.6		± 1.3
Range	< 0.1 - 16		
Muscle			
$\bar{x}$ (# of animals)	5.5 (11)	No tissue	1.3 (27)
SD	± 3.6		± 0.22
Range	< 0.1 - 12		
Selenium, MATURE			
Liver			
$\bar{x}$ (# of animals)	18.2 (15)	10.2 (15)	47 (46)
SD	± 12.4	± 30.4	± 26
Range	5.5 - 36	1.42 - 120	

Table 4. (continued)

	Affected Dolphins	NVSL Normal Dolphins	Literature Dolphins
Kidney			
$\bar{x}$ (# of animals)	4.59 (12)	No tissue	5.5 (43)
SD	± 3.8		± 2.1
Range	0.41 - 15.7		
Muscle			
$\bar{x}$(# of animals)	0.49 (12)	No tissue	2.24 (73)
SD	± 0.23		± 1.8
Range	0.26 - 1.29		
Selenium, IMMATURE			
Liver			
$\bar{x}$ (# of animals)	4.01 (16)	No tissue	1.97 (9)
SD	± 6.3		± 1.0
Range	0.41 - 24		
Kidney			
$\bar{x}$ (# of animals)	2.16 (15)	No tissue	5.8 (26)
SD	± 0.91		± 0.93
Range	0.909 - 4.05		
Muscle			
$\bar{x}$ (# of animals)	0.41 (11)	No tissue	0.88 (36)
SD	± 0.09		± 0.25
Range	0.31 - 0.56		

Table 5. Age, Sex, Location and Date[a] of Stranding for Affected Dolphins

Animal #	Sex	Age	Stranding Location	Date
WAM 209	F	1 year	Virginia Beach, VA	8-8-87
WAM 253	M	22 years	Fort Story, VA	8-29-87
WAM 258	M	Immature	Sandbridge, VA	9-1-87
WAM 264	F	13 years	Camp Pendelton, VA	9-4-87
WAM 269	F	2 years	Virginia Beach, VA	9-6-87
CWP 271	F	Immature	Virginia Beach, VA	9-25-87
CWP 272	F	< 3 mo.	Virginia Beach, VA	9-25-87
CWP 273	F	15 years	Little Creek, VA	9-27-87
VB 87 004	F	Mature	Virginia Beach, VA	10-5-87
VB 87 005	F	29 years	Virginia Beach, VA	10-6-87
VB 87 009	F	7 years	Virginia Beach, VA	10-6-87
VB 87 012B	M	16 years	Virginia Beach, VA	10-7-87
VB 87 014	F	1 year	Virginia Beach, VA	10-8-87
S 88 Tt 01	F	Immature	Atlantic Beach, FL	1-1-88
S 88 Tt 04	M	Mature	Amelia Island, FL	1-2-88
S 88 Tt 07	M	Immature	Daytona Beach, FL	1-4-88
S 88 Tt 10	M	Immature	St. John's County, FL	1-8-88
S 88 Tt 11	F	Mature	Volusia County, FL	1-10-88

[a]F = female M = male
Mature - > 5 years Immature - < 5 years
All affected animals are Atlantic bottlenose dolphins (Tursiops truncatus)

Table 5. (continued)

Animal #	Sex	Age	Stranding Location	Date
S 88 Tt 19	F	19 years	Fort Clinch Park, FL	1-13-88
S 88 Tt 27	M	13 years	St. John's County, FL	1-18-88
S 88 Tt 32	F	3 years	St. John's County, FL	1-19-88
S 88 Tt 33	M	1 year	St. Augustine Beach, FL	1-19-88
S 88 Tt 34	M	6 years	Atlantic Beach, FL	1-20-88
S 88 Tt 39	F	10 years	Ormond, FL	1-28-88
S 88 Tt 44	M	2 years	Ormond, FL	2-1-88
S 88 Tt 48	F	< 3 mo.	Volusia County, FL	2-7-88
S 88 Tt 49	F	1 year	Volusia County, FL	2-7-88
S 88 Tt 50	M	2 years	Ormond, FL	2-9-88
S 88 Tt 51	M	Immature	St. John's County, FL	2-10-88
S 88 Tt 55	F	7 years	Canaveral, FL	2-17-88
S 88 Tt 57	M	7 years	Canaveral, FL	2-19-88

Table 6. Information on NVSL Normal Dolphins

The dolphins listed below are NVSL normals. They are captive dolphins of unknown age, sex and species.

MH 82 227

MH 78 08

MH 75 16

MH 79 179

MH 83 216

The dolphins listed below are NVSL normals. They are harbor porpoises (Phocoena phocoena) of unknown age and sex.

MH 87 460

MH 86 250

MH 87 462

MH 87 479

MH 87 468

MH 87 455

MH 87 454

MH 87 453

MH 87 472

MH 87 456

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