Mercury and Selenium in Blood of Bottlenose Dolphins (*Tursiops truncatus*): Interaction and Reference to Life History and Hematologic Parameters

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Introduction

Mercury (Hg) contamination of freshwater and marine ecosystems is a grave environmental concern, particularly in the southeastern United States. In 2004, 218 fish consumption advisories were published for Florida, and *all* of the state's fresh and coastal waters were included in fish consumption advisories for mercury (USEPA, 2005). Although mercury occurs naturally, anthropogenic releases (primarily through burning of fossil fuel and waste incineration) have been variously estimated to account for roughly half of global mercury emissions (Pacyna & Pacyna, 2002; Pacyna et al., 2001; UNEP, 2002). Mercury released into the environment may inter-convert among various inorganic forms through physicochemical processes and eventually undergo biotic or abiotic methylation (Porcella, 1994). The resultant methyl mercury (MeHg) then enters the food web where it can both biomagnify and bioaccumulate (Gray, 2002). Consequently, MeHg may present an increased risk of toxicosis to upper level trophic organisms, particularly species with comparatively long lifespans such as humans and some piscivorous marine mammals. Because Se antagonizes Hg in laboratory studies, the coincident increase of Se and Hg in certain tissues of marine mammals (principally Odontoceti) implicates a protective role for Se against Hg toxicity (Wang et al., 2001). Unlike Hg, which has no known function in mammals, Se is an essential element, and as such, its levels are controlled tightly (homeostasis). As a component of the amino acid selenocysteine, Se is a constituent of numerous proteins, including the glutathione peroxidases (GSHPxs), which possess potent antioxidant properties, especially with respect to various hydroperoxides (Brigelius-Flohé, 1999).

Initial data from free-ranging bottlenose dolphins (*Tursiops truncatus*) inhabiting Sarasota Bay, FL, USA, indicated whole blood mean total Hg (THg) concentrations in excess of 500 µg/L. This concentration is approximately 10 times the 58 µg/L benchmark dose level for human cord blood (USEPA), and 100 times the level at which adverse neurodevelopmental effects can occur (5.8 µg/L in human cord blood) (Trasande et al., 2005). We embarked upon the current study with the following objectives: 1) to monitor and to explore relationships between levels of Hg, Se, and GSHPx in biopsies of blood and epidermis from Sarasota Bay *Tursiops*, and 2) to relate tissue Hg and Se concentrations to specific environmental, hematological, or morphometric parameters, including season, age, and trophic level as assessed by stable isotopic signatures of N.

Materials and Methods

For brevity, many analytic procedures are concisely presented and cited as published work.

Field Sampling

Blood samples from Sarasota Bay bottlenose dolphins were collected during February and June of 2004 and 2005. All sampling protocols were approved through the Mote Marine Laboratory and University of Alaska Fairbanks (Protocol Number: 04-60) Institutional Animal Care and Use Committees and conducted under National Marine Fisheries Service Scientific Research Permit #522-1569, issued to R. Wells. Dolphins were visually located, encircled by a seine net and immobilized via physical restraint. Immediately thereafter, blood was obtained by venipuncture of the ventral fluke vessels with a 19 ga. 3/4" butterfly catheter using standard antiseptic technique. First, blood from each dolphin destined for blood Hg, Se and GSHPx determinations was collected in two royal blue Vacutainer™ tubes with Na_2EDTA anticoagulant (Fisher Scientific International, http://www.fisherscientific.com) intended for trace element and toxicologic analyses. Additional blood was collected in Vacutainer™ red top serum tubes for analysis of serum Se. At each capture event, blanks were made in the field by drawing deionized water through a butterfly catheter into appropriate Vacutainer™ tubes, which were treated in the same fashion as the corresponding blood samples. In the field, all blood samples were placed on ice immediately after collection. Upon return to Mote Marine Laboratory (Sarasota, FL), blood from one royal blue Vacutainer™ tube was decanted into an acid-washed glass jar with Teflon®-lined polypropylene cap (Quality Environmental Containers, Inc., http://www.qecusa.com) and frozen at -20° C., while the other Vacutainer™ was refrigerated. Red top Vacutainers™ were centrifuged for approximately 5 minutes at 3000rpm. Serum was pipetted into polypropylene tubes and frozen at -20° C. Samples were shipped on ice overnight to the University of Idaho (Moscow, ID, USA) for analysis of GSHPx activity, blood total Hg, and blood and serum Se. Blood and serum remaining after these analyses was refrozen and shipped on ice overnight to the Wildlife Toxicology Laboratory at the University of Alaska Fairbanks (Fairbanks, AK, USA) for analysis of methyl mercury and stable isotopes.

Hematological indices were analyzed at Cornell University College of Veterinary Medicine Diagnostic Laboratory, Ithaca, NY, USA.

Data on the dolphins' age and sex were supplied by the Chicago Zoological Society's Sarasota Dolphin Research Program. Ages were determined via counts of dental cementum annuli (Randall Wells, pers. comm.).

Determination of GSHPx activity
GSHPx activity is measured as a modified procedure of Carmagnol et al. (1983).

Total mercury, methyl mercury and selenium analyses

Total Hg and Se were measured at the University of Idaho (Moscow), and total Hg and methyl Hg were determined at the University of Alaska Fairbanks following similar methods and QA/QC as reported in Woshner et al. (2001A and B), and Dehn et al. (2005 and 2006).

Analysis of stable isotopes

Stables isotopes of C and N were analyzed and reported as described in Dehn et al. (2005 and 2006).

Statistical analysis

Summary statistics, Student's t-test and linear regression analyses were performed using Excel® 2000 (Microsoft Corporation). Cluster analysis and stepwise regression was executed with Systat® 8.0 software (SPSS, Inc.).

Results

During 2004 and 2005, we sampled 46 dolphins (24 females; 22 males). Three dolphins were captured twice; for these, both samples were combined into a mean value for all variables examined. A few significant differences were noted among blood parameters in comparisons between females and males (Table 1). Although females had higher plasma protein and total protein than males, neither albumen nor globulin levels were significantly different between sexes. However, when summed (and taking rounding error into consideration), the differences in albumin and globulin appear to account for the 0.4 g/dl mean difference observed between sexes in both total and plasma protein (Table 1). Data were pooled for all subsequent analyses.

Mean age of 39 animals was 10.2 years (SD=7.77; range: 1.5 – 30.5). Summary data for selected blood parameters analyzed are presented in Table 2. On average, Se concentration in serum was approximately 50% the concentration of whole blood. Because the mean percentage of THg present as MeHg was 109±30 (range: 60–190), we concluded that virtually all of the Hg in the blood was present as methyl mercury and used THg in further statistical analyses.

We attempted to discriminate relative degrees of connection between the above variables through hierarchical clustering. Initial analysis showed that several basic hematologic indices clustered together (not shown). These parameters were removed from the subsequent cluster analysis (Figure 1).

Blood GSHPx activity correlated linearly (poor fit) with Se in whole blood ($P=0.03$; $F=5.06$; $R^2=0.13$), but not with serum Se ($P=0.12$; $F=2.54$; $R^2=0.07$) nor with blood THg ($P=0.36$; $F=0.86$; $R^2=0.02$). Blood Se correlated only marginally with serum Se or with blood THg. However, blood THg concentrations were related linearly (poor fit) to serum Se ($P=0.01$; $F=7.41$; $R^2=0.18$) as well as to serum albumen ($P=0.02$; $F=5.56$; $R^2=0.14$)

and trophic level as represented by $\delta^{15}N$ (P=0.03; F=5.19; R^2=0.14). Each of the following variables increased linearly as a function of Age: THg (P=<0.001); MeHg (P=<0.001); blood Se (P=0.007); serum Se (P=0.006); and GSHPx (P=0.04). A forward stepwise regression indicated that increase in blood THg in dolphins was linearly (good fit) related to the interaction of $\delta^{15}N$ and age (GLM, F=79.9, P<0.001, R^2 =0.727).

Discussion

Because all parameters presented here were measured in blood, we anticipated spurious associations among various analytes. Nevertheless, as a protein component, Se may be a determinant in levels of several of the hematological parameters assayed. Other selenoproteins in the blood (besides GSHPx) include type I iodothyronine deiodinase, the enzyme which converts the circulating form of inactive thyroid hormone, thyroxine (T_4), to the active 3,3′,5- triodothyronine (T_3). This conversion and its consequent influence on metabolic rate have been linked to Se availability (Hawkes and Keim, 2003). In a similar fashion, Hg has not only a strong affinity for Se, but for sulfhydryls as well; thus Hg concentration may relate to numerous blood parameters. Despite these numerous interrelationships and confounding effects inherent to the blood matrix, we can draw a few inferences from the preliminary data presented above. The grouping of serum Se, blood THg and blood lipid most likely reflects the contemporaneous uptake of Se, MeHg and lipids from food. Absorption of MeHg from food is virtually complete, with almost all absorbed MeHg in the blood contained within erythrocytes (Ancora et al., 2002). The fact that blood THg was intimately associated with age via hierarchical clustering is almost assuredly due to the selection of larger fish (i.e., older fish feeding on other smaller fish), which have bioaccumulated higher concentrations of mercury, by older dolphins. This connection is corroborated by the forward stepwise regression, which indicated that increase in blood THg in dolphins was linearly related to the interaction of $\delta^{15}N$ and age.

Previous researchers have surmised that odontocetes detoxify dietary MeHg through a process of demethylation, followed by subsequent binding of inorganic Hg to Se, and hepatic sequestration of the resultant mercuric selenide (HgSe) complexes (Martoja and Berry, 1980). Because these HgSe complexes apparently accumulate in the liver (and other internal organs such as kidney and brain) throughout life, the *percentage* of hepatic methyl mercury declines with age, even as total mercury increases (Joiris et al., 2001; Woshner et al., 2001A). In contrast, Hg in muscle is predominantly MeHg, with some research documenting slightly higher levels among older animals (Joiris et al., 2001; Woshner et al., 2001A). Generally speaking, the dynamics of Hg in cetacean muscle are not well grasped, and on balance the proportion of Hg existing as MeHg in internal tissues is small, except in very young animals. Thus, while it seems unlikely that Hg amassed in tissue may have served as a significant source of circulating Hg in bottlenose dolphins of this study, a contribution to circulating Hg by tissue pools cannot be ruled out.

In contrast to parameters reflecting recent dietary intake, blood Se and GSHPx levels are more meaningful manifestations of overall Se nutritional status, with correlations

between the two reported in various species (Koller et al., 1984; Whanger et al., 1988; Flueck, 1991). However, while dolphin blood Se levels are approximately 2 to 3 times those reported in terrestrial mammals, blood GSHPx activities were within ranges similar to terrestrial mammals (Koller et al., 1984; Whanger et al., 1988; Flueck, 1991; Puls, 1994). This contrasts markedly with the findings of Filho et al. (2002), who reported a three-fold greater level of GSHPx in marine mammals as compared to terrestrial species. Some researchers have noted that with high dietary Se, GSHPx levels asymptote as saturation levels are reached (Whanger et al., 1988). If this is so, then despite blood Se levels that are high (in relation to terrestrial species), GSHPx demand (and by extrapolation oxidative stress) is great enough to prevent such saturation in the Sarasota dolphins, since these two parameters were correlated in a linear fashion (albeit a poor fit, R2 = 0.13). Overall, these observations may connote that GSHPx levels in Sarasota dolphins, while not deficient, are not at a maximum, and may be sub-optimal due to high dietary Hg exposures, even in the face of adequate Se on a total concentration basis. Additional data and ongoing analyses will help us to better describe the complex relationships among Hg, Se, GSHPx and other life history and hematologic indices.

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Table 1. Parameters with significant differences between sexes, as well as albumen and globulin, as determined by Student's *t*-test.

Parameter	$Mean_{Females}$ (n)	$Mean_{Males}$ (n)	P (two-tail)	*t*-statistic
THg (µg/L)	702 (19)	389 (17)	0.01	2.03
Age (years)	13.25 (16)	8.10 (17)	0.04*	1.72
Plasma Protein (g/dl)	8.4 (22)	8.0 (22)	0.009	2.03
Total Protein (g/dl)	7.7 (24)	7.3 (22)	0.03	2.02
Albumin (g/dl)	4.6 (24)	4.6 (22)	0.39†	2.02
Globulin (g/dl)	3.1 (24)	2.8 (22)	0.11†	2.03

*One-tail
†Non-significant

Table 2. Summary statistics for various parameters in blood of bottlenose dolphins in Sarasota Bay, FL, USA, including total mercury (THg), methyl mercury (MeHg), selenium (Se), glutathione peroxidase (GSHPx), stable nitrogen isotope enrichment ($\delta^{15}N$), and selected hematologic indices.

Blood Parameter	mean	SD	Range	n
Se_{blood} (µg/ml)	0.77	0.17	0.46 – 1.30	36
Se_{serum} (µg/ml)	0.41	0.07	0.22 – 0.53	35
GSHPX (U/g Hgb)	98	25	59 – 170	46
THg_{blood} (µg/L)	554	378	110 – 1600	36
$MeHg_{blood}$ (µg/L)	545.50	442.53	90.50 – 2262.75	45
Mean $\delta^{15}N$ Atm-air (‰)	10.67	0.67	9.38 – 11.98	45
Hematocrit (%)	42	3	31 – 48	46
Hemoglobin (g/dl)	14.4	1.1	10.3 – 16.2	46
Plasma Protein (g/dl)	8.2	0.5	7.4 – 9.6	44
Total Protein (g/dl)	7.5	0.6	6.4 – 9.2	46
Lipid (mg/dl)	18	16	0 – 75	46
Albumin (g/dl)	4.6	0.2	3.9 – 5.4	46
T_3 (ng/dl)	1.72	0.43	0.89 – 2.90	46
T_4 (µg/dl)	13.05	2.27	7.29 – 18.23	42
Free T_4 (ng/dl)	2.71	0.86	1.30 – 5.32	42
Total Iron (TIRON) (µg/dl)	137	49	41 – 282	42
Total Iron Binding Capacity (TIBC) (µg/dl)	341	71	217 – 473	46

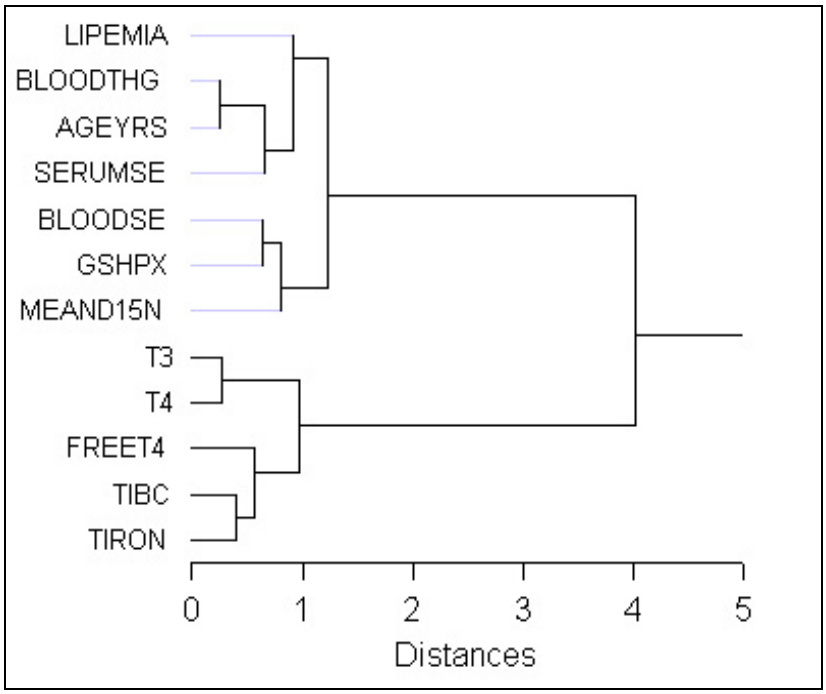

Figure 1. Hierarchical clustering of age and selected parameters (please refer to Table 2 for abbreviations; lipemia=blood lipid, mean15N= mean δ15N) in blood of bottlenose dolphins in Sarasota Bay, FL, USA, sampled 2004-2005. Distance metric is 1-Pearson correlation coefficient; Ward minimum variance method.