

Evaluation of circulating eosinophil count and adrenal gland function in California sea lions naturally exposed to domoic acid

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Objective—To determine the effect of natural exposure to domoic acid (DA) on eosinophil counts and adrenal gland function in California sea lions (*Zalophus californianus*).

Design—Cross-sectional prospective study.

Animals—39 California sea lions.

Procedures—Adult female sea lions admitted to a rehabilitation hospital during 2009 were classified into 1 of 3 groups (acute DA toxicosis, chronic DA toxicosis, or no DA exposure) on the basis of clinical signs, DA concentration in urine or feces, and hippocampal morphology. Endoparasite burden, eosinophil count, and serum cortisol and plasma ACTH concentrations were determined for each sea lion. For a subset of 8 sea lions, fecal glucocorticoid concentration after IM administration of cosyntropin was determined.

Results—Sea lions exposed to DA (acute DA toxicosis, n = 11; chronic DA toxicosis, 19) had higher eosinophil counts and lower serum cortisol concentrations, compared with values for sea lions with no DA exposure (9). Eosinophil count was not associated with endoparasite burden. Serum cortisol concentration was associated with plasma ACTH concentrations in sea lions from the no DA exposure group but not in sea lions in the acute or chronic DA toxicosis groups. Following cosyntropin injection, fecal glucocorticoid concentrations increased in all sea lions evaluated except 1.

Conclusions and Clinical Relevance—In adult sea lions, eosinophilia may be a cost-effective biomarker for DA exposure and may reflect alterations in hypothalamic, pituitary gland, or adrenal gland function. Domoic acid exposure may have subtle health effects on marine animals in addition to induction of neurologic signs. (*J Am Vet Med Assoc* 2012;241:943–949)

Domoic acid is a potent marine neurotoxin produced by some diatom species, especially *Pseudo-nitzschia australis*, which is being found more frequently along the California coast.^{1,2} The increasing incidence of DA-producing diatoms along the California coast has caused concern for marine animal health because DA is an excitatory amino acid that has a high affinity for the α -amino-5-hydroxy-3-methyl-4-isoxazole propionic acid and kainate subclasses of glutamate receptors in these animals. The interaction of DA and those glutamate receptors

ABBREVIATIONS	
CA	Cornu ammonis
CV	Coefficient of variance
DA	Domoic acid

causes cell depolarization, dysfunction, and death.³ In humans, DA toxicosis is the cause of amnesic shellfish poisoning, first recognized in Canada in 1987 among people who had consumed DA-contaminated shellfish.⁴ The clinical signs associated with DA toxicosis in humans include gastrointestinal distress, seizures, coma, and death.

Domoic acid toxicosis was first reported in wildlife in 1991 in pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) that died in Monterey Bay, Calif.⁵ It was diagnosed in California sea lions (*Zalophus californianus*) in 1998,⁶ and every year since then, sea lions with severe neurologic signs associated with acute and chronic exposure to DA have become stranded along the California shoreline.^{7,8} Domoic acid toxicosis has been diagnosed in northern fur seals (*Callorhinus ursinus*), and DA exposure has been confirmed in a variety of cetaceans in the Atlantic Ocean.^{9–12} Histopathologic lesions in sea lions and a fur seal that died of acute DA toxicosis were characterized by ischemic neuronal necrosis in

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the hippocampus, especially in CA areas CA4, CA3, and CA1 and the dentate gyrus.¹³ In sea lions that had clinical signs of DA toxicosis for at least 15 days before death (ie, chronic DA toxicosis), histopathologic lesions also included neuronal loss with parenchymal atrophy and gliosis.⁸

Although the clinical signs associated with DA toxicosis are predominantly neurologic, degenerative cardiomyopathy may also develop.¹⁴ In sea lions, the myocardial lesions are likely a result of the overstimulation of glutamate receptors in the myocardium.¹⁵ To date, the only hematologic abnormality found in sea lions with DA toxicosis has been a mild eosinophilia,⁷ the pathogenesis of which is unknown. An eosinophilia of unknown pathogenesis has also been detected in bottlenose dolphins (*Tursiops truncatus*) from the northern portion of the Gulf of Mexico that were exposed to DA; these dolphins also had decreased T-lymphocyte proliferation and increased neutrophil phagocytosis.^{12,16} Whether DA caused the eosinophilia in those dolphins could not be determined because of concurrent parasitosis. Results of a study¹⁷ conducted in laboratory animal species indicate that DA can bind to glutamate receptors in multiple organs, which suggests that in addition to its neurologic effects, DA may have various other subtle effects on animal health. Thus, DA exposure may affect circulating eosinophil counts via DA binding to glutamate receptors in tissues other than the hippocampus. Generally, eosinophilia is associated with parasitosis. However, eosinophilia has also been associated with low serum cortisol concentrations, and in the 1940s, it was used as a biomarker for adrenal gland dysfunction prior to development of the ACTH stimulation test.^{18,19} The purpose of the study reported here was to evaluate the concurrent endoparasite burden and adrenal gland function in California sea lions naturally exposed to DA in an effort to further elucidate the pathogenesis of the eosinophilia associated with DA toxicosis.

Materials and Methods

Animals—The study population included California sea lions found stranded along the California coast between San Luis Obispo and Humboldt Counties and then taken to The Marine Mammal Center in Sausalito, Calif, for clinical examination and treatment during 2009. For each sea lion, sex was determined on the basis of genital morphology, and age class was estimated by a combination of body length, tooth size, and stage of sagittal crest development.²⁰ Only adult female sea lions were included in the study reported here because those are the animals most commonly affected by DA toxicosis, presumably because of seasonal feeding behavior.^{2,6,7} Following an initial physical examination, any sea lion that was deemed to have a poor prognosis was euthanized via IV administration of a solution^a of 39% pentobarbital sodium and 5% phenytoin sodium (1 mL/5 kg [1 mL/11 lb]) into a subclavian vein. Sea lions that were not euthanized were individually housed in a pen with a pool of freshwater, observed hourly from 7 AM through 11 PM, and fed thawed herring 3 times

daily. These sea lions were treated as described,⁷ but despite clinical care, all died within 8 weeks after becoming stranded. All treatment and study procedures were authorized by the National Marine Fisheries Service and approved by The Marine Mammal Center's Internal Animal Care and Use Committee.

Study design—Each sea lion was classified into 1 of 3 groups: acute DA toxicosis, chronic DA toxicosis, or no DA exposure. The acute DA toxicosis group included sea lions that were observed to have seizures or were comatose at the time of initial physical examination, had detectable concentrations of DA in a urine or fecal sample that was collected within 48 hours after stranding, or, if no urine or fecal sample was available for determination of DA concentration, had acute hippocampal necrosis on histologic examination. The chronic DA toxicosis group included sea lions that had intermittent seizures, undetectable DA in a urine or fecal sample that was collected within 48 hours after stranding, and hippocampal atrophy on histologic examination. The no DA exposure group included sea lions that had undetectable DA in a urine or fecal sample that was collected within 48 hours after stranding and had no hippocampal abnormalities on histologic examination. For each sea lion, a CBC was performed to determine the circulating eosinophil count; also evaluated were serum cortisol and plasma ACTH concentrations, urine or fecal DA concentration, endoparasite burden, and gross and histologic abnormalities. An ACTH stimulation test was performed on a subset of 8 sea lions (acute DA toxicosis group, n = 2; chronic DA toxicosis group, 4; and no DA exposure group, 2), and fecal glucocorticoid concentrations were determined. The ACTH stimulation tests were performed to determine the potential site of physiologic changes in cortisol metabolism in animals affected by DA toxicosis. Because cortisol release from the adrenal gland inhibits ACTH release from the pituitary gland via negative feedback on the hypothalamic-pituitary axis, suppression of cortisol release by the adrenal gland should be reflected by a negative correlation between plasma ACTH and serum cortisol concentrations, and the ACTH stimulation test was expected to cause an increase in fecal glucocorticoid concentration only in sea lions in the no DA exposure group. Conversely, for sea lions with DA toxicosis, changes in serum cortisol concentration caused by pathological changes in the hypothalamus or pituitary gland should be reflected by a positive correlation between plasma ACTH and serum cortisol concentration following cosyntropin injection.

ACTH stimulation test—To each of the 8 sea lions on which an ACTH stimulation test was performed, cosyntropin^c (10 µg/kg [4.5 µg/lb], IM) was administered once. Fecal samples were opportunistically obtained from the pen floor of each sea lion (without the animals being restrained) for up to 3 days after cosyntropin administration. Fecal samples were frozen and stored at -70°C until analysis.

Sample collection—During initial physical examination, each sea lion (even a sea lion that was comatose) was restrained by 3 animal handlers and a conical net

to ensure both animal and handler safety. A 1.5-inch, 21-gauge needle was used to collect blood samples from a caudal gluteal vein directly into an evacuated serum separator tube^b (5 mL) and 2 tubes^b (5 mL each) containing EDTA. Blood samples (1 tube of blood mixed with EDTA and blood in the serum separator tube from each animal) were centrifuged at 1,500 \times g for 15 minutes within 30 minutes after collection to obtain plasma or serum, which was then frozen and stored at -70°C .

Fecal samples were obtained from the pen floor or from the rectum during necropsy. Urine samples were obtained from the urinary bladder during necropsy. Urine and fecal samples obtained for DA analysis were frozen immediately after collection and stored at -70°C .

A complete necropsy was performed on all sea lions, and representative tissue specimens of brain and all visceral organs were obtained and fixed in neutral-buffered 10% formalin. Formalin-fixed tissue specimens were embedded in paraffin, cut into sections with a thickness of 4 to 5 μm , placed on a microscope slide, deparaffinized, and stained with H&E stain for histologic evaluation.

CBC—Complete blood counts were performed via a hemacytometer^d on blood samples that were collected into tubes that contained EDTA. Manual WBC differential counts were performed on blood smears stained with Wright-Giemsa stain^e by the same technician throughout the study. Eosinophils were identified on the basis of the presence of segmented nuclei and eosinophilic granules similar to those observed in the eosinophils of domestic dogs.

Serum cortisol and plasma ACTH concentrations—Frozen serum and plasma samples were shipped on dry ice to the Clinical Pathology Laboratory at the Purdue University Veterinary Teaching Hospital for determination of serum cortisol and plasma ACTH concentrations. Serum cortisol concentrations were determined via an automated chemiluminescent immunoassay^f as described.^{21,22} Intra-assay variation was calculated with the results obtained from 19 replicates for each of 2 control samples (10.5 and 14.0 mg/dL) and ranged from 13.3% to 14.5%. Interassay variation was calculated with the results obtained from 19 replicates for each of 3 control samples (3.6, 11.8, and 25.5 mg/dL) and ranged from 7.4% to 8.0%.

Plasma ACTH concentrations were determined via a solid-phase, 2-site chemiluminescent enzyme immunoassay,⁸ which was used to measure the intact ACTH molecule (amino acids, 1 to 39) via a mouse monoclonal capture antibody directed against the 18 to 39 C-terminal region and an enzyme-labeled rabbit polyclonal antibody directed against the 1 to 24 N-terminal region of the ACTH molecule. On the basis of results of previous studies,^{21,22} ACTH-related peptides, ACTH (22 to 29), and melanocyte-stimulating hormone do not interfere and ACTH (1 to 18) and ACTH (1 to 24) cause only minimal interference with the performance of the chemiluminescent enzyme immunoassay, whereas ACTH (18 to 39), a corticotropin-like intermediate lobe peptide, has some cross-reactivity (12% to 17%) with the chemiluminescent enzyme immunoassay. Information supplied by the assay's manufacturer in-

dicated that the detection limit of the assay (defined as the ACTH concentration at 2 SDs above the ACTH concentration of a blank sample) was 5 pg/mL with a working range of 12 to 1,250 pg/mL. The intra-assay variability was determined via calculation of the CVs of 8 replicates for each of 3 samples, which had low (16 pg/mL), moderate (247 pg/mL), or high (450 pg/mL) ACTH concentrations. The intra-assay CVs were considered acceptable (ie, $< 10\%$) and were 8.22%, 4.09%, and 4.26% for low, moderate, and high ACTH concentrations, respectively. The interassay variability was determined via calculation of the CVs of 4 replicates for each of the same 3 samples used for the calculation of intra-assay variability.²² The interassay CVs were considered acceptable (ie, $< 15\%$) and were 6.01%, 4.62%, and 14.8% for low, moderate, and high ACTH concentrations, respectively.

Urine and fecal DA concentration—Frozen urine and fecal samples were shipped on dry ice to the Northwest Fisheries Science Center. The DA concentration in each sample was determined via an ELISA^h and high-performance liquid chromatography as described.⁹

Fecal glucocorticoid concentration—Frozen fecal samples were lyophilized in a freeze dryer for 48 hours to remove all moisture and then homogenized. Two samples (approx 0.1 g each) of dry fecal powder were each extracted with 15 mL of 70% ethanol in distilled water and placed on a pulsing vortexerⁱ for 30 minutes and then centrifuged at 1,200 \times g for 20 minutes. The supernatants from the 2 samples were combined, and a portion (5 mL) was stored at -20°C for up to 3 months prior to glucocorticoid testing. The glucocorticoid concentration in the sample was determined via a corticosterone double-antibody ¹²⁵I radioimmunoassay^j performed in accordance with the manufacturer's instructions. Intra-assay variation was 3.93%, and interassay variation was 13.0%.

Endoparasite burden—Gross evaluation of the lung for *Parafilaroides* spp, stomach for Aniskidae, intestine for cestodes, and liver for *Zalophotrema* spp was performed by 1 investigator (FMDG) during the necropsy of each sea lion to determine its endoparasite burden. The parasite burden of each sea lion was graded on a scale of 1 to 4. Sea lions that had no evidence of endoparasite infestation were assigned a score of 1. A mild endoparasite infestation was scored as 2 and defined as a sea lion in which *Parafilaroides* spp were identified in 1 of 4 areas of lung tissue examined by scraping the parenchyma with a scalpel, 1 to 20 Anisakidae were observed in the lumen of the stomach, cestodes were identified in 1 of 4 areas of the jejunum examined, or 1 to 10 *Zalophotrema* spp were observed in the biliary tree. A moderate endoparasite infestation was scored as 3 and defined as a sea lion in which *Parafilaroides* spp were identified in 2 of 4 areas of lung tissue examined by scraping the parenchyma with a scalpel, 21 to 100 Anisakidae were observed in the lumen of the stomach, cestodes were identified in 2 of 4 areas of the jejunum examined, or 11 to 50 *Zalophotrema* spp were observed in the biliary tree. A severe endoparasite infestation was scored as 4 and was defined as a sea lion in which *Parafilaroides* spp were

identified in > 2 of 4 areas of lung tissue examined grossly by scraping the parenchyma with a scalpel, > 100 Anisakidae were observed grossly in the lumen of the stomach, cestodes were identified in > 2 of 4 areas of the jejunum examined, or > 50 *Zalophotrema* spp were observed in the biliary tree.

Statistical analysis—For each DA classification group (acute DA toxicosis, chronic DA toxicosis, and no DA exposure), the distribution of the respective data for eosinophil count, serum cortisol concentration, plasma ACTH concentration, urine and fecal DA concentration, and fecal glucocorticoid concentration were evaluated for normality via the Kolmogorov-Smirnov test. A logarithmic transformation was used to normalize the distribution of any data that were not normally distributed prior to statistical analyses; 1 was added to values of 0 so that the logarithmic transformation could be performed. Differences in the geometric mean eosinophil counts, geometric mean serum cortisol concentrations, and arithmetic mean plasma ACTH concentrations among the DA classification groups were evaluated via generalized linear regression and 1-way and multivariable ANOVA. Fecal glucocorticoid concentration was evaluated via a generalized additive mixed model to account for the longitudinal nature of the data. All analyses were performed with statistical software,^k and values of $P < 0.05$ were considered significant.

Results

Animals—Thirty-nine adult female California sea lions were found stranded along the California coast between San Luis Obispo and Humboldt Counties from February 1 through September 30, 2009, and were in-

cluded in the present study. Acute DA toxicosis was diagnosed in 11 of those sea lions, and chronic DA toxicosis was diagnosed in 19. The 9 sea lions that were classified in the no DA exposure group had osteomyelitis ($n = 3$), leptospirosis (2), or carcinoma (4). Three sea lions (1 from each classification group) were comatose during the initial physical examination. Six sea lions were euthanized immediately after the initial physical examination because they had poor prognoses. The remaining 33 sea lions died within 8 weeks after becoming stranded despite clinical care.

Eosinophil count—The WBC counts for all sea lions were within the reference range²⁴ (3.4×10^9 WBCs/L to 11.3×10^9 WBCs/L) for captive adult California sea lions. The geometric mean circulating eosinophil counts for each classification group were summarized (Table 1). Sea lions in the acute and chronic DA toxicosis groups had eosinophilia (reference range,²⁴ 0×10^9 eosinophils/L to 0.68×10^9 eosinophils/L). The geometric mean eosinophil count differed significantly ($P = 0.014$) among the 3 classification groups. The geometric mean eosinophil count did not differ significantly ($P = 0.481$) between the acute DA toxicosis group and the chronic DA toxicosis group; therefore, the data for both groups were combined and the median eosinophil count (0.925×10^9 eosinophils/L) for the combined DA toxicosis group was significantly ($P = 0.007$) higher than that for the no DA exposure group.

Serum cortisol and plasma ACTH concentrations—The serum cortisol concentrations were characterized by high variability, and the geometric mean serum cortisol concentrations differed significantly ($P < 0.001$) among the 3 classification groups (Table 1). Similar to eosinophil count, geometric mean se-

Table 1—Geometric mean \pm SD, median, and range for eosinophil count and serum cortisol concentration and arithmetic mean \pm SD, median, and range for plasma ACTH concentration for 39 adult female California sea lions (*Zalophus californianus*) that became stranded along the California coast between San Luis Obispo and Humboldt Counties from February 1 through September 30, 2009, and were classified as having acute DA toxicosis ($n = 11$), chronic DA toxicosis (19), or no DA exposure (9).

Variable	Sea lion classification group	Mean \pm SD	Median	Range
Eosinophil count ($\times 10^9$ cells/L)	Acute DA toxicosis	1.09 \pm 0.59	1.09	0.10–2.07
	Chronic DA toxicosis	0.97 \pm 0.89	0.81	0.00–2.94
	No DA exposure	0.28 \pm 0.44*	0.00	0.00–1.13
Cortisol (mg/dL)	Acute DA toxicosis	18.77 \pm 25.54	12.00	6.40–90.80
	Chronic DA toxicosis	11.19 \pm 3.04	10.70	7.58–17.20
	No DA exposure	56.94 \pm 40.38*	50.00	7.09–133.00
ACTH (pg/mL)	Acute DA toxicosis	68.45 \pm 72.25	32.70	13.20–247.00
	Chronic DA toxicosis	50.31 \pm 37.86	34.50	25.60–169.00
	No DA exposure	88.83 \pm 52.49	82.50	28.30–164.00

The acute DA toxicosis group included sea lions that were observed to have seizures or were comatose at the time of initial physical examination, had detectable concentrations of DA in a urine or fecal sample that was collected within 48 hours after stranding, or, if no urine or fecal sample was available for determination of DA concentration, had acute hippocampal necrosis on histologic examination. The chronic DA toxicosis group included sea lions that had intermittent seizures, undetectable DA in a urine or fecal sample that was collected within 48 hours after stranding, and hippocampal atrophy on histologic examination. The no DA exposure group included sea lions that had undetectable DA in a urine or fecal sample that was collected within 48 hours after stranding and had no hippocampal abnormalities on histologic examination. A logarithmic transformation was applied to the data for eosinophil count and serum cortisol concentration, respectively, to normalize the distributions of the data for statistical analyses; 1 was added to data with values of 0 so the logarithmic transformation could be performed.

*Value differs significantly ($P < 0.05$) from the respective values of the other 2 classification groups.

rum cortisol concentrations did not differ between the acute DA toxicosis group and the chronic DA toxicosis group; therefore, the data from the 2 groups were combined. The geometric mean serum cortisol concentration for the combined DA toxicosis group was significantly ($P < 0.001$) lower than that for the no DA exposure group.

Data for the plasma ACTH concentrations were normally distributed; therefore, a logarithmic transformation was not applied to those data. Similar to the serum cortisol concentration, the plasma ACTH concentrations were characterized by high variability. The arithmetic mean plasma ACTH concentration did not differ significantly among the 3 classification groups (Table 1). Results of a multivariable ANOVA indicated a significant ($P < 0.001$) positive association between plasma ACTH concentration and serum cortisol concentration for sea lions in the no DA exposure group; however, a similar association was not identified for sea lions in the acute or chronic DA toxicosis groups (Figure 1).

Association between eosinophil count and serum cortisol concentration—Eosinophil counts for sea lions in the acute and chronic DA toxicosis groups differed greatly, whereas the serum cortisol concentrations were < 20 mg/dL for all except 1 sea lion in the acute DA toxicosis group. Conversely, most of the sea lions in the no DA exposure group had no detectable circulating eosinophils and cortisol concentrations > 30 mg/dL. Despite these trends within classification groups, a linear relationship between eosinophil count and cortisol concentration was not identified (Figure 2). Results of multivariable ANOVA indicated that classification group was a significant predictor of eosinophil count and cortisol concentration; sea lions in the no DA exposure group generally had lower eosinophil counts and higher serum cortisol concentrations than did sea lions in the acute and chronic DA toxicosis groups.

ACTH stimulation test—Fecal glucocorticoid concentrations differed among the 8 sea lions on which an ACTH stimulation test was performed. Although glucocorticoid concentrations in the fecal samples collected prior to ACTH injection differed substantially among individual sea lions, all but 1 sea lion had a $> 110\%$ increase in glucocorticoid concentration between the fecal sample with the lowest glucocorticoid concentration (at or immediately following ACTH injection) and the subsequent fecal sample with the peak glucocorticoid concentration. The 1 exception (a sea lion in the chronic DA group) had a fecal glucocorticoid concentration that was among the highest observed (1,556 ng/g) at the time of cosyntropin administration, which decreased thereafter. Peak fecal

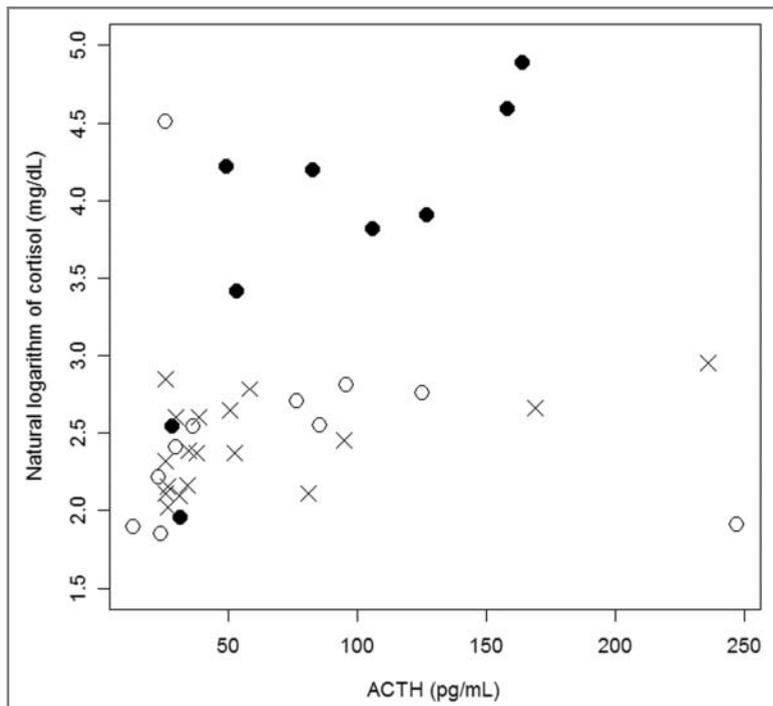


Figure 1—Scatterplot of the natural logarithm of serum cortisol concentrations versus plasma ACTH concentrations for 39 adult female California sea lions (*Zalophus californianus*) that became stranded along the California coast between San Luis Obispo and Humboldt Counties from February 1 through September 30, 2009; the lions were classified as having acute DA toxicosis ($n = 11$; white circles), chronic DA toxicosis (19; crosses), or no DA exposure (9; black circles). The acute DA toxicosis group included sea lions that were observed to have seizures or were comatose at the time of initial physical examination, had detectable concentrations of DA in a urine or fecal sample that was collected within 48 hours after stranding, or, if no urine or fecal sample was available for determination of DA concentration, had acute hippocampal necrosis on histologic examination. The chronic DA toxicosis group included sea lions that had intermittent seizures, undetectable DA in a urine or fecal sample that was collected within 48 hours after stranding, and hippocampal atrophy on histologic examination. The no DA exposure group included sea lions that had undetectable DA in a urine or fecal sample that was collected within 48 hours after stranding and had no hippocampal abnormalities on histologic examination. A logarithmic transformation was applied to the data to normalize the distributions of the data for statistical analyses; 1 was added to data with values of 0 so the logarithmic transformation could be performed.

glucocorticoid concentrations and the magnitude of change from trough to peak fecal glucocorticoid concentration after cosyntropin injection did not differ significantly among DA classification groups. Peak fecal glucocorticoid concentration was detected between 28 and 50 hours after cosyntropin administration in all except 1 sea lion, in which peak fecal glucocorticoid concentration was detected 160 hours after cosyntropin injection.

Endoparasite burden and histologic evaluation—Endoparasite burden score was not significantly associated with eosinophil count or serum cortisol concentration. Histologic evaluation revealed no clinically relevant abnormalities in adrenal gland or pituitary gland morphology for any of the sea lions evaluated. However, morphology of the hippocampus differed among the sea lions and ranged from no detectable abnormalities to necrosis or severe atrophy.

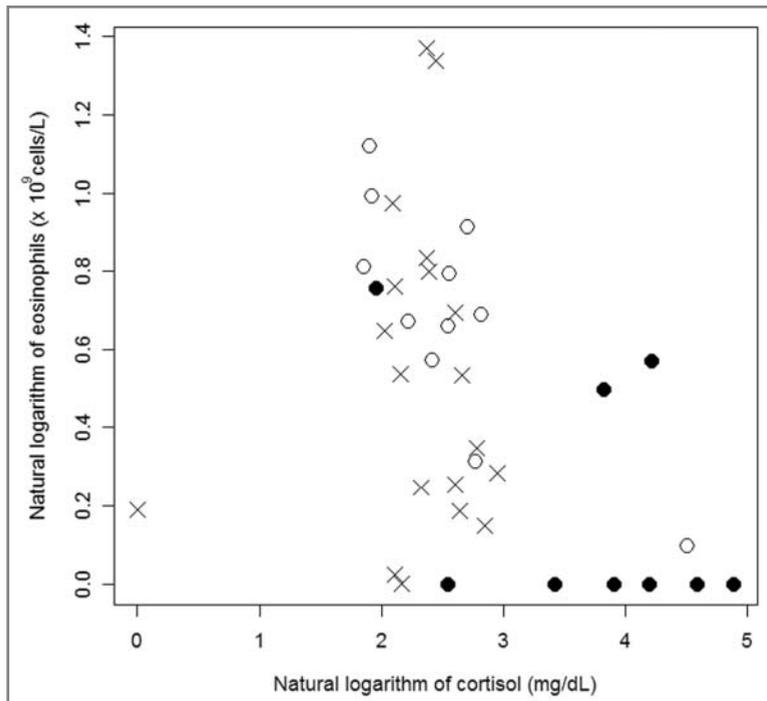


Figure 2—Scatterplot of the natural logarithm of eosinophil counts versus the natural logarithm of serum cortisol concentrations for the same 39 California sea lions in Figure 1. See Figure 1 for remainder of key.

Discussion

Results of the present study indicated that sea lions affected with DA toxicosis develop eosinophilia and are consistent with the results of another study.⁷ In the present study, the eosinophilia in sea lions affected with DA toxicosis was associated with a low serum cortisol concentration but was not associated with endoparasite burden. Although a reference range for serum cortisol concentration in California sea lions has not been established, the serum cortisol concentrations detected in the sea lions with DA toxicosis in the present study were generally lower than the serum cortisol concentrations detected in clinically normal Steller sea lions (*Eumatopias jubatus*), a taxonomically similar species.²³ Results of that study²³ also indicate that fecal corticosterone concentrations in California sea lions increase for 48 hours after manual restraint. In the present study, all sea lions were restrained in the same manner for blood sample collection; therefore, it is unlikely that the stress of restraint caused the differences in eosinophilia counts and serum cortisol concentrations detected among the classification groups (acute DA toxicosis, chronic DA toxicosis, and no DA exposure). Moreover, stressed cetaceans often develop neutropenia and eosinopenia,²⁴ conditions not observed in the sea lions of the present study despite the fact that all the sea lions were likely stressed by disease and rehabilitation.

The lack of an association between eosinophil count and endoparasite burden in the present study may suggest that DA is more effective in inducing eosinophilia than are parasites. However, endoparasites are ubiquitous in wild sea lions; thus, the reference range that has been established for eosinophil counts in California sea lions may not accurately reflect the

eosinophil count for sea lions not infested with endoparasites.²⁴ On the basis of results of the present study, we cannot determine whether the low serum cortisol concentration caused the eosinophilia detected in the sea lions from the acute and chronic DA toxicosis groups or whether DA toxicosis caused the eosinophilia independent of the serum cortisol concentration. Interestingly, in humans, circulating eosinophil counts were used as a measure of adrenal gland function for the Thorn test prior to the advent of the ACTH stimulation test, which suggests that there may be a causal relationship between serum cortisol concentration and eosinophilia.^{18,19}

Domoic acid may cause eosinophilia independent of serum cortisol concentration. Subclinical exposure to DA was associated with eosinophilia in wild bottlenose dolphins,^{12,16} and exposure to DA can alter other hematologic components.²⁵ Investigators of an *in vitro* study²⁵ reported that exposure of peripheral blood leukocytes obtained from clinically normal California sea lions to DA increases conavalin A–induced T-lymphocyte proliferation, but the mechanism by which DA caused that effect is unknown. It was recently hypothesized²⁶ that excessive activation of glutamate receptors by DA causes astrocyte damage; the damaged astrocytes release arginine, which stimulates an increased turnover and proliferation rate of eosinophils to uptake the arginine. Also, cytokine-induced eosinophilia has been associated with hypereosinophilic syndromes that have been detected in other animal species without exposure to DA.²⁷ Future research conducted to determine the pathogenesis of eosinophilia induced by DA in marine animals should focus on the evaluation of glutamate receptors in tissues other than the hippocampus as well as the potential role of arginine and various cytokines.

The fact that the mean plasma ACTH concentration did not differ significantly among the 3 classification groups and the lack of an association between serum cortisol and plasma ACTH concentrations in sea lions with acute or chronic DA toxicosis suggest that the low serum cortisol concentration may be the result of an inadequate adrenal gland response to ACTH. However, the ACTH stimulation test results were comparable among all DA classification groups; thus, the low serum cortisol concentration in the sea lions with acute and chronic DA toxicosis was likely the result of abnormal function of the hypothalamus or pituitary gland. On the basis of the results of the present study, we were unable to determine whether this inadequate adrenal gland response was caused by hypothalamic-pituitary-adrenal gland exhaustion or DA activation (ie, binding) of glutamate receptors in the endocrine glands, and it is possible that changes occur at multiple sites following exposure to DA. The lack of consistent morphological changes in the adrenal and pituitary glands of sea lions with DA toxicosis did not help elucidate which possibility was more likely. Adrenal gland insufficiency as-

sociated with acute conditions such as septic shock has been described in domestic animals and humans and is referred to as critical illness-related corticosteroid insufficiency.²⁸ However, in the present study, 2 severely ill sea lions with conditions other than DA toxicosis (ie, trauma or cancer) did not have adrenal gland insufficiency as evidenced by increased fecal glucocorticoid concentrations after cosyntropin administration. To further evaluate cortisol concentrations in sea lions with DA toxicosis, ACTH stimulation tests should be performed on sea lions with peracute DA toxicosis. Future research should also focus on the characterization and distribution of glutamate receptors in the hypothalamus and adrenal and pituitary glands.

Results of the present study indicated that natural exposure of California sea lions to DA altered both hematologic and endocrine variables. Thus, even though most animals with DA toxicosis have severe neurologic deficits, clinicians should also consider subtle health effects caused by changes in hormone concentrations when making decisions regarding prognosis and treatment of animals exposed to DA. This is especially important because the number of marine animals exposed to DA is likely to increase as the presence of DA-producing algae along coastlines increases.

- a. Beuthanasia-D Special, Schering-Plough Animal Health Corp, Union, NJ.
- b. Becton, Dickinson and Co, Franklin Lakes, NJ.
- c. Cosyntropin for Injection, Bioniche Pharma, Rosemont, Ill.
- d. Vet ABC, SCIL America, Gurnee, Ill.
- e. EMD Chemicals Inc, Gibbstown, NJ.
- f. Immulite immunoassay system, Diagnostics Product Corp, Los Angeles, Calif.
- g. Immulite ACTH, Diagnostics Product Corp, Los Angeles, Calif.
- h. Okadaic acid (DSP) ELISA kit, Biosense Laboratories, Bergen, Norway.
- i. Pulsing vortexer, Glas-Col LLC, Terre Haute, Ind.
- j. Corticosterone double antibody-125I RIA Kit, MP Biologicals, Solon, Ohio.
- k. R, version 2.14.0, R Foundation for Statistical Computing, Vienna, Austria. Available at www.r-project.org. Accessed Jan 31, 2010.

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