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A comparison of published brevetoxin tissue levels in West Indian manatee, bottlenose dolphin and double-crested cormorants in southwest Florida

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Abstract

This study compares reported brevetoxin concentrations in liver, kidney, brain, lung and stomach contents of West Indian manatees *(Trichechus manatus latriostris),* bottlenose dolphins (*Tursiops truncates*), and double-crested cormorants (*Phalacrocorax auritus*) that died due to exposure to brevetoxin in southwest Florida, to identify differing responses to brevetoxicosis. Liver, kidney, and brain of both manatee and cormorants had significantly higher levels of brevetoxin compared to dolphins, with manatee liver concentrations significantly higher compared to cormorants. Dolphins had significantly higher loads in their stomach contents compared to both manatee and cormorants, which were also significantly different from each other. Lung concentrations of brevetoxin were similar in all species. Manatee and cormorants had similar responses to brevetoxin, while dolphins appeared to be more vulnerable. Ingestion appears as the primary route of brevetoxin exposure, but inhalation is possible in all three species. [JMATE. 2012;5(1):20-27]

Keywords: *Karenia brevis*; muscle; blubber; lung; kidney

Introduction

Since the late 1800s, mortality in various marine animals connected to algal blooms have been reported on the lower west coast of Florida (19). The main culprit has been identified as *Karenia brevis* – an unarmoured dinoflagellate that can easily lyse in turbulent water, releasing brevetoxin (37). Brevetoxin is a neurotoxin that can remain in the local environment well after *K.brevis* blooms, also known as red tide, have terminated (26). In choppy water, the toxin can become aerosolized and remain above the water surface, affecting a wider range of species (7,35). This phenomenon is mostly isolated to marine ecosystems in or near southwest Florida, as the only other *Karenia* species that produces brevetoxin is New Zealand's *Karenia concordia* (16).

Marine animal species that are significantly affected by brevetoxicosis include the West Indian manatee (*Trichechus manatus latriostris*), bottlenose dolphin (*Tursiopsis truncates*) and double-crested cormorant (*Phalacrocorax auritus*). Manatee and dolphins are marine mammals that have completely different diets, herbivorous versus piscivorous respectively, and are the most commonly encountered marine mammal species in southwest Florida. Dolphins and cormorants eat many of the same fish species that can accumulate high levels of brevetoxin during a bloom, while manatee and cormorants display many of the same symptoms indicating brevetoxicosis. All three species were found to have uniquely large morbidity/ mortality events during past *K.brevis* blooms when compared to other local species. Whether they exhibit differences in their tissue accumulation of brevetoxin is less clear.

West Indian manatee strandings are well documented and individuals have stranded either alive or dead. The live individuals have allowed for observation of certain behaviours that have become clinical indicators of brevetoxicosis such as disorientation, inability to submerge or maintain horizontal position, back flexing, and laboured breathing (5,29). From February to April 1982, a *K.brevis* bloom of 9.6×10^6 cells/L in the area of the lower Caloosahatchee River and nearby waters of southwestern Florida caused 39 manatee deaths (29) which appeared to be primarily due to ingestion of contaminated seagrass which remained coated long after bloom termination (26). From March to May of 1996, there was a mass mortality event of 149 manatees during a bloom of 23.3×10^6 cells/L located in Charlotte Harbour (5,16). This event appeared to include aerosolized brevetoxins as evidenced by inflammatory lesions present on the respiratory tracts of many individual animals.

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Bottlenose dolphins (*Tursiops truncatus*) have also been widely affected by ingesting contaminated fish in southwest Florida and in the Florida panhandle (10,15,16). In 2004 alone, Flewelling investigated 69 dolphin deaths in southwest Florida and 107 deaths in the Florida panhandle that occurred during a 2 month period (15,16). Surprisingly there was no *K.brevis* bloom at the time but stomach contents of these carcasses revealed amounts of brevetoxin as high as 12,151 ng/g dry weight (12). Undigested fish (*Brevoortia* menhaden spp.) that were recovered whole and tested separately were also heavily contaminated with brevetoxin (15).

Like dolphins, double-crested cormorants (*Phalacrocorax auritus*) experience brevetoxin poisoning and have a largely piscivore diet, which consists of local species like pinfish. In fact, cormorants make up the bulk of marine birds admitted to rehabilitation centres in southwest Florida during *K.brevis* blooms (2). Unlike dolphins who died of brevetoxicosis and yet showed minimal abnormal findings at necropsy (16,17), cormorants had reported lesions such as multi-organ congestion, haemorrhaging and hemosiderosis, all also common to manatee with brevetoxicosis (5,25). Affected cormorants also had atrophied musculature and reduced body weight (2), a feature not seen in manatees (29). With treatment and care, both manatees and cormorants have been shown to recover fully within days (25,29). Since dolphins with brevetoxicosis have not been witnessed live, no information is available on whether dolphins could be successfully treated for brevetoxicosis (10).

This review compares published brevetoxin tissue levels in various organs of West Indian manatee (*Trichechus manatus latriostris*), bottlenose dolphin (*Tursiopsis truncates*) and double-crested cormorant (*Phalacrocorax auritus*) in order to identify whether species differences exist.

Methods

Brevetoxin concentrations (ng/g dry weight) for liver, kidney, brain, lung and stomach contents for each of the three target species were compiled from the existing scientific literature (Table 1). The data included in this review were from animals who either died of confirmed brevetoxicosis or died in the midst of an algae bloom greater than 10^4 cells/L. The tissue analysis (ng/g dry weight) used by all studies was enzyme-linked immunoabsorbant assay (ELISA) as described by Naar (27). Any concentrations that were reported as below the detectable limit were set to 0 ng/g dry weight.

Mean concentration and standard deviation were calculated for each organ data set within a species. Analysis of Variance (ANOVA) with Bonferroni correction for multiple group comparisons was used to compare across organs within a species and then across species within an organ. Significance was taken at $P \leq 0.05$. Significance was also confirmed using alternate statistical tests including unpaired T-test with Welch correction, Mann-Whitney, and to account for any deviation from a normal distribution, the Kruskal-Wallis with Dunn correction. The latter is especially relevant for non-parametric data and confirmed the results as reported.

Results

Liver tissue comparisons (Figure 1) showed that manatee accumulated the most brevetoxin at 158.4±69.9 ng/g , cormorants accumulated second most at 66.4±51.6 ng/g, and dolphins the least at 32.0±27.0 ng/g. Manatee liver brevetoxin levels were significantly higher when compared to cormorants, while dolphin livers had significantly less than both manatees and cormorants.

Species	Fresh Samples		Archived Samples		Reference #
	# died	year stranded	# died	year stranded	
West Indian manatee	26	2002	61	1996	16
bottlenose dolphin	83	1994-2003; 1996-2006	37	2001-03; 2004	10; 11; 16 ; Fire,pers comm)
double-crested cormorant	39	2005; 2002-06	none	none	36; 2

Table 1: Source of data included in paper. Both fresh and archived tissues were assessed.

In kidneys (Figure 2), manatee accumulated the most brevetoxin at 34.7±14.6 ng/g, cormorants second most at 34.5±31.1 ng/g and dolphins the least at 17.4±17.9 ng/g. Manatee and cormorant kidney tissue brevetoxin levels were similar whereas dolphins had significantly less than both manatee and cormorants.

When comparing brain tissue (Figure 3), cormorants revealed the highest brevetoxin concentration at 15.8±15.8 ng/g, manatee second at 13.9±3.7 ng/g, and dolphins the least at 4.7±5.3 ng/g.

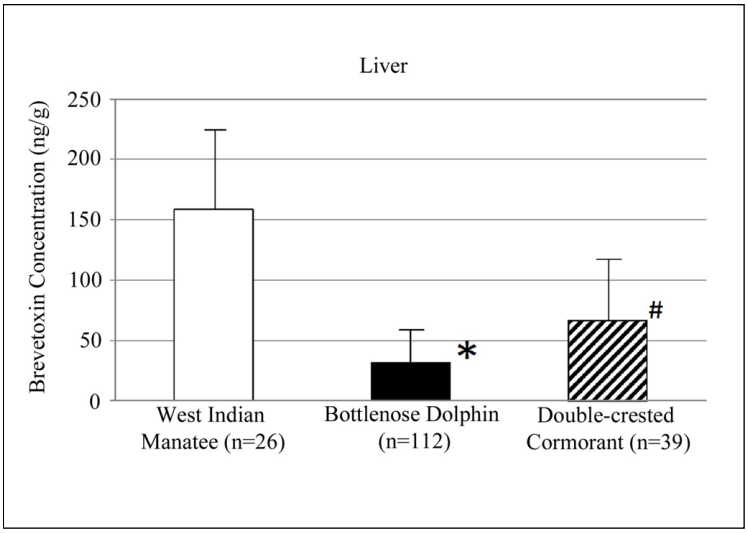

*Figure 1: Brevetoxin concentration (ng/g dry weight) found in liver tissue samples of West Indian manatee, bottlenose dolphin, and double-crested cormorant (mean±SD). *significantly different from both manatee and cormorant (P<0.0001); # significant different from manatee (P<0.0001).*

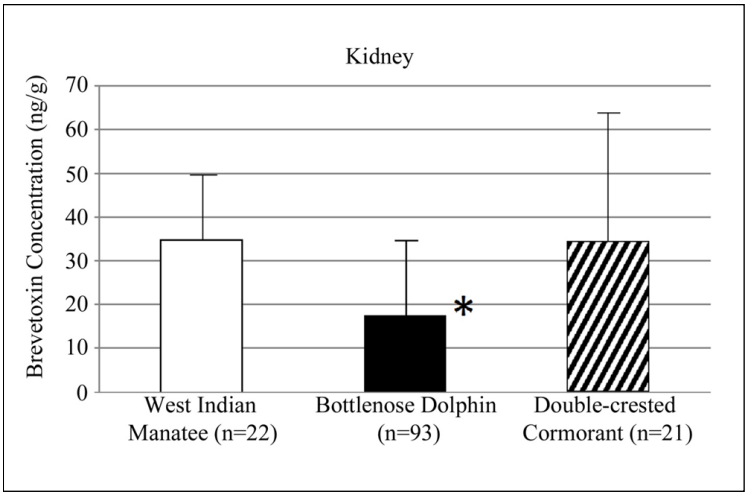

*Figure 2: Brevetoxin concentration (ng/g dry weight) found in kidney tissue samples of West Indian manatee, bottlenose dolphin and double-crested cormorant (mean±SD); *significantly different from both manatee (P<0.0001) and cormorant (P<0.001).*

Brevetoxin brain concentrations in manatee and cormorants were not significantly different, however, dolphin brains had significantly less than both those of manatees and cormorants.

Comparisons of stomach contents (Figure 4) showed that dolphins had the highest brevetoxin concentration at 1095.1±1525.4 ng/g, manatee second at 425.7±292.3 ng/g, and cormorants the least at 162.1±377.7 ng/g. Brevetoxin levels in manatee stomach contents was significantly higher than cormorants, while

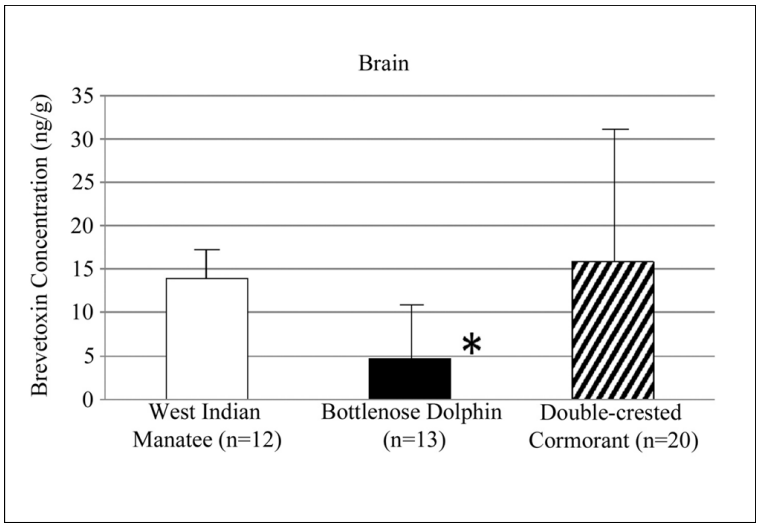

*Figure 3: Brevetoxin concentration (ng/g dry weight) found in brain samples of West Indian manatee, bottlenose dolphin, and double-crested cormorant (mean±SD); *significantly different from both manatees (P<0.0001) and cormorants (P<0.05).*

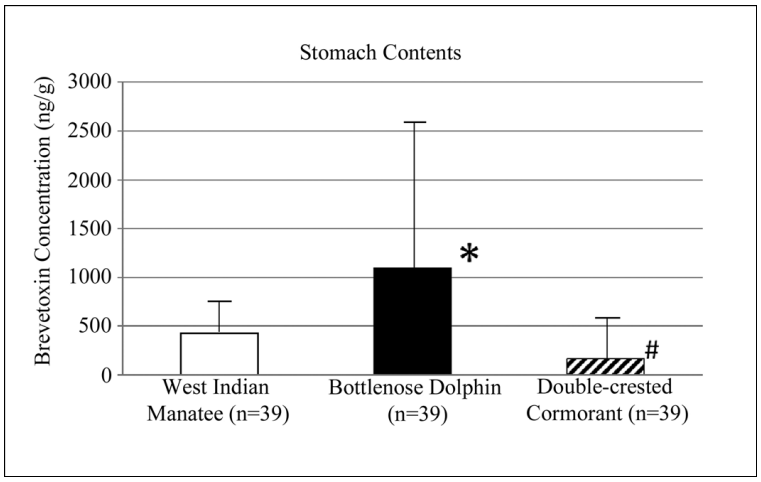

*Figure 4: Brevetoxin concentration (ng/g dry weight) found in stomach contents tissue samples of West Indian manatee, bottlenose dolphin, and double-crested cormorant (mean±SD); *significantly different from both manatee(P<0.01) and cormorant (P<0.001); #significantly different from manatee (P<0.001).*

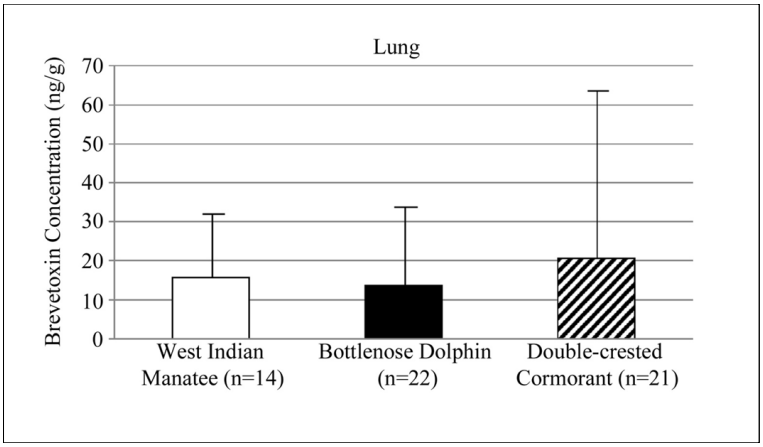

Figure 5: Brevetoxin concentration (ng/g dry weight) found in lung tissue samples collected from West Indian manatee, bottlenose dolphin, and double-crested cormorant (mean±SD); no groups were significantly different (P=0.73).

dolphin levels were significantly higher than both manatee and cormorants.

Lastly, when lung tissue levels were compared (Figure 5), cormorants presented the most brevetoxin at 20.6±42.6 ng/g, manatee second at 15.7±15.1 ng/g, and dolphins the least at 13.7±18.2 ng/g. However, these values were not significantly different from each other.

Discussion

There is an apparent difference in how these three species respond to brevetoxin, when comparing the reported concentration of the toxin found in various organs. The fact that the difference mostly lies between dolphins and the other two species is surprising given that ingestion appears to be the major route of exposure to brevetoxin and manatees are herbivores and dolphins and cormorants are piscivores. Despite this difference, the overall amount of brevetoxin each organ contained when compared against each other was consistent in all species: liver>kidney>lung>brain. Similar results were noted in studies that measured brevetoxin concentrations in several species of fish (13,21,27), rats (30), and turtles (22). Given this consistent hierarchy in brevetoxin accumulation within an individual, it is surprising that manatees and cormorants would present so similarly, while dolphins present vastly different amounts of brevetoxin. There are limitations to this study that may skew the data comparisons, mostly in regards to the fact that the data came from tissues recovered from different blooms. However, the blooms related to the data presented had over 10^4 cells/L and took place on the coast of central west Florida. Despite this, significant species differences in toxin levels were found.

The livers of manatees had significantly higher concentrations of brevetoxin compared to cormorants, and both had significantly higher levels than dolphins. Necropsies on manatees and cormorants revealed that their livers had hemosiderosis and, in manatees only, were severely congested (5,25). Interestingly, in both studies, none of these findings were determined to be the primary cause of death. In contrast, affected bottlenose dolphins did not show liver pathology at necropsy (16,17) and their liver brevetoxin levels were below half that seen in the next lowest liver in the cormorant. It appears that not only do livers in general contain a large amount of brevetoxin at death, but that manatees, and to a lesser extent, cormorant livers were exposed to a greater load of the toxin. Dolphin livers did not accumulate as large an amount of brevetoxin and did not show the same pathology as manatees and cormorants.

In the kidney, manatees and cormorants had similar amounts of brevetoxin, while dolphin kidneys exhibited half the levels seen. Manatee kidney accumulated one fifth the levels seen in their liver, while cormorant kidney levels were half that seen in their liver. Interestingly, both species display features in their kidneys associated with brevetoxicosis such as congestion, nephritis, and profuse bleeding, while dolphin kidneys, having the lowest toxin levels, did not show obvious pathology (5,16,25). It is interesting to note that although past dolphin mortality events related to *K.brevis* blooms were significantly large, dolphin livers and kidneys had little brevetoxin amounts compared to manatee and cormorants and presented as relatively healthy despite presence of brevetoxin. Once again, organ data shows that dolphins might succumb to the effects of brevetoxin before tissue levels can raise to a more directly harmful level.

In brain tissue, manatees and cormorants had similar brevetoxin concentrations, which were far less than those seen in their kidneys. Dolphin brain levels were, again, significantly less compared to the other two species but one third less than that of its kidneys. Brevetoxin can pass through the blood-brain barrier, accumulate in the brain, and cause problems in the central and peripheral nervous system (1,28). Both

manatee and cormorants in the wild, and rats in lab studies, have been observed with symptoms indicating brevetoxicosis. For example, in manatees these include ataxia, respiratory impairment, back flexing, an inability to maintain a horizontal position, and cormorants exhibit problems flying (2,4,5,29). In some animals that underwent rehabilitation, these symptoms passed and their recovery was complete (25,29). Lymphocytic infiltrates, mild hemmoraging, and lesions - evidence of direct interaction with brevetoxin – were found in the brains of manatee that underwent necropsy after the 1996 red tide event (5,34). In contrast, despite having similar brain levels of brevetoxin, lesions on any brain or brain stem sample of cormorants could not be found (25). Impaired behaviour has yet to be observed in affected dolphins (10). Of the 13 dolphin brain samples, seven did not have any brevetoxin (16) and observations of lesions of any kind were not found. The reason as to why manatee brains show significant pathology with brevetoxicosis, but dolphin and cormorant brains do not, requires further study. This is especially relevant because cormorant brains had similar toxin loads to manatees, and in liver and kidney demonstrated pathologies similar to manatees.

Brevetoxin acts specifically on site 5 of the alpha subunit of voltage-sensitive sodium channels which shifts the channel activation to increasingly negative potentials, eventually creating a complete blockade of neuronal excitability (4). In a study on rat cerebellar granule neurons, it was noted that while neurotoxicity of brevetoxin is dependent on activation of these sodium channels, neurotoxicity was actually dependent on activation of NMDA (*N*-methyl-D-aspartate) receptors (4). Brevetoxin studies on lemon sharks showed that individuals exposed to brevetoxin had reduced levels of acetylcholinesterase and increased muscarinic cholinergic receptor levels, which can result in impaired behaviour (28). Though brevetoxin has a large neuronal effect on specific receptors, enzymes, and channels, it is still unclear as to how and what degree these effects occur in the brains of manatees, dolphins, and cormorants. As cormorants did not display lesions in the brain, it may be that brevetoxin affects species differently. In addition, although dolphins have never been observed with behaviours indicative of brevetoxicosis, it cannot be ruled out. For example, dolphins may experience impaired behaviours severe enough to cause mortality which were not witnessed. This could be another potential reason as to why dolphins seem to have significantly less brevetoxin in their organs yet comparatively more in their stomach contents at time of death. They may simply be more susceptible to severe acute brevetoxicosis that results in rapid mortality such that no visible pathology has time to manifest. Closer observation of bottlenose dolphin behaviour during *K.brevis* blooms may shed some light on these issues.

There are at least nine different types of brevetoxin molecules with different potencies and, as noted in prior rat studies, different possible pathways of metabolism (4,30). Whether this applies to marine animals is, as yet, unclear. In one study, rats that were injected intravenously with brevetoxin type-3 metabolised the majority of the toxin in the liver (30). However, in another study using rats, it was noted that the kidneys were also capable of eliminating brevetoxin type-3 (9). A third study exposed rats to brevetoxin type-2 and noted that brevetoxin type-2 was mostly eliminated through urine, demonstrating that the kidney might be the principal route of metabolism for the type-2 molecule (31). Assuming that brevetoxin type-2 is eliminated primarily by the kidneys, and brevetoxin type-3 through the liver, it is possible that, in relation to the findings in this paper, manatees contain more brevetoxin type-3 molecules in their liver, while both cormorants and manatees are equally capable of collecting brevetoxin type-2 molecules in their kidneys. The type-3 molecule is also far more potent than type-2 which might explain the increased liver lesions seen at necropsy in manatees compared to cormorants, but an equal tolerance to type-2. Unfortunately the very nature of ELISA makes it impossible to identify and quantify the type of brevetoxin molecules present in individual tissues, so further study is required to determine if the type of brevetoxin molecule has any bearing on how a marine animal copes with brevetoxicosis.

Several studies have pointed to trophic transfer, or the transfer of toxins through the food chain, as being the ultimate cause of brevetoxin poisoning in several marine species (21,26,33). Based on the current observed several fold higher concentration of the toxin in stomach contents compared to that found in lung tissue,

ingestion, rather than inhalation, was most likely the primary route of exposure. However the stomach contents of all three species had significantly different concentrations of brevetoxin, which was surprising given that while manatees eat sea grasses, dolphins and cormorants both eat mostly fish, most particularly pinfish, pigfish, and toadfish (13, 36). The higher concentration of brevetoxin in stomach contents of dolphins together with the comparatively lower organ brevetoxin toxin levels versus manatees and cormorants suggests that in dolphins, acute attacks of brevetoxin occur rather than the chronic but lethal exposure to brevetoxin which has been noted in manatees (5,14). It is possible to calculate the approximate amount of brevetoxin each species consumes daily. West Indian manatees weigh between 370-460 kg and consume 8-15% of their own body weight in seagrass daily (8). Flewelling noted that seagrass in southwest Florida had up to 1000 to 3100 ng brevetoxin per gram of plant material (15). Therefore manatees, which had the second highest concentration of brevetoxin in stomach contents, could receive a maximum daily dose of 465 µg brevetoxin per kg body weight. Fire *et al* calculated that bottlenose dolphins that fed on exclusively pinfish - measured to have a mean brevetoxin content of 433 ng/g dry weight - would receive a maximum daily dose of only 29 µg brevetoxin per kg body weight (13). Double-crested cormorant average weight is around 2.27 kg and they consume 22% or 0.49 kg of fish daily (18,23). If cormorants fed exclusively on pinfish then they would receive a maximum dose of 93 µg brevetoxin per kg body weight. So dolphins would potentially be exposed to a smaller daily brevetoxin dose than both manatees and cormorants, and yet their stomach contents had the highest concentration of brevetoxin and their liver, kidney, and brain had the lowest concentrations. As to why this is the case remains to be clarified, however altered metabolism or reduced uptake may well play a role.

In rat models, brevetoxin has been shown to be capable of reaching the lungs via the vascular system (30). However if the vascular route was the only way the brevetoxin could get to the lungs, dolphin lungs would have most likely demonstrated the same pattern seen in liver, kidney, and brain. That is, dolphin lungs would have had significantly less brevetoxin than manatees and cormorants. Instead our comparative review of reported data shows that all three species had similar amounts of brevetoxin in lung tissue. This is clear evidence that during a bloom, as might be expected, brevetoxin becomes aerosolized above the water and then inhaled. Bossart *et al* noticed lesions on upper respiratory tracts of manatees that died in the 1996 mortality event including congestion, inflammation, edema, and an immune response to brevetoxin (5). In rats that repeatedly inhaled brevetoxin, it was noted that they also had a similar respiratory pathology (3,32). Further studies are required to prove whether or not a brevetoxin induced immune response in the respiratory system is lethal. Immune responses in lung tissue of cormorants were absent, but they did display mild congestion (25). Records of lung injury in association to brevetoxin in dolphins could not be found and interestingly, lung levels of toxin in all three species were quite similar to that seen in their brains.

The results of this paper show that in all three species, the hierarchy of brevetoxin each organ contains is consistent. The liver collected a large amount of brevetoxin, with manatee livers sequestering the most, cormorant livers second, and dolphin livers the least. Kidneys contained much less brevetoxin than liver, with significantly higher concentrations found in manatee and cormorant kidneys compared to dolphin kidney. Brain tissue in the three species accumulated the least amount of brevetoxin and while manatee and cormorant brain tissue brevetoxin levels were similar they were significantly higher than dolphin brains. Stomach contents had the highest amount of brevetoxin, suggesting that ingestion is one critical route for brevetoxin to enter the system via trophic transfer of brevetoxin. It was dolphin stomach contents that had the highest brevetoxin concentrations compared to levels found in manatee and cormorant stomach contents. This result was in complete contrast to the maximum daily doses predicted for each species. Concentrations of brevetoxin found in lung tissue in all three species were similar which indicated that inhalation by all three species most likely occurs to a similar degree. Manatee and cormorants appear to suffer from more chronic brevetoxin exposure and absorb similar amounts in various organs while dolphin tissues harbor much less brevetoxin yet have higher brevetoxin in their stomach

contents and still succumb in large numbers. This might be due to the fact that dolphins either succumb to symptoms of brevetoxicosis farther out at sea where they are less easily observed or less likely to strand alive on shore or they have a greater sensitivity to brevetoxin.

Clearly different species respond to brevetoxin in different ways, and while some are able to function for a time with significant toxin in their system, others such as dolphins appear more vulnerable.

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