The Impact of Harmful Algal Blooms on Wildlife

Symposium on Diagnostic Pathology of Diseases of
Aerial, Terrestrial and Aquatic Wildlife

C.L. DAVIS, DVM FOUNDATION
MADISON, WISCONSIN

April 9-11, 2008

Valerie Shearn-Bochsler, DVM, MPH
Diplomate, American College of Veterinary Pathologists
USGS National Wildlife Health Center
6006 Schroeder Rd
Madison, Wisconsin, 53711

I. Introduction to harmful algal blooms (HABs)

A. Occur in both marine and freshwater settings, and are defined as a rapid increase in the population of toxic algae in an aquatic system.

B. Commonly referred to as “red tide” events in marine settings, algal blooms often, but not always, cause discoloration of water resulting from the high density of pigmented cells present.

C. Causative agent may be truly algal (phytoplankton such as dinoflagellates or diatoms) or may be cyanobacterial (also known as blue-green algae).

D. Algal bloom concentrations are measured in cells per milliliter of water, and also in biomass, which is expressed in terms of chlorophyll concentration. These measures, along with the identification of the species present, are used in monitoring the bloom event.

E. Potent toxins are produced by one or several dominant species in a harmful algal bloom and are responsible for morbidity and mortality in exposed wildlife species.

F. The cessation of a harmful algae bloom may bring about its own problems. As algal or cyanobacterial species die, many release large amounts of toxin. Also, the accumulating dead biomass provides nutrients for bacteria, which may grow rapidly and consume large amounts of oxygen. The result is a “dead zone”, an area of hypoxic water which cannot support aquatic life.

II. Algal toxins

A. DOMOIC ACID (DA)

1. Toxic principle: Excitatory neurotoxin; binds to neurons via glutamate receptors in the hippocampus and causes excitotoxic cell death

2. Source: Blooms of multiple species of marine diatoms including *Pseudonitzschia australis*.

3. Route of exposure: Ingestion and trophic transfer through the food chain. Toxin is ingested by filter feeders (clams, mussels, etc) and by small fish such as sardines and anchovies, which are then consumed by species higher on the food chain, such as marine mammals and fish-eating birds.

4. Public health significance: Cause of Amnesic Shellfish Poisoning in people consuming contaminated shellfish (symptoms: gastroenteritis, disorientation, short-term memory loss (may be permanent), seizures; has caused fatalities; may cause long-term neurologic complications such as epilepsy.

5. Distribution: North Atlantic, Pacific, Gulf of Mexico, Sea of Japan, and European and New Zealand coastal waters. Increasingly common seasonally off the coast of central California.

6. Wildlife species currently suspected or proven to be affected: Sea lions, northern fur seals, sea otters, dolphins, grey whales, pelicans and cormorants, and other fish-eating birds.

7. Diagnostic samples:

   For domoic acid (DA) analysis in mammals: Collect serum, urine, feces, stomach contents, liver, kidney, brain, lung – all tissues and fluids can be stored frozen until analyzed. Urine and feces are the most reliable samples used for detection of domoic acid (DA); DA has a very short half-life in serum. Stomach contents are of value both in determining what prey species is involved, and for measuring DA levels in the ingesta. Collect amniotic fluid in pregnant animals.

   For DA analysis in birds: Stomach and cloacal content

   All major organs from any species should be saved in formalin for histopathology.

In addition, for all suspected DA-associated die-offs, collect samples of prey species and water containing plankton from the location of the die-off where possible. Plankton samples may be preserved in Lugol's solution or in 2% gluteraldehyde. For toxin analyses in whole water, surface water should be collected, protected from light, and refrigerated.

8. Diagnostic tests:

Biotoxin detection: Rapid receptor-binding assays and ELISAs are available; if positive, the result should be confirmed by HPLC-MS. Mouse bioassays may also be used.

Immunocytochemical techniques are becoming available for detection of domoic acid in fixed or frozen tissue.

Identification of *Pseudonitzschia* diatoms in stomach content or feces is also evidence of exposure.

Domoic acid poisoning in California sea lions (*Zalophus californias*)

1. Exposure: Ingestion of toxin-laden fish (particularly anchovies)

2. Clinical signs:

Acute poisoning: Seizures (unilateral or bilateral), ataxia, muscle tremors, head-weaving, decreased responsiveness to stimuli, scratching, vomiting. Symptoms are continuous, and affected animals either recover (with treatment) or die. Adult females may be over-represented.

Chronic disease: Animals may present with chronic disease, or may develop chronic disease after apparent recovery from acute disease. Chronic disease is characterized by intermittent seizures, periods of lethargy and inappetence, vomiting, muscular twitching, blepharospasm, behavioral changes, and an increased risk of stranding. Seizures may progress from simple partial to generalized

over time, and may become refractive to treatment, resulting in death or euthanasia. Behavioral changes may include stranding in unusual locations, repetitive behaviors, and abnormal aggression. EEGs often show numerous epileptiform discharges, even when the animal is at rest.

Reproductive failure: Abortion, premature live birth, *in utero* death (due to death of mother)

2. Clinical pathology, acute poisoning:

Increased serum creatine kinase (likely due to seizure activity)

3. Pathologic findings:

Brain, acute injury: Hippocampus: Zonal vacuolation of the hippocampal neuropil, affecting the strata radiatum, lauculosm, and moleculare of both the hippocampus and dentate gyrus; most severe in the anterior ventral hippocampus. Neuronal necrosis in the granule cell layer of the dentate gyrus and pyramidal cells in sectors CA4, CA3, and CA1. Surviving animals may develop bilateral hippocampal atrophy with neuronal loss and gliosis; mild nonsuppurative inflammation; loss of laminar organization in affected areas; and pyriform lobe malacia.

Brain, chronic disease: Hippocampus and parahippocampal gyrus: Parenchymal atrophy due to neuronal loss and astrocytosis, occasionally with active neuronal necrosis; the dentate gyrus and sector CA3 are most consistently and severely affected. Lesions are inconsistently symmetric.

Heart: Myocardial necrosis/fibrosis with variable fibrinous epicarditis and hemorrhage.

Lung: Diffuse pulmonary edema and congestion.

Gastrointestinal: Mild superficial hemorrhages.

Reproductive tract: Uterine rupture/ torsion; placental necrosis and hemorrhage

Eye: Fibrinous ophthalmitis in acute cases.

Domoic acid poisoning in Southern sea otters (*Enhydra lutris nereis*):

1. Exposure likely due to ingestion of toxin-laden invertebrates (especially the spiny mole crab).

2. Possible link with lymphocytic myocarditis and dilated cardiomyopathy.

3. Biotoxin detection tests for this species are less reliable than for sea lions at this time.

Domoic acid poisoning in fish-eating birds:

(Best documented in brown pelicans (*Pelicanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*)):

1. Exposure: Ingestion of toxin-laden fish.

2. Clinical signs:

   Brown pelicans: Central nervous system signs including side-to-side head weaving, tremors, ventroflexion of the head, scratching of the pouch, clenched toes, torticollis, loss of awareness of surroundings, loss of righting reflex. Vomiting is also common.

   Brandt's cormorants: Docile when approached and handled.

2. Clinical pathology:

   Elevated serum creatinine kinase, blood urea nitrogen, and uric acid.

3. Pathologic findings: Hemorrhage in the muscles of the limbs; in some birds, multifocal skeletal muscle necrosis.

B. BREVETOXIN (PbTx)

1. Toxic principle: Brevetoxins are lipid-soluble polyether excitatory neurotoxins. Brevetoxins lower the activation threshold of voltage dependent sodium channels and enhance polarization of nerve cells, leading to uncontrolled Na+ influx into the cell. Brevetoxins also have hemolytic properties.

   Brevetoxins have also been shown to inhibit cysteine cathepsins, a class of lysosomal proteases important in antigen presentation. This may be a mechanism for toxin-associated immunosuppression.

2. Source: Blooms of the dinoflagellate *Karenia brevis.*

3. Route of exposure: Inhalation and/or ingestion.

4. Public health significance:

   Neurotoxic Shellfish Poisoning: Occurs when people ingest toxic filter-feeding shellfish. Gastrointestinal and neurologic symptoms result. Rarely, if ever, fatal.

   Respiratory and ocular irritation may occur through inhalation of toxic aerosols. May induce asthma-like attacks in sensitive individuals. Occasional contact dermatitis has also been reported.

5. Distribution: Mexico, Texas, Louisiana, Mississippi, Alabama, Florida, South Carolina and North Carolina; also in Gulf Stream up to mid-Atlantic states. Regular occurrence in Gulf of Mexico; highest risk area is west coast of Florida from Tampa Bay to Sanibel Island.

6. Wildlife species currently suspected or proven to be affected: Fish, fish-eating birds, manatees, bottle-nosed dolphins, sea turtles. *G. breve* red tides are often associated with massive fish kills.

7. Diagnostic samples:

   For brevetoxin analysis in manatees: Stomach content, liver, kidney, lung.

   For brevetoxin analysis in dolphins: Stomach content, liver, kidney,

lung, urine, feces, blood.

For brevetoxin analysis in birds: Stomach content, liver, cloacal content.

All major organs should be saved in formalin for histopathology/immunohistochemistry.

8. Diagnostic tests:

   Biotoxin detection: Rapid receptor-binding assays and ELISAs are available; if positive, the result should be confirmed by HPLC-MS.

   Immunohistochemistry may be performed on fixed tissues for species in which it is available.

   Collect live-caught prey species of suspect poisoned animals for toxin assays. Water samples for plankton analysis may be collected as described for domoic acid

Brexetoxin poisoning in West Indian manatees (*Trichechus manatus longirostris)*

1) Exposure may be due to either inhalation of toxic aerosols at the ocean surface, or ingestion of toxin-laden tunicates and/or seagrass.

2) Clinical signs: Muscle fasciculations, incoordination, loss of righting reflex; disorientation, inability to properly submerge; labored breathing.

3) Clinical pathology: Not reported.

4) Pathologic findings: Exposure may occur through either inhalation or ingestion (or both). Lesions may differ dependent upon the route of exposure; changes in the upper respiratory tract are suspected to be due to inhalation, and share some similarities with inhalation-related symptoms in humans.

   Gross lesions: Nasopharyngeal and pulmonary edema, congestion, and hemorrhage. Congested liver, kidneys, meninges.

   Microscopic lesions: Marked catarrhal rhinitis, tracheitis, and

bronchitis; pulmonary hemorrhage and edema; multi-organ hemosiderosis; and mild nonsuppurative leptomeningitis (primarily in the cerebellum).

Immunohistochemistry on tissues from affected animals has shown the presence of brevetoxin in lymphocytes and macrophages in the lung, liver, and secondary lymphoid tissues, as well as areas of inflammation in the nasal mucosa and meninges.

Brevetoxin poisoning in bottle-nosed dolphins (*Tursiops truncates*)

(Strongly suspected but less well-documented than in manatees)

1) Exposure postulated to be due to ingestion of toxin-laden fish; or through ingestion of milk in calves nursing from affected mothers.

2) Clinical signs: Increased numbers of dead, stranded dolphins.

3) Clinical pathology: None reported; however blood, urine, and feces are potentially good samples for brevetoxin level analysis.

4) Pathologic findings:

   May be found with first chamber of stomach full of whole or partially digested fish. Findings are variable, and acute or sublethal, chronic effects might be seen. Dead animals may occur at times and places distant from a known bloom.

   Potential findings include: Lymphoplasmacytic oropharyngitis and tracheitis; evidence of lymphoid depletion and secondary infections, especially bacterial septicemias and skin lesions; extensive ulceration of pulmonary bronchiolar epithelium and replacement by fibrous tissue containing multiple foci of mineralization; hepatic lipidosis; fibrosis of liver, lung, pancreas, heart; adhesions of abdominal and thoracic viscera.

   Immunohistochemistry may reveal positively-staining lymphocytes and macrophages within lymph nodes, and in the lung and spleen.

Brevetoxin poisoning in fish-eating (and perhaps other) birds

(Best studied in double-crested cormorants (*Phalacrocorax auritus*);

1. Exposure: Ingestion of toxin-laden fish prey.

2. Clinical signs: Severe cerebellar ataxia (broad-based stance, truncal incoordination, hypermetric gait, intention tremors of head). Also exaggerated responses to stimuli and often, positional vertical nystagmus. Immature birds overrepresented. Many recover with supportive treatment.

2. Clinical pathology: Anemia and hypoproteinemia often seen.

3. Pathologic findings: No pathognomic lesions. Diagnosis may be aided by positive immunohistochemical results from lymphoid cells of the spleen and macrophages in the spleen and lung, as well as tracheal mucosa, heart, and brain. However, results of IHC have been inconsistent.

C. SAXITOXIN (Stx) and its derivatives

1. Toxic principle: Paralytic neurotoxins; bind to voltage-dependent sodium channels on neurons and block influx of sodium into excitable cells, restricting transmission between neurons. Saxitoxins are nitrogenous heterocyclic guanidines. There are at least 18 different compounds that make up the "saxitoxin family" and vary in virulence.

2. Source: Marine dinoflagellates (including *Alexandrium* sp., *Gymnodinium catenatum, Pyrodinium bahamense*) and multiple species of freshwater cyanobacteria (ex. *Anabena circinalis* and *Cylindrospermopsis raciborskii*)

3. Route of exposure: Ingestion of zooplankton, filter-feeding fish, crustaceans, or shellfish which have accumulated the toxin.

4. Public health significance: Cause of Paralytic Shellfish Poisoning (PSP) in people consuming contaminated shellfish. There is a rapid onset of gastrointestinal and neurologic symptoms; death may result from respiratory paralysis (15% mortality rate).

5. Distribution: Worldwide in both marine and freshwater environments. In North America, shellfish poisoning occurs seasonally on the east coast (from Newfoundland to Massachusetts) and west coast (from Alaska to California).

6. Wildlife species known or suspected to be affected: Fish, fish-eating birds, humpback whales, North Atlantic right whales, sea otters, monk seals, turtles

7. Diagnostic samples: Stomach content; liver; kidney; feces or cloacal content

8. Diagnostic tests: Mouse bioassay; ELISA; HPLC-mS is gold standard

Saxitoxin poisoning in wild birds

1. Exposure: Ingestion of toxin-laden fish or shellfish. Evidence for mortality has mostly been circumstantial yet is still highly suggestive.

2. Multiple species have been reported to have died in mortality events suspected to have been caused by Stx poisoning. The most commonly reported include gulls, cormorants, terns, black ducks, shorebirds, and seaducks (scoters and eiders). However any fish-eating bird, especially, may be considered vulnerable. There has been one report of mortality presumed due to Stx in penguins in South America.

3. Clinical signs: Best documented in common terns (*Sterna hirundo*) and herring gulls (*Larus argentatus*). Vomiting, paralysis, and acute death reported. Illness develops rapidly (as soon as one hour) after ingestion of contaminated food. Some birds may recover, and may learn to avoid contaminated food in the future.

4. Clinical pathology: Not reported.

5. Pathologic findings: No pathognomic lesions. In common terns, females near egg-laying have been over-represented. Inflamed intestines have been sporadically reported.

Saxitoxin poisoning in humpback whales (*Megaptera novaeangliae*) (suspected)

1. Exposure: Consumption of Atlantic mackerel (*Scomber scombrus*) containing measurable saxitoxin in liver.

2. Clinical signs: Stranded, dead humpback whales were found off the coast of New England in 1997 and 2003. Death appeared to have been acute, as one whale was observed behaving normally and then found dead 90 minutes later.

3. Clinical pathology: Not reported.

4. Pathologic findings: Whales were in good nutritional condition. Many whales had partially digested mackerel in their stomachs. Testing of the mackerel found saxitoxin present in fish liver and kidney (via mouse bioassay and HPLC). Testing of whale tissues found saxitoxin present in liver and kidney (via mouse bioassay; however HPLC results were negative).

Saxitoxin poisoning in North Atlantic right whales (Eubalaena glacialis) (postulated)

Exposure only has been documented at this time. The primary food source of North Atlantic right whales is the zooplankton *Calanus finmarchicus.* Saxitoxin was detected in whale feces and in *Calanus finmarchicus* in the summer of 2001, during a bloom of the toxic dinoflagellate *Alexandrium fundyense* in the Bay of Fundy. North Atlantic right whales are a critically endangered, declining species with a decreasing reproductive rate; exposure to algal toxins has been hypothesized as one reason for their decline.

Saxitoxin poisoning in Mediterranean monk seals (*Monachus monachus*) (suspected)

1. Exposure: Consumption of contaminated fish (one event recorded)

2. Clinical signs: Lethargy, motor incoordination and paralysis in the water. Death occurred relatively quickly after the onset of clinical signs and was believed to be due to drowning. Adult seals primarily affected, juveniles relatively spared.

3. Clinical pathology: Not reported.

4. Pathologic findings: Congested lungs; airways filled with fluid. Good nutritional status. Saxitoxin detected by mouse bioassay and HPLC in monk seal prey fish species in the area where the affected seals were found. Saxitoxin in several forms was detected in seal brain, liver, kidney, and skeletal muscle samples by mouse bioassay, HPLC, and mass spectrometry. Saxitoxin was also detected in monk seal brain sections by immunoassay using polyclonal antibodies to saxitoxin.

Saxitoxin and Alaskan sea otters (*Enhydra lutris*)

1. Potential exposure: Ingestion of contaminated butter clams (*Saxidomus giganteus*)

2. It has been suggested that the range of Alaska's sea otter population is determined in part by the otters' avoidance of areas where saxitoxin is known to accumulate in butter clams. Butter clams in inside passages of south-east Alaska (but not elsewhere) accumulate saxitoxin and

remain toxic year-round; and no seas otters are found in these areas. Elsewhere, butter clams are not exposed to HABs producing Stx and do not accumulate toxins, and in these areas they are the main prey of co-existing sea otter populations. Feeding studies have shown that sea otters have the ability to detect and avoid consumption of butter clams containing even small amounts of saxitoxin.

D. CIGUATOXIN (Ctx-1)

1. Toxic principle: Structurally related to brevetoxins; mode of action is the same; but ciguatoxins are far more potent.

2. Source: Marine dinoflagellate, *Gambierdiscus toxicus*, which grows as an epiphyte on macroalgae associated with coral reefs.

3. Route of exposure: Ingestion of contaminated fish (herbivorous or carnivorous).

4. Public health significance: Causes illness in people consuming fish which have consumed the toxic dinoflagellate, or fish that have consumed other fish that have consumed toxic dinoflagellates. Most often the fish implicated in human illness are large carnivorous fishes associated with coral reefs, such as barracuda, grouper, and snapper. The illness in people is called ciguatera. Gastrointestinal, neurologic, and respiratory symptoms may result. Death is uncommon but may occur via respiratory paralysis.

5. Distribution: Associated with warm-water coral reefs.

6. Wildlife species suspected to be affected: Hawaiian monk seal (*Monachus schauinslandi*). Cigautoxins have been found in fish species preyed upon by this highly endangered seal species, and are postulated to play a role in its decline. However definitive proof is lacking.

7. Diagnostic samples: Fish prey species and stomach content. Further investigation is needed to determine which, if any, tissue samples might yield positive results.

8. Diagnostic tests: Tests of fish extracts must be able discriminate between ciguatoxin and brevetoxin. Combination of a sodium channel receptor binding assay and a ouabain/veratridine-dependent cytotoxicity assay has been described and found to successfully

discriminate between these two similar toxins.

E. OKADAIC ACID (OA) AND DINOPHYSISTOXINS (DTX)

1. Toxic principle: Acidic polyether toxins; inhibit ser/thr protein phosphatases

2. Source: Marine dinoflagellate species including *Dinophysis sp, Procentrum lima,* and *Procentrum concavum.*

3. Route of exposure: Consumption of contaminated shellfish (humans); consumption of macroalgae and seagrasses where *Procentrum* sp. are present as epiphytes (sea turtles)

4. Public health significance: Cause of Diarrhetic Shellfish Poisoning, a relatively mild intoxication with rapid onset of vomiting and diarrhea that resolve in 2-3 days. In addition, okadaic acid has been identified as a tumor promoter.

5. Distribution: Likely worldwide; cases of poisoning in people usually occur in temperate regions.

6. Wildlife species currently suspected to be affected: Hawaiian green sea turtles. A spatial association has been found between high-risk areas for sea turtle fibropapillomatosis and the occurrence of *Procentrum* sp epiphytes on sea turtle forage. In addition, okadaic acid has been found in sea turtle tissues. It is suspected that OA may act as a tumor promoter in this species and may have a role in the etiology of fibropapillomatosis.

7. Diagnostic samples: Water and tissue samples.

8. Diagnostic tests: Protein phosphatase inhibition tests, ELISA tests, and HPLC-MS.

III. **Cyanobacterial toxins**

MICROCYSTINS (MCYSTs)

1. Toxic principle: Hepatotoxic cyclic heptapeptides; protein phosphatase inhibitors and tumor promoters.

2. Source: Multiple cyanobacterial species including *Microcystis, Arthospira, Anabena, Planktothrix sp.* (and others).

3. Route of exposure: Ingestion of contaminated water most common.

3. Public health significance: Cause of "Caruaru syndrome", named after an incident occurring at a hemodialysis center in Caruaru, Brazil, in 1996. In this incident, water used for routine renal dialysis was contaminated by microcystins-YR, -LR, and -AR, and 116 of 131 patients became ill; clinical signs included headache, blurred vision, nausea, and vomiting. 100 patients developed acute liver failure, and 76 died. Severe hepatomegaly, jaundice, and bleeding diatheses were seen.

   Chronic exposure suspected to be carcinogenic.

5. Distribution: Lakes, rivers, and streams world-wide.

6. Wildlife species currently suspected or proven to be affected: Equal opportunity killer especially when combined with anatoxins. Many reports of suspected domestic and wild mammalian, avian, and fish mortalities. Hepatotoxic.

7. Diagnostic samples: Testing of water samples for cyanobacteria and for cyanobacterial toxins is currently the only practical testing available. Tests for cyanobacterial toxins in tissue are very difficult and not widely available.

8. Diagnostic tests: Protein phosphatase inhibition tests, ELISA tests, and HPLC-MS for the presence of microcystin in water samples are available. LC/MS for measuring MYCYSTs in animal tissue has been described but testing is not readily available outside a research setting.

ANATOXIN-A

1. Toxic principle: Neurotoxic amine alkaloid; post-synaptic cholinergic nicotine agonist (acetylcholine analog); not degraded by cholinesterase; causes death by blockage of neuromuscular transmission, resulting in respiratory paralysis.

2. Source: Cyanobacteria, including *Anabaena sp., Aphanizomenon sp., Cylindrospermum sp., Planktothrix sp*.. Anatoxin-A seldom occurs as a lone cyanotoxin but instead occurs typically in mixed cyanobacterial blooms with multiple toxins.

3. Route of exposure: Ingestion of contaminated water

4. Public health significance: Acts like an organophosphate compound if contaminated water is ingested.

5. Distribution: Lakes, rivers, and streams world-wide.

6. Wildlife species currently suspected or proven to be affected: Mortality events where anatoxins are suspected to play a role typically occur when anatoxin is present in combination with other cyanobacterial toxins; it is therefore often difficult to be certain of the relative significance of each toxin. Avian species reportedly affected include waterbirds, including gulls, coots, ducks, and lesser flamingos (*Phoeniconaias minor Geoffroy*). Mammalian species reportedly affected include fox squirrels, muskrats, skunk, mink, and bats, as well as domestic species (livestock, dogs). There is one report of suspected mortality in white rhinoceros in South Africa. Signs of poisoning in wild and domestic animals may include salivation, staggering, muscle fasciculations, gasping, convulsions, and in birds, opisthotonus. Death occurs by respiratory arrest.

7. Diagnostic samples: Testing of water samples for cyanobacteria and for cyanobacterial toxins is currently the only practical testing available. Tests for cyanobacterial toxins in tissue are very difficult and not widely available.

8. Diagnostic tests: Acetylcholinesterase inhibition assays, ELISAs, and HPLC-MS for the presence of anatoxin-A in water samples are available. HPLC-LC/MS for measuring anatoxins in animal tissue has

been described but is not readily available outside a research setting.

NODULARIN

1. Toxic principle: Cyclic pentapeptide hepatotoxin; protein phosphatase inhibitor and tumor promoter.

2. Source: Cyanobacteria *Nodularia spumigena*.

3. Route of exposure: Ingestion of contaminated water; may also accumulate in shellfish.

4. Public health significance: It is likely that human exposure to nodularins carries similar risk as exposure to microcystins.

5. Distribution: Primarily brackish waters in New Zealand, Australia, and the Baltic Sea

6. Wildlife species known or suspected to be affected: Livestock, canine, and wildlife mortalities have been reported. The toxicity and pathogenicity of nodularin is very similar to that of microcytsin.

7. Diagnostic samples: See microcystins.

8. Diagnostic tests: Protein phosphatase inhibition tests, ELISA tests, and HPLC-MS for the presence of microcystin in water samples are available. LCMS for measuring nodularins in animal tissue has been described but testing is not readily available outside of a research setting.

CYLINDROSPERMOPSIN (cyn)

1. Toxic principle: Hepatotoxic alkaloid; cyn is metabolized by cytochrome P450 in the liver to a more toxic metabolite.

2. Source: The cyanobacterium *Cylindrospermopsis raciborskii.* Note: *C. raciborskii* can also produce other toxins, including saxitoxin, which may lead to mixed clinical and pathologic findings in the field. The multiple toxins produced by different strains of this cyanobacterium have likely not yet been fully described. This cyanobacterium is considered an emerging disease agent in the USA.

3. Route of exposure: Primarily by ingestion of contaminated water. Recent studies have documented cyn bioaccumulation in snails, fish, and crayfish, suggesting alternate routes of exposure.

4. Public health significance: Causes hepatoenteritis in people ingesting contaminated water. Can also cause contact dermatitis after recreational or occupational exposure to contaminated water. Potentially carcinogenic.

5. Distribution: Mainly in tropical and subtropical regions of Africa, Australia, Cuba, India, Indonesia, and South America; but is also found in temperate regions of Europe, Central Asia, and North America

6. Wildlife species currently suspected or proven to be affected: Cane toad tadpoles

7. Pathologic findings: Reported in only a few species at this time.

   Mice: Diffuse lipidosis, centrilobular to massive hepatocellular necrosis; lymphoid depletion; ulceration of the esophageal part of the gastric mucosa; variable proximal tubular epithelial cell necrosis; scattered adrenal epithelial cell necrosis; subepicardial and myocardial hemorrhages; and an increased tendency for multiple vascular thromboses. At very high doses, may not cause hepatic necrosis prior to animal death.

   Cattle: Gross lesions reported include a pale swollen liver, distended gallbladder, subserosal small intestinal and omental hemorrhages, and epicardial hemorrhages. Histologic lesions include extensive hepatic degeneration and necrosis, with fibrosis and bile duct proliferation.

Cane toad tadpoles: Multiple organ tissue injuries reported, with the liver, intestine, nephric ducts, and gill epithelia most severely damaged.

8. Diagnostic samples: Testing of water samples for cyanobacteria and for cyanobacterial toxins is currently the only practical testing available. Tests for cyanobacterial toxins in tissue are very difficult and not widely available.

9. Diagnostic tests: HPLC-MS on surface water samples; mouse bioassays; rat hepatocyte assays. LCMS on animal tissue has been described but is not readily available outside of a research setting.

***PFIESTERIA PISCICIDA* AND *PFIESTERIA*-LIKE ORGANISMS**

(controversial and still poorly understood)

1. Toxic principle: Recently described toxin is a highly labile, radical forming organic-ligated metal (copper and iron) complex. It is believed to act as an exotoxin. Toxicity is due to metal-mediated free radical production. Sunlight and metal exposure (particularly the copper speciation present in estuarine waters) may initiate *Pfiesteria piscicida* toxicity during an algal bloom.

2. Source: *Pfiesteria piscicida* and *Pfiesteria*-like organisms (estuarine dinoflagellates). This organism is difficult to identify and has multiple life stages.

3. Route of exposure: People: dermal contact with affected water or inhalation of aerosolized toxin; fish: contact with affected water.

4. Public health significance: Possible cause of Estuarine-Associated Syndrome, which includes one or more of the following symptoms: cognitive dysfunction, and especially memory loss, plus some combination of skin rash, eye irritation, GI upset, muscle cramps

5. Distribution: Estuaries (warm, calm, brackish waters with elevated nutrients) on the East coast of the USA from North Carolina to Virginia (mid-Atlantic region). Usually occur between the months of April-October. Some believe that eutrophication of coastal waters through intensive swine and poultry production in North Carolina and

Maryland has contributed to the occurrence of *Pfiesteria* sp. at toxic levels. Occurrence ceased in North Carolina after Hurricane Floyd hit North Carolina in 1999.

6. Wildlife species currently suspected or proven to be affected: Fish; suspected cause of Ulcerated Disease Syndrome in affected fish; there may be microbial cofactors; may cause massive fish kills (Atlantic menhaden, southern flounder, mullet, others).

7. Diagnostic samples: Water from area of fish kill.

8. Diagnostic tests: Verify the presence of the dinoflagellate species. The toxin is difficult to identify and isolate and is unstable in purified form, and is therefore impractical to measure routinely at this point in time.

III. Syndromes suspected to be linked to algal toxins:

Avian vacuolar myelinopathy

References:

1) Anderson DM and White AW. Toxic dinoflagellates and marine mammal mortalities: proceedings of an expert consultation held at the Woods Hole Oceanographic Institution. *Woods Hole Oceanog. Inst. Tech. Rept, WHOI-89-36 (CRC-89-6).* (1989)

2) Ballot A et al. Cyanobacteria and cyanobacterial toxins in three alkaline Rift Valley lakes of Kenya – Lakes Bogoria, Nakuru, and Elmenteita. *J Plankton Res* 26(8):925-935 (2004).

3) Bossart GD et al. Brevetoxicosis in manatees (*Trichechus manatus latirostris*) from the 1996 epizootic: Gross, histologic, and immunohistochemical features. *Toxicol. Pathol*. 26:276-282 (1998).

4) Brode EC et al. Domoic acid causes reproductive failure in California sea lions (*Zalophus californianus*). *Marine Mammal Sci* 22(3):700-707 (2006).

5) Carmichael W et al. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. *Environ Health Perspect* 109(7) 663-668 (2001).

6) Costas E and Lopez-Rodas V. Paralytic phycotoxins in monk seal mass mortality. *Vet Record* 142:643-644 (1998).

7) Dechraoui MY et al. Use of two detection methods to discriminate ciguatoxins from brevetoxins: application to great barracuda from the Florida Keys. *Toxicon* 46(3):261-270. (2005).

8) Dierauf LA and Gulland FMD (eds.) *CRC Handbook of Marine Mammal Medicine.* (CRC press, 2001).

9) Doucette GJ et al. Paralytic shellfish poisoning (PSP) toxins in North Atlantic right whales *Eubalaena glacialis* and their zooplankton prey in the Bay of Fundy, Canada. *Mar Ecol Prog Ser* 306:303-313 (2006).

10) Durbin E et al. North Atlantic right whales, *Eubalaena glacialis*, exposed to paralytic shellfish poisoning (PSP) toxins via a zooplankton vector, *Calanus finmarchicus. Harmful Algae* 1(3):243-251 (2002).

11) Edwards M et al. Regional climate change and harmful algal blooms in the northeast Atlantic. *Limnol. Oceanogr*. 51(2):820-829 (2006)

12) Fleming et al. Emerging harmful algal blooms and human health: *Pfiesteria* and related organisms. *Tox Pathol* 27(5):573-581 (1999).

13) Flewelling LJ et al. Brevetoxicosis: Red tides and marine mammal mortalities. *Nature* 435:755-756 (2005)

14) Geraci JR. Clinical investigation of the 1987-88 mass mortality of bottlenose dolphins along the U.S. central and southern Atlantic coast. *Final report to National Marine Fisheries Service and U.S. Navy, Office of Naval Research and Marine Mammal Commision* (1989).

15) Geraci JR et al. Humpback whales (*Megaptera novaeangeliae*) fatally poisoned by dinoflagellate toxin. *Can J Fish Aquat Sci* 46:1895-1898 (1989).

16) Gilbert et al. The role of eutrophication in the global proliferation of harmful algal blooms. *Oceanography* 18(2):198-209 (2005).

17) Gilmartin WG et al. An investigation into an unusual mortality event in the Hawaiian monk seal, *Monachus schauinslandi*. *Proceedings of the symposium on the status of resource investigations in the Northwestern Hawaiian Islands*. UNIHI-SEAGRANT report No. MR-80-04, 10 pp. (1980)

18) Glasgow HB et al. Field ecology of toxic *Pfiesteria* complex species and a conservative analysis of their role in estuarine fish kills. Environ Health Perspect (109) Supplement 5: *Pfiesteria*: From biology to public health. Pp 715-730 (2001).

19) Gochfeld M and Burger J. Apparent paralytic shellfish poisoning in captive herring gulls fed commercial scallops. *Toxicon* 36(2):411-415 (1998).

20) Goldstein T et al. Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): an increasing risk to marine mammal health. *Proc. R. Soc. B* 275:267-276 (2008)

21) Hallegraeff GM. Harmful algal blooms: a global overview. In: Hallegraeff GM, Anderson DM, Cembella AD (eds.) *Manual on harmful marine microalgae*, pp 25-49. UNESCO publishing, Paris (2003)

22) Hernanfez et al. Did algal toxins cause monk seal mortality? *Nature* 393:28-29 (1998).

23) Kinnear SH et al. Multiple-organ toxicity resulting from cylindrospermopsin exposure in tadpoles of the cane toad (*Bufo marinus*). *Environ Toxicol* 22(6):550-558 (2007).

24) Kreuder C et al. Clinicopathologic features of suspected brevetoxicosis in double-crested cormorants (*Phalacrocorax auritus*) along the Florida gulf coast. *J Zoo Wild Med* 33(1):8-15 (2002).

25) Kreuder C et al. Patterns of mortality in Southern sea otters (*Enhydra lutris nereis*) from 1998-2001. *J Wild Dis* 39(3):495-509 (2003).

26) Kreuder C et al. Evaluation of cardiac lesions and risk factors associated with myocarditis and dilated cardiomyopathy in southern sea otters (*Enhydra lutris nereis*). *AJVR* 66(2):289-299 (2004).

27) Krienitz L et al. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. *FEMS Microbiol Ecology* 43:141-148 (2003)

28) Kvitek RG et al. Paralytic shellfish poisoning toxins mediate feeding behavior of sea otters. *Limnol and Oceanogr* 36(2):393-404 (1991).

29) Kvitek RG. Sequestered paralytic shellfish poisoning toxins meditate glaucous-wing gull predation on bivalve prey. *The Auk* 108:381-392 (1991).

30) Landsberg JH et al. The potential role of natural tumor promoters in marine turtle fibropapillomatosis. *J Aquat Anim Health* 11:199-210 (1999)

31) Kvitek RG et al. Diet and foraging behavior of sea otters in southeast Alaska. *Mar Mamm Sci* 9(2): 168-181 (1993).

32) Landsberg JH. The effects of harmful algal blooms on aquatic organisms. *Reviews in Fisheries Science* 10(2):113-390 (2002)

33) Landsberg JH at al. Algal biotoxins. In: Thomas NJ, Hunter DB, Atkinson CT (eds.) *Infectious diseases of wild birds*, pp 431-455.

Blackwell publishing, IA (2007).

34) Moeller PDR et al. Metal complexes and free radical toxins produced by *Pfiesteria piscicida*. Environ Sci Technol 41:1166-1172 (2007).

35) Neilan BA et al. Phylogeography of the invasive cyanobacterium *Cylindrospermopsis raciborskii*. *Molec Ecology* 12(1):133-140 (2003).

36) News@Nature (04 Aug 2003), doi: 10.1038/news030804-1, News

37) Nisbet ICT. Paralytic shellfish poisoning: effects on breeding terns. *Condor* 85:338-345 (1983).

38) Ott JL and Carmichael WW. LC/ESI/MS method development for the analysis of hepatotoxic cyclic peptide microcystins in animal tissues. *Toxicon* 47:734-741 (2006).

39) Pybus MJ and Hobson DP. Mass mortality of bats due to probable blue-green algal toxicity. *J Wild Dis* 22(3):449-450 (1986).

40) Rose ET. Toxic algae in Iowa lakes. *Proc. Iowa Acad. Sci*. 60:738-745 (1953).

41) Saker ML et al. Cattle mortality attributed to the toxic cyanobacterium *Cylindrospermopsis raciborskii* in an outback region of North Queensland. *Environ Toxicol* 14(1): 179-182 (1999).

42) Seawright AA et al. The oral toxicity for mice of the tropical cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska). *Environ Toxicol* 14:135-142 (1999).

43) Scholin CA et al. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature* 403:80-84 (2000).

44) Shumway SE et al. Marine birds and harmful algal blooms: sporadic victims or under-reported events? *Harmful Algae* 2:1-17 (2003).

45) Sierra BA et al. Sea bird mortality at Cabo San Lucas, Mexico: evidence that toxic diatom blooms are spreading. *Toxicon* 35:447-453 (1997).

46) Silvagni PA et al. Pathology of domoic acid toxicity in California sea lions (*Zalophus californianus*). *Vet Pathol* 42:184-191 (2005).

47) Smith, M. The cyanobacterium *Cylindrospermopsis raciborskii,* and its related toxin, cylindrospermopsin. *Austr J Ecotoxicol* 3:7-23 (1997).

48) Soll MD and Williams MC. Mortality of a white rhinoceros (*Ceratotherium simum*) suspected to be associated with the blue-green algae *Microcystis aeruginosa*. *J South African Vet Assoc* 56(1):49-51 (1985).

49) Todd ECD. Domoic acid and amnesic shellfish poisoning – a review. *Journal of Food Protection* 56:69-83 (1993).

50) Work TM et al. Epidemiology of domoic acid poisoning in brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) in California. *J Zoo Wild Med* 24(1):54-62 (1993).