Chapter 28: Cyanobacterial poisoning in livestock, wild mammals and birds – an overview

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Abstract

Poisoning of livestock by toxic cyanobacteria was first reported in the 19th century, and throughout the 20th century cyanobacteria–related poisonings of livestock and wildlife in all continents have been described. Some mass mortality events involving unrelated fauna in prehistoric times have also been attributed to cyanotoxin poisoning; if correct, this serves as a reminder that toxic cyanobacteria blooms predate anthropogenic manipulation of the environment, though there is probably general agreement that human intervention has led to increases in the frequency and extent of cyanobacteria blooms. Many of the early reports of cyanobacteria poisoning were anecdotal and circumstantial, albeit with good descriptions of the appearance and behaviour of cyanobacteria blooms that preceded or coincided with illness and death in exposed animals. Early necropsy findings of hepatotoxicity were subsequently confirmed by experimental investigations. More recent reports supplement clinical and post–mortem findings with investigative chemistry techniques to identify cyanotoxins in stomach contents and tissue fluids.

Introduction

Planktonic blooms and surface scums appear to have been recognised for over two thousand years, and indigenous peoples of North America, Africa and Australia may have been aware of the poisonous nature of cyanobacteria (Codd et al. 2005). Detailed studies – including microscopy – of the behaviour of planktonic cyanobacteria blooms began in the late 19th century. Surface scums that were concentrated by wind activity were observed, having a sudden onset and dissipation (Phillips, 1884). The world's first scientific report of the toxic nature of cyanobacteria was written by George Francis, who described in 1878 the rapid death of stock animals at Lake Alexandrina, a freshwater lake at the mouth of the Murray River in South Australia (Francis 1878). Francis described a wind–blown surface scum of the brackish water cyanobacterium *Nodularia spumigena*, incidental consumption of which resulted in the death of sheep, horses, dogs and pigs within periods of 1 to 24 hours. Francis also experimentally dosed a sheep with fresh bloom material. Necropsy examination showed a significant pericardial effusion, "two pints of yellow serum" (i.e. most likely ascitic fluid) in the abdominal cavity, and normal–looking liver, lungs and kidneys (Francis 1878). Codd et al (1994) note that the observations of Francis are entirely in agreement with the modern understanding of *Nodularia*–associated intoxication through its associated cyanotoxin, nodularin. Beasley et al (1989a, 1989b) suggest that another cyanotoxin may have been involved, given that Francis did not report any hepatic pathology characteristic of nodularin intoxication, however the liver lesion caused by nodularin may not have been apparent or obvious to the inexperienced observer. The description of ascitic fluid in this context is strongly suggestive of acute liver damage. Beyond Francis's (likely) observation of ascitic fluid, and the necropsy findings of (possibly) a single animal, it is probably unwise to attempt a reinterpretation of Francis's findings (Francis did report opening "many sheep that died", but it is not clear from the report whether his examinations of those field deaths extended beyond his obvious interest in the apparent absence of bloom material from the stomachs) (Francis 1878).

Since Francis's report in the last quarter of the 19th century there have been numerous descriptions of mammal and bird mortalities associated with exposure to cyanobacteria. The relevant literature throughout the 20th century represents all inhabited continents. Anecdotal and case reports have been collated and/or reviewed by many authors, e.g. (Schwimmer and Schwimmer 1955, 1968; Schwimmer and Schwimmer 1964; Hammer 1968; Codd and Beattie 1991; Carmichael and Falconer 1993; Ressom et

al. 1994; Duy et al. 2000; Briand et al. 2003; Codd et al. 2005; Falconer 2005). Many of the earlier reports are circumstantial, noting a temporal association between the presence of cyanobacterial blooms in a waterbody and otherwise unexplained mortalities. Early necropsy findings of hepatotoxicity were subsequently confirmed by experimental toxicology studies into isolated and purified cyanotoxins. More recent reports supplement clinical and post–mortem findings with investigative chemistry techniques to identify cyanotoxins in stomach contents and organs.

Discussion of wild and domestic animal poisonings can be found in most reviews of toxic cyanobacteria, and mention of field deaths often served to introduce and contextualise reports of experimental toxicological investigations into cyanobacterial suspensions, extracts or isolates. As the number of primary anecdotal and case reports and reviews into cyanobacteria–related mortality of birds and terrestrial mammals is extensive, a systematic, comprehensive review has not been conducted in recent times. Such a task is also beyond the scope of this review; therefore we will present some selected discussion of early reports, including palaeontologic findings, then we will discuss more recent investigations that incorporate modern investigative chemistry techniques to supplement clinical and post–mortem findings in order to diagnose cyanotoxin poisoning.

Early reports (pre–1960s)

Lake Alexandrina in South Australia, as mentioned in the Introduction, was a trouble–spot for cyanobacteria–related poisonings, with several hundred deaths associated with a number of bloom events in the latter part of the 19th century. The local Aboriginal people were apparently aware of poisonings in the lake at around 1850 (Codd et al. 1994).

Another early locus was South Africa, where Steyn (1945) suggested that "many thousands" of cattle and sheep mortalities over the preceding 25–30 years were attributable to cyanobacterial poisoning; horses, mules, donkeys, dogs, hares, poultry and waterbirds found dead near affected waterbodies were also thought to be similarly affected. A toxic bloom on the Vaal Dam in 1942 covered an estimated 98 per cent of the reservoir, this being at the time 120km x 24km (at its broadest point) with a 1 teralitre capacity (Stephens 1949). *Microcystis* sp. were identified in the water; fulminant hepatic disease was observed in some animals, as were subacute cases with death ensuing after some two weeks and chronic cases with weeks or months of morbidity preceding either death or recovery. Necropsy findings in fulminant cases included pulmonary haemorrhage, hae-

moperitoneum, hepatomegaly (with livers dark red or black in colour, and friable), splenomegaly, and occasional "bloody patches…on the mucous membranes of [the stomach and intestines]." Findings in subacute cases were haemo–serous exudate in the pericardium, thorax and peritoneal cavity, the liver being soft or friable and yellow–coloured. Experimental dosing of animals with dam water produced identical clinical signs and post–mortem lesions. Secondary photosensitivity was described in detail: painful inflammation, fissuring of affected epithelium, purulent discharge, sloughing of skin. Unpigmented areas are mainly affected – muzzle, udder and teats, ears (Steyn 1943).

Cyanobacteria–related poisonings were reported in North America also from the latter half of the 19th century – see citations in Codd et al (2005). Howard and Berry (1933) discussed *Anabaena*–related cattle and other animal deaths at an Ontario lake in 1924. Storm Lake in Iowa experienced dramatic bloom events in 1952: associated with *Anabaena flos–aquae* blooms were estimated deaths of 5–7,000 gulls, 560 ducks, 400 coots, 200 pheasants, 50 squirrels, 18 muskrats, 15 dogs, 4 cats, 2 hogs, 2 hawks, 1 skunk, 1 mink, plus "numerous" songbirds. These figures were taken from the recorded number of animals buried by a field worker at the time, so were most probably underestimates of the total number of deaths. Signs of neurotoxicity were seen: prostration and convulsions preceded death; milder cases displayed restlessness, weakness, dyspnoea and tonic spasms. 57 weak and partially paralysed mallards recovered following gastric lavage. A dog drinking the water when sick birds were being gathered then died near the lake shore when trying to escape from workers. Botulism was excluded from the diagnoses, and necropsy findings were unremarkable. Experimental oral or parenteral dosing of bloom filtrate to laboratory rodents and chickens resulted in rapid death – within minutes, though delayed mortalities were reported: one chicken lived for 48 hours, and guinea pigs exposed to bloom filtrate orally or by i.p. injection died "within 24 hours" (Firkins 1953, Rose 1953).

While some of the aforementioned reports were dramatic in terms of the number of animals affected by specific bloom events, equally compelling were early descriptions of the rapidity with which large animals succumbed after exposure to (presumably) neurotoxic cyanobacteria. McLeod and Bondar (1952) noted that a horse, "several" calves, two pigs and a cat all died within an hour of consuming cyanobacteria–affected water at Lake Dauphin, Manitoba in 1945. At the same site in 1951, nine dogs and a horse died within one hour; the horse showed signs of progressive muscular weakness and paralysis, with profuse sweating. Experimental dosing of laboratory rodents was conducted using bloom material (99% *Aphanizomenon flos–aquae*) collected from the lake six days after the last re-

ported poisoning event. Interestingly, oral or i.p. injections of lake water filtrate were not toxic, whereas unfiltered lake water, various preparations of cyanobacterial residue or filtrate from disrupted cellular material resulted in deaths ranging from under 12 hours to around 65 hours. Clinical signs were those of "loss of equilibrium followed by progressive paralysis." Later signs were clonic muscular spasms and respiratory dysfunction leading to pronounced cyanosis. Post–mortem findings from these experimental animals were not reported (McLeod and Bondar 1952).

Prehistoric animal mortalities

The first detailed descriptions of the behaviour of cyanobacteria blooms and cyanobacterial toxicity were written in the latter part of the 19th century (see Introduction), but there is no reason to suppose that both phenomena were not present at earlier stages of our history. Codd et al (1994) suggest that Pliny the Elder may have described a cyanobacterial or algal scum on the River Dnieper in AD77, and Höger (2003) implies that an Old Testament reference may be a description of a piscicidal *Planktothrix* bloom in the River Nile. These contemporaneous descriptions reach modern researchers through the device of written language. However, two reports from the palaeontology literature suggest that cyanobacterial toxicity may be a prehistoric phenomenon. Braun and Pfeiffer (2002) present their hypothesis that at Neumark–Nord in Germany, a Pleistocene (1.8 million – 11,000 years ago) lake assemblage of >70 deer, as well as forest elephant, rhinoceros, auroch (ox) and cave lion skeletons may represent a cyanotoxin–related mass mortality event. The preservation features and antler development suggest rapid, catastrophic death in autumn. Calcified layers in the sediment are thought to represent decomposed cyanobacteria, and absorption spectra of cyanobacteria–specific carotenoids were identified in sediment extracts. The authors used HPLC with UV detection to determine the presence of microcystins in methanol extracts of sediment; a similar (but not identical) UV spectrum to that obtained from an extract of a *Microcystis aeruginosa* strain with an unspecified microcystin congener profile was seen. Other possible explanations for the deaths at Neumark–Nord – butchery by early humans, or a gas eruption – are discussed by the authors but thought to be unlikely (Braun and Pfeiffer 2002). Considering further the cyanotoxin hypothesis, it would be interesting to see further confirmatory investigations for microcystins in the sediment extracts using a second method such as mass spectrometry. Looking beyond the search for microcystins in the sediment, it would seem that sudden, catastrophic

death due to ingestion of a cyanobacterial neurotoxin could equally explain these events.

An older mammal and bird assemblage from the Middle Eocene epoch (49 – 37 million years ago) also has features suggestive of a mass poisoning event. The Messel oil shale pit in Germany was an ancient freshwater lake. While the sediment layers are more compacted than those of Neumark–Nord, and chemical and physical transformation of sediments by diagenesis is likely, Koenigswald et al (2004) suggest that there are marked similarities across both sites, and thin layers of siderite at Messel probably represent the decomposed remains of sunken cyanobacteria blooms. Well–preserved skeletons of horses, turtles, bats and birds are found; most were in good health, and stomach contents show that they were well nourished. Apparent features of seasonal death are seen: of some 50 dawn horse skeletons, five are of pregnant mares. The foetuses are all in late stages of development, which again suggests seasonal mortality, as predator pressure on newborn ungulates drives seasonal parturition, usually in late spring. These skeletons were found in different layers, so the picture is that of seasonal deaths occurring in different years. The frequent presence of winged animal skeletons at Messel, which is unusual in lake deposits, also supports a toxic waterbloom hypothesis (Koenigswald et al. 2004). The authors also discuss a finding of aquatic turtle specimens in which two animals are preserved as pairs in close proximity to each other. "At least" five slabs of shale featuring such pairs of turtles have been found; photographs of two pairs are presented in the paper. The authors cite another worker's interpretation of this finding as representing sudden death during mating, which would support a toxic cyanobacteria bloom hypothesis (Koenigswald et al. 2004). However the photographs show two pairs of turtles apparently fused posteriorly but facing away from each other, which is not the normal copulatory position adopted by turtles. The male mounts the female, though infrequent reports of "belly–to–belly" position can be found in the literature (Goode 1967, Kuchling 1999).

If these reports do indeed describe ancient cyanobacteria–related intoxications, they serve as a reminder that toxic cyanobacteria blooms are natural events; they predate recent anthropogenic manipulation of the environment, and may indeed predate human arrival. However, most cyanobacteriologists would acknowledge that the problem of harmful cyanobacteria is exacerbated in modern times due to cultural eutrophication, and may worsen in future with the additional impact of human activity–related climate warming and other population–related pressures.

Recent developments

While many early reports and reviews of cyanobacteria–related poisonings are impressive because of their comprehensive nature and empirical observations supplemented by simple experimental techniques, more recent reports of mammal and bird poisonings reveal the advances in analytical techniques that allow definitive identification of well–characterised cyanotoxins. The following section will present discussion of recent case studies that investigated animal poisonings caused – or likely to have been caused – by cyanobacteria that produce toxins from each of the main cyanotoxin groups that have been characterised to date. Clinical presentation, necropsy findings that reflect the current understanding of the mechanisms of toxicity, and modern investigative chemistry procedures that confirm the diagnosis are discussed. These examples are by no means exhaustive, as different analytical approaches may be applied, and factors such as dose, time period of exposure and ingestion of more than one type of cyanotoxin may complicate the diagnosis.

The experimental toxicology of these isolated cyanotoxins has been well documented, and many excellent reviews are available, e.g. (Ressom et al. 1994, Codd et al. 1999, Carmichael 2001, Falconer 2005). Peracute microcystin and nodularin poisoning presents principally with fulminant haemorrhagic liver injury; pathologic changes can also be seen in the gut, kidney, heart and lungs. Cylindrospermopsin intoxication is characterised by fatty degeneration of the liver, cholestasis, injury to renal tubules and thymic atrophy. Experimental dosing with cylindrospermopsin–producing cyanobacterial extracts results in pathological effects on other tissues and organ systems, including the gastrointestinal tract and spleen.

Cyanobacterial neurotoxins cause death by respiratory failure through various mechanisms: anatoxin–a by a depolarising block at the neuromuscular junction; anatoxin–a(s) is an anticholinesterase, thus preventing the enzymatic degradation of acetylcholine which results in uncontrolled hyperstimulation of muscles. Hypersalivation and lacrimation, which are signs of parasympathetic stimulation, also characterise anatoxin–a(s) poisoning. The saxitoxin group are sodium channel blocking agents, thus interfering with axonal conduction. Death is very rapid – within minutes – when purified cyanobacterial neurotoxins are administered parenterally, particularly from anatoxin–a or saxitoxins. Post–mortem and histology findings are generally unremarkable.

Microcystins

Case study: duck deaths in Japan, 1995 (Matsunaga et al. 1999)

Shin–ike pond in Hyogo Prefecture had become eutrophic from the entry of untreated sewage following an earthquake in January 1995, when a sewage treatment plant was damaged. A bloom of *M. aeruginosa* was evident. In September of that year, some 20 ducks died at the site; Oo–ike pond, 1km distant, also had a cyanobacteria bloom, but no unusual bird deaths were reported from that site. Water samples were collected from both ponds. Necropsy of one of the affected ducks showed a liver that was necrotic and "severely jaundiced…" Preliminary toxicity testing with sonicated cell suspensions using a mouse bioassay resulted in unspecified signs of *Microcystis* toxicity and death within two hours from the Shin–ike pond material, whereas that from Oo–ike pond did not produce signs of acute toxicity. Quantification of microcystins by HPLC revealed that Shin–ike pond lyophilised bloom material contained 318μg/g MC–RR, and 161μg/g MC–LR. Oo–ike pond cyanobacteria contained 29μg/g MC–RR and no detectable MC–LR (Matsunaga et al. 1999). While tissues from affected birds were not analysed for microcystins, the combination of necropsy, bioassay and microcystin quantification, along with differential findings between the suspect pond and a nearby "control" site lend strong support to the presumptive diagnosis of acute microcystin intoxication in this case.

Nodularin

Case study: dog death in South Africa, 1994 (Harding et al. 1995).

In March 1994 a bull terrier was admitted to a Cape Town veterinary hospital with a history of lethargy, vomiting and loss of appetite after drinking from an urban lake the previous day. The lake is known to be eutrophic and the phytoplankton is normally dominated by *M. aeruginosa*. Antibiotic, corticosteroid, antiemetic and choleretic pharmacotherapy was administered, as well as vitamin B complex and intravenous fluids. Serum alanine transaminase was markedly elevated, indicating acute hepatic dysfunction. The dog died on the fifth day after admission; necropsy was not conducted, but liver samples were collected and prepared for histopathological examination. Water and scum samples from the lake were col-

lected two days after the dog drank from it. Microscopic examination revealed a bloom of 95% *Nodularia spumigena*, with the remainder being *M. aeruginosa*. Scum material was acutely toxic in a mouse bioassay, with liver damage suggestive of cyanobacterial hepatotoxin poisoning. Methanol extracts of bloom material analysed by HPLC with photodiode array and UV detection showed a major peak that increased when spiked with nodularin standard, and a UV spectrum that matched that of nodularin. Lyophilised cyanobacteria contained 3.5mg/g nodularin; no evidence for the presence of microcystins in the bloom material was found. Histology findings suggested toxic liver injury, with hepatocyte degeneration and indications of cholestasis (Harding et al. 1995). Again, while tissues were not examined for the presence of cyanotoxins, the combination of clinical presentation, diagnostic tools – liver function tests, histopathology, mouse bioassay – and investigative chemistry strongly supported the diagnosis of nodularin poisoning.

Cylindrospermopsin

Case study: cattle deaths in Australia, 2001 (Shaw et al. 2004).

Two separate poisoning incidents were investigated in Central and Northwest Queensland involving a total of 55 cows. Affected animals were lethargic and recumbent for three or four days ante–mortem. Post–mortem findings were typical of cylindrospermopsin intoxication, with pallid livers and cholecystomegaly. Histopathology showed hepatocyte degeneration and necrosis, nephrosis and multifocal cardiomyopathy. Farm water samples, rumen contents, liver, kidney and muscle were analysed by HPLC-tandem mass spectrometry for cylindrospermopsin, which was found in all samples except muscle. Water and rumen samples contained cylindrospermopsin concentrations in excess of 1mg/L (Shaw et al. 2004). The principal cylindrospermopsin–producing cyanobacterium in tropical and subtropical Queensland is *Cylindrospermopsis raciborskii*. Associated stock animal deaths and human water supply–related acute and chronic poisonings have long been suspected there (Hayman 1992, Thomas et al. 1998, Griffiths and Saker 2003), and livestock industry publications seek to inform farmers, veterinarians and government workers of the risks from this cyanotoxin (Berry 2001).

Anatoxin–a

Case study: dog deaths in France, 2003 (Gugger et al. 2005).

Two dogs died in separate incidents in September 2003 shortly after drinking from the shore of a river in the Jura region. Clinical signs were vomiting, hind limb paresis and respiratory failure preceding death. The smaller dog (2.5kg) sickened and died shortly after emerging from the water, whereas the larger dog (25kg) had a delayed onset of signs and died within five hours. Stomach contents, intestinal contents and liver were sampled as well as water column and benthic biofilm from the river; stomach contents and field samples were examined for phytoplankton identification. An initial screen for three potential cyanotoxins was conducted on biofilm samples: protein phosphatase 2A inhibition assay for microcystins, mouse neuroblastoma *in vitro* assay for saxitoxins, and HPLC with photodiode array for anatoxin–a. No microcystins or saxitoxins were detected, but a compound with similar chromatographic characteristics to anatoxin–a was seen, so subsequent investigations were directed to that end. A total of seven Oscillatorealean cyanobacterial species and genera were identified across field and gastric samples; three of these were isolated and one isolate, *Phormidium favosum*, was found to produce anatoxin–a. This benthic species was found in the biofilm covering river sediments, stones and macrophytes, as well as from the stomach contents of both dogs – *P. favosum* being the dominant cyanobacterium found in the smaller dog's stomach. Identification of anatoxin–a in biofilm, liver and gastro–intestinal contents was confirmed by tandem mass spectrometry; the authors draw attention to the necessity for confirmation of identity by fragmentation ion spectral assignments to allow definitive identification of anatoxin–a, as the amino acid phenylalanine has an identical mass spectrum peak to that of anatoxin–a when single ion monitoring is used. This was also the first report of anatoxin–a in French waters, and the first identification of an anatoxin–a–producing *Phormidium* species. The authors suggest that neurotoxic signs in 37 dogs (with 26 deaths) in 2002 and 2003 in the south of France may also have been associated with anatoxin–a intoxication (Gugger et al. 2005). This comprehensive investigation utilised well–equipped facilities, use of anatoxin–a and phenylalanine standards, and combined expertise in the fields of investigative chemistry, phytoplankton identification and isolation, so it probably represents the current state of the art for the diagnosis of anatoxin–a poisoning.

Anatoxin–a(s)

Case study: waterbird deaths in Denmark, 1993 (Henriksen et al. 1997, Onodera et al. 1997).

Cyanobacteria–associated animal deaths have been reported at Lake Knud sø since 1981. In 1993, two grebes and a coot that died when a cyanobacterial bloom was evident were collected and frozen. Stomach contents were examined microscopically to identify cyanobacteria; *Anabaena lemmermannii* were found in all three birds. This material was lyophilised for further toxin analysis. Four neurotoxic strains of *A. lemmermannii* were isolated and cultured from bloom material collected from the lake. Bloom material, cyanobacterial cultures and stomach contents were analysed for anticholinesterase activity with a colorimetric assay and for microcystins by ELISA. Only low levels of microcystins (ng/kg range) were found in bird gastric contents. Neither anatoxin–a nor saxitoxins were detected by HPLC. Intraperitoneal injection of bloom material into mice produced signs of acute neurotoxicity, including the muscarinic signs of salivation, lacrimation and urinary incontinence that typify anatoxin–a(s) intoxication. Anticholinesterase activity was significant in bloom samples (2.3mg anatoxin–a(s) equivalents/g); *A. lemmermannii* isolates and stomach contents also showed anticholinesterase activity (29–743μg/g, and 2–90μg/kg body weight respectively). Subsequent work using mass spectrometry, nuclear magnetic resonance and circular dichroism definitively identified anatoxin–a(s) in bloom material (Henriksen et al. 1997, Onodera et al. 1997).

Saxitoxins

Case study: sheep deaths in Australia, 1994 (Negri et al. 1995).

Thirteen ewes and one ram died next to or within 150m of a farm dam. Observed signs were trembling, recumbency and crawling. The ram, which was ataxic, was caught by the owner and "immediately died in his arms." Necropsy and histopathology findings were unremarkable. The dam was found to contain a bloom of *Anabaena circinalis*. Mouse bioassay of bloom material caused death within 4–11 minutes, with clinical signs of staggering, gasping, leaping and respiratory failure. High levels of saxitoxins were found in bloom extracts and intestinal contents of one sheep by HPLC with fluorescence detection; principal components were C–toxins

and gonyautoxins, with low levels of saxitoxin and decarbamoyl saxitoxin. Neither anatoxin–a nor microcystins were found using GC/MS and HPLC respectively; trace amounts of microcystin–like activity were found with a protein phosphatase assay (Negri et al. 1995). Saxitoxin–producing *Anabaena circinalis* is a particularly problematic cyanobacterium in Australia (Humpage et al. 1994). A 1000 km-long riverine bloom in the spring and summer of 1991 was associated with significant stock losses; one farmer alone reportedly lost over 1,100 sheep during the six–week period of the bloom (NSW Blue–Green Algae Task Force 1992, Smith 2000).

Lesional neurological disease and cyanobacteria?

The case studies summarised above represent investigations into animal deaths associated with the well–characterised cyanotoxins. However, other less well–understood cyanotoxins may be capable of killing animals, and are being actively researched. Wilde et al. (2005) and Williams et al (2007) report on avian vacuolar myelinopathy (AVM), a recently–described wildlife disease. AVM was reportedly responsible for more than 100 bald eagle deaths, and "untold numbers" of waterbirds. Clinical signs of the disease are loss of coordination on land and in water, and difficulty in flying. Necropsy reveals a diffuse vacuolation of myelinated neuronal tissue. Epidemiological investigations suggest that a neurotoxin is responsible, with exposure being seasonal, linked to dietary intake by herbivorous waterfowl from AVM–positive waterbodies, and moving up the food chain (i.e., from waterfowl prey to eagle predator). Current evidence suggests that AVM is not contagious. A common factor at AVM–positive sites – and absent or rare at AVM–negative sites – appears to be an epiphytic cyanobacterium in the order Stigonematales. The authors demonstrate the potential of veterinary epidemiology to advance the understanding of cyanobacteria–related toxicity by use of experimental techniques that are not available in the field of human epidemiology. A mallard duck sentinel trial was conducted, where 20 farm–raised birds were tagged for identification, had wing feathers clipped and were released into an AVM–suspect pond. The site was regularly monitored, symptomatic birds were captured and sacrificed, and at the end of the six–week trial, remaining sentinels were captured and sacrificed. Five symptomatic ducks and ten birds captured at the end of the trial – these 15 being all birds able to be recaptured from the pond – all showed AVM lesions in brain tissues (Wilde et al. 2005). Williams et al (2007) have isolated and cultured the suspect cyanobacterium from environmental samples, and have developed PCR as-

says to aid detection and identification. Further epidemiological field work using suitable control sites would be of great interest.

"Unusual" animal deaths and cyanobacteria

The previous discussion has concentrated on cyanotoxin–related deaths in common domestic, stock and wild animals. Some other animals reportedly poisoned by cyanobacteria are:

- **Flamingos:** Cyanobacteria–related mortalities have been reported in three flamingo species, both wild and captive (see summary by Codd et al (2003)). Lesser Flamingos in Kenyan soda lakes have been the subject of research interest, with four microcystin congeners and anatoxin–a found in cyanobacterial mats and stomach contents of dead birds at Lake Bogoria. Microcystins and anatoxin–a were also detected in faecal pellets collected from lake shorelines (Krienitz et al. 2003). The same cyanotoxins have been found in feathers taken from poisoned flamingos, with a dietary origin most likely (Metcalf et al. 2006). Mass die–offs of tens of thousands of birds have been reported in these crater lakes, with implications for management and regional and national economies, as flamingos are a significant tourist attraction (Krienitz et al. 2003, Ndetei and Muhandiki 2005). Isolated strains of *Arthrospira fusiformis* from two crater lakes have been shown to produce both microcystin–YR and anatoxin–a, while an *A. fusiformis* strain from a third lake produces anatoxin–a (Ballot et al. 2004, Ballot et al. 2005). Similar Lesser Flamingo poisonings have been reported from alkaline lakes in Tanzania, with toxic *A. fusiformis* implicated (Lugomela et al. 2006). The implications of these findings are significant for several reasons, as A*rthrospira* sp. (also known as *Spirulina*) are the principal food source of Lesser Flamingos, and *Spirulina* spp. are used as a dietary supplement by humans and as a feed additive for livestock. There is a significant body of literature that suggests that consumption of *Spirulina* is not harmful (Ciferri 1983, Belay et al. 1993, Hayashi et al. 1994, Qureshi et al. 1996, Salazar et al. 1998, Abdulquader et al. 2000, Al–Batshan et al. 2001), though presumably these studies refer to non-toxic strains of *Arthrospira* used for both commercial mass production and from wild harvesting. Investigations to determine the relative contribution of *A. fusiformis* to the production of cyanotoxins in Kenyan soda lakes would be of great interest. Some of these lakes are periodically dominated by more well–known toxigenic cyanobacteria such as *Anabaena* and *Microcystis* spp. (Ndetei and Muhandiki 2005),

so it will be important to estimate the production capacity of cyanotoxins by various cyanobacteria in field situations when harmful levels of cyanotoxins are present.

- **Insectivorous bats:** Staff at a campground in Alberta, Canada, in the summer of 1985, counted 500 dead bats and estimated over 1,000 deaths on the leeward side of a lake. At least 24 dead mallards were also reported. The lake area was covered with a "thick white scum" that had a blue–green sheen. Examined animals were covered with a green slime; necropsy did not reveal any abnormalities. An alkaloid was extracted from the material covering the carcases. This alkaloid was identified by GC/MS and found to be anatoxin–a (known at the time as Very Fast Death Factor) (Pybus et al. 1986).
- **Rhinoceros:** Four white rhinoceroses were introduced to a South African game reserve in May, 1979. Two months later, two of them were found dead but were unable to be examined. Approximately one week later, another rhino was found dead after being seen to be active the previous day. Macroscopic and microscopic findings were typical of acute hepatotoxicity: hepatomegaly, ascitic fluid, coagulopathy seen in various tissues, severe hepatic necrosis and loss of hepatic architecture. At the time of death, a severe bloom of *M. aeruginosa* covered the park dam, with a surface scum of 4–12cm (Soll and Williams 1985).
- **Honeybees:** In the summer of 1971, "almost total" mortality of bees from 84 hives was associated with the insects watering on the leeward edge of a lake in New South Wales, Australia. That area of the lake was affected by a windborne scum of *A. circinalis*; an apiary on the windward shore was unaffected (May and McBarron 1973).

Exposure routes, avoidance behaviour

Exposure to cyanotoxins in the field is presumably either through contaminated drinking water or by direct consumption of benthic cyanobacteria. Codd et al (2005) also note suggestions that cyanotoxins may move along the food chain to poison wild animals. This would seem to be a plausible interpretation: there is a significant section of the literature devoted to experimental and observational studies of cyanotoxin bioaccumulation and trophic transfer. Much of the literature is concerned with potential human exposure to cyanotoxins through consumption of molluscs and fish; short-term (days, weeks) accumulation of hazardous levels of cyanotoxins in aquatic prey is certainly feasible, and predator animals may be more at risk

than humans in some circumstances, since they are more likely to consume the viscera in which cyanotoxins are found in highest concentrations. Several bioaccumulation studies are inconclusive; for some positive examples see: (Negri and Jones 1995, Saker and Eaglesham 1999, Magalhães et al 2001, Sipiä et al 2002, Saker et al 2004, Sipiä et al 2004, Smith and Haney 2006).

Experimental work has shown that the inhalation route may be an efficient method for microcystin–LR to access the circulation (Creasia 1990, Fitzgeorge 1994, Ito 2001), but there is no evidence that cyanotoxins can be effectively aerosolised to toxic concentrations under field conditions. The topic of inhalational exposure to cyanotoxins is clearly under–researched at present.

Codd et al (1992) outlined the premise that exposure to toxic cyanobacteria may not always be coincidental; dogs may actively seek out and consume benthic cyanobacteria. Some descriptions in the report of Hamill (2001) also suggest that dogs may actively consume toxic cyanobacteria. The work of Gugger et al (2005) also appears to support this concept: while descriptions from the field were that two dogs died "soon after drinking water from the shoreline of the…river…" analysis of phytoplankton in the dogs' stomachs revealed the presence of biofilm–associated cyanobacteria that were not seen in water column samples. This thick biofilm reportedly covered sediment, stones and macrophytes at the water's edge (Gugger et al. 2005); it is at least conceivable that those animals were also actively consuming toxic cyanobacteria for their final meal.

As for planktonic cyanobacteria, there are suggestions that wild and domestic animals will avoid drinking from cyanobacterial scums if less–affected water is accessible. Falconer (2005 p.80) notes that detailed investigations of cyanobacteria–related stock animal deaths often reveal that cleaner water was unavailable because of fence lines, thus forcing the animals to consume toxic water. Other workers have reported avoidance behaviour in cattle (Codd 1983) and flamingos (Ndetei and Muhandiki 2005), but Carbis et al (1994) report that sheep did not discriminate between hepatotoxic cyanobacteria–contaminated water and a readily available alternate supply. Lopez Rodas and Costas (1999) report observing ungulates, birds, wasps and unspecified wildlife failing to demonstrate avoidance behaviour, consuming concentrated *M. aeruginosa* scum in Spanish reservoirs. Their subsequent experiments showed that laboratory mice demonstrated an ultimately fatal preference for dense cultures of microcystin–producing *M. aeruginosa* over tap water, and they did not discriminate between toxic and non–toxic strains of *M. aeruginosa* in their drinking water. Steyn (1943) noted that pregnant animals and dairy cows displayed a preference for consuming *Microcystis* scums, suggesting that a

relative nutritional deficiency may explain higher mortalities than seen in dry cows.

Treatment of affected animals; research needs

As no complete pharmacological antagonists against cyanotoxins are available, treatment of affected animals is largely supportive. Purgatives and enemas may be helpful; unguents and restriction to shaded areas should be considered for animals suffering secondary photosensitization (Steyn 1943). Other supportive therapies include emesis, activated charcoal and bathing to remove bloom material from the fur (Corkill et al. 1989, Roder 2004). Blood transfusion and correction of electrolyte imbalance has been recommended (Beasley et al. 1989a). Aggressive intervention with fluid replacement and steroids has been suggested for cases of cyanobacterial hepatotoxin poisoning (Roder 2004). While such an approach was unsuccessful in the case (discussed above) of the bull terrier presumed to have died from nodularin intoxication (Harding et al. 1995), there is clearly a need for controlled experiments to evaluate interventional therapies. Pre–treatment with pharmacologic doses of hydrocortisone prevented acute and delayed MC–LR–related mortality in mice (Adams et al. 1985), and dexamethasone and indomethacin pre–treatment blocked renal toxicity caused by MC–LR (Nobre et al. 2001). Pre–treatment with steroids is obviously not a practical therapeutic intervention, but these findings suggest that there is much more to be learned about the immunotoxicology of the cyanobacterial hepatotoxins.

Artificial ventilation has been suggested in cases of cyanobacterial neurotoxin poisoning (Beasley et al. 1989a, Beasley et al. 1989b, Carmichael and Falconer 1993, Roder 2004). While the practical application of this technique in the field would present significant challenges, experimental work to evaluate the benefits of intermittent positive pressure ventilation and other intensive therapy may have implications for the field of human medical care as well as for economically valuable equine and livestock animals. Work conducted in the 1970s on calves and rodents dosed with anatoxin–a–producing cyanobacteria suggested that artificial ventilation is unlikely to be a practical intervention, with one calf failing to achieve effective spontaneous respiration after being ventilated for 30 hours, and rats similarly afflicted after maintenance ventilation of up to eight hours (Carmichael et al. 1975, Carmichael et al. 1977). However, anatoxin–a doses producing up to 95% neuromuscular blockade in rats were reportedly reversible with respiratory support (Valentine et al. 1991). Artificial ventila-

tion was conducted on three rats during experimental administration of anatoxin–a(s); they survived a greater than 4–fold lethal dose in the short term (less than one hour), but the study was not designed to follow through to recovery (Cook et al. 1990). Interventional strategies to treat saxitoxin poisoning using antitoxin or the potassium–channel blocker 4–aminopyridine and artificial ventilation have shown promise, though the toxicodynamics of saxitoxin are complex and the combined neurological, cardiovascular and respiratory effects are incompletely understood (Chang et al. 1993, Benton et al. 1994, Chang et al. 1996, Chang et al. 1997, Benton et al. 1998).

Management of seizures, presumably anticonvulsant pharmacotherapy, has been suggested in cases of cyanobacterial neurotoxin poisoning (Roder 2004), though this may be a moot point if, as suggested at least for anatoxin–a, seizures result from hypoxia–related cerebral ischaemia (Carmichael 1994). Cyanobacterial neurotoxin–related seizures are not well described in the literature, mostly being referred to as "convulsions", though there are references to clonic spasms (McLeod and Bondar 1952), clonic seizures (Cook et al. 1989, Cook et al. 1998, Carmichael 2001), tonic spasm (Firkins 1953) and tonic seizures (Beasley et al. 1989a, Beasley et al. 1989b). The electrophysiology of these events has not been investigated. Opisthotonus is often described (Carmichael et al. 1975, Gorham and Carmichael 1988, Cook et al. 1989, Carmichael 2001, Codd et al. 2003); this increase in tone of the musculature of the neck and shoulders has some similarity to the clinical manifestation of a tonic seizure, though again the priority from a management perspective would appear to be restoration of oxygen to the tissues. Anticonvulsants have also been administered for management of seizures in cases of cyanobacterial hepatotoxin poisoning, but with little apparent benefit (Corkill et al. 1989).

Further empirical studies are warranted to assess various emergency interventions, including pharmacotherapies, for cases of cyanotoxin poisoning. However, these are potent, rapidly acting toxins, so prevention will likely remain the most effective intervention for the foreseeable future. In the short term this involves restricting access to cyanotoxin–affected drinking water, with longer–term strategies of nutrient reduction to mitigate blooms. Dissemination of information to farmers and the livestock industry is always important; beyond the academic research literature there are some industry and government partnerships that provide helpful resources, e.g. Australia's Animal Health Surveillance Report (see for example Berry 2001, Elliott 2001), and the Animal Health Expositor from Canada (now Animal Health Perspectives) (Yong 2000a, Yong 2000b). Comprehensive economic analyses of the costs of cyanotoxin–related livestock and wild

animal poisonings to the agricultural and tourist industries would be valuable.

Concluding remarks

Cyanotoxins in the environment continue to be hazardous to livestock and wild animals throughout the world. While much is known about the most potent, acutely toxic neurotoxins and hepatotoxins, there is still more to learn about the toxicokinetics and toxicodynamics of even the well–characterised cyanotoxins. Cyanobacteria are rich sources of biologically active compounds, and it is likely that some yet–to–be–described cyanobacterial products will be found to be harmful. Current investigations into the subacute disease avian vacuolar myelinopathy hint at this possibility. While controlled dosing with purified cyanotoxins under laboratory conditions has been an essential endeavour that allows these materials to be characterised and understood, exposures in the field may be much more complex. Subacute or chronic exposures, particularly to hepatotoxic cyanobacteria, may result in clinical presentations characterised by concomitant degenerative and regenerative changes. Expert veterinary clinicians and pathologists, as well as specialist investigative chemistry laboratory services, are invaluable resources for the diagnosis and management of these complex poisonings.

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