Acknowledgements: Researchers….. Colleagues, Grad Students, Postdocs, & More

Collaborating Scientists:

- Wayne Carmichael, PhD
- Wanda Haschek, BVSc, PhD, D. ACVP
- Phil Solter DVM, PhD, D. ACVP
- Ken Holmes, PhD
- Ken Rinehart, PhD
- Ken-Ichi Harada, PhD
- Michio Namikoshi, PhD

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- Randall Lovell, DVM, PhD, D. ABVT
- Steve Hooser, DVM, MS, PhD, D. ABVT
- Bill Cook, DVM, MS, PhD, D. ABVT
- Richard Stotts, DVM, PhD
- Safdar Khan, DVM, MS, PhD, D. ABVT
- Mark Wickstrom, DVM, PhD

Postdocs

- Bill Valentine, PhD, DVM, D. ABVT
- Sushmita Ghosh, PhD

Many Student & Technical Assistants…

Individuals Who Provided Recent Findings/ Insights for this Presentation:

- Wayne Carmichael, PhD
- Michael Knight DVM, D. ABVT
- Rick Fredrickson DVM, MS
- Deon van der Merwe DVM, PhD, D. ABVT
- Wilson Rumbeiha DVM, PhD, D. ABVT

Cyanobacterial (Blue-Green Algal) Toxins & Domestic Animal Concerns

Springfield, Illinois
January 16, 2013

Val Beasley DVM, PhD, Diplomate, ABVT
Professor Emeritus of Veterinary, Wildlife, & Ecological Toxicology
Founder & Former Executive Director, Envirovet Program in Wildlife & Ecosystem Health
College of Veterinary Medicine
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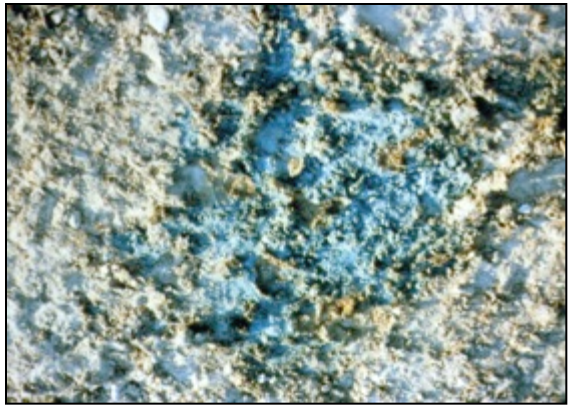
A grad student in his initial study of his fellow prokaryotes.

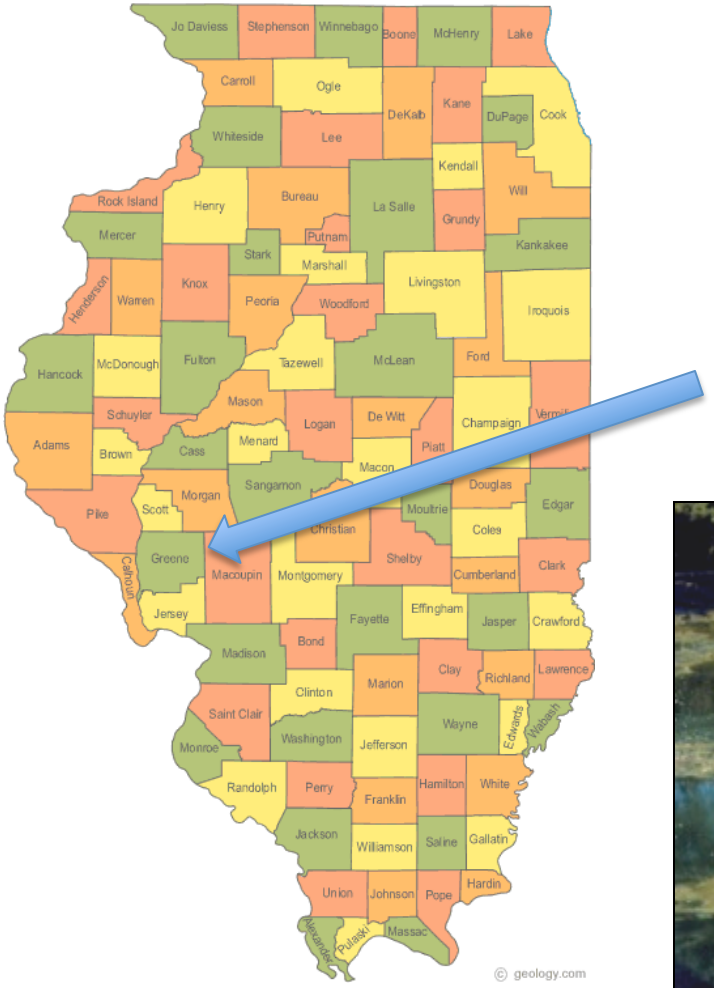
Author on August 21, 1981,when he could still transcribe DNA coding for hair & melanin!

Pigs were not eating *Datura*

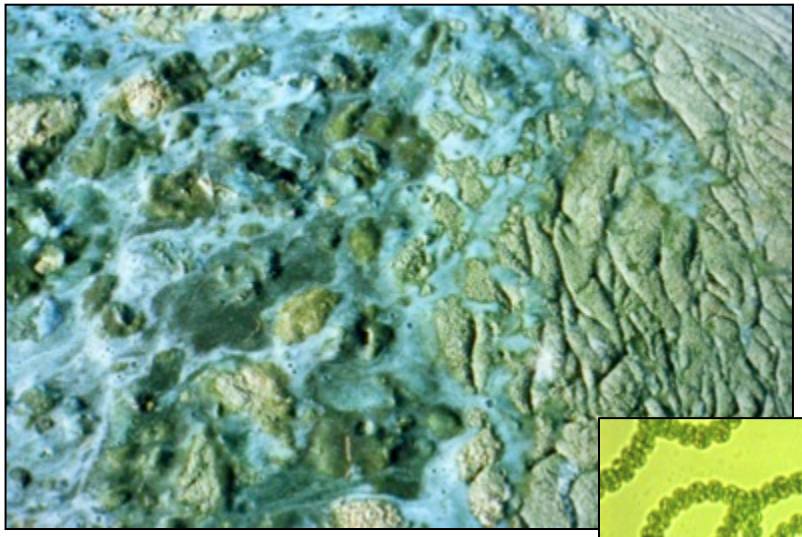


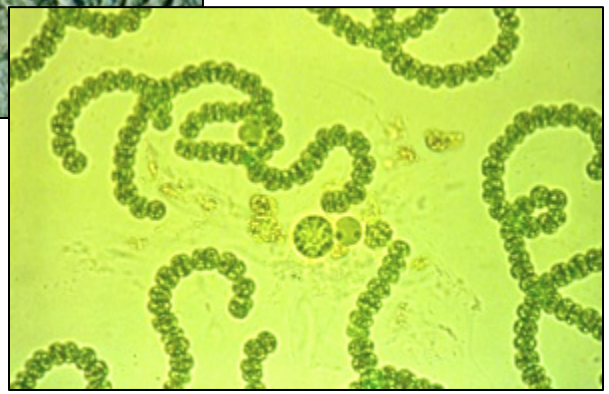
Vomitus containing *A. spiroides*

- Case call came in through the Animal Poison Control Center regarding sows dying at a farm pond in Greene, County, Illinois



Call taken at 11:45 AM.
We prepared for departure via aircraft, & arrived on site at 3:45 PM the same day.





Anabaena spiroides had been ingested by swine shortly before their deaths in 1981.

- Cyanobacteria associated with deaths, but toxins not confirmed.
  - We were becoming toxicologists & new to cyanobacterial toxinology.
  - Anatoxin-A structure was known, but Wayne Carmichael was nowhere to be found.
  - Anatoxin-a(s) was yet to be isolated & structurally characterized.

Apparent blue-green algae poisoning in swine subsequent to ingestion of a bloom dominated by *Anabaena spiroides*

V. R. Beasley, DVM, R. W. Coppock, DVM, J. Simon, DVM, PhD, R. Ely, DVM, PhD, W. B. Buck, DVM, MS, and R. A. Corley, BS, College of Veterinary Medicine, University of Illinois, Urbana, IL 61801; D. M. Carlson, DVM, 703 Sycamore St, Greenfield, IL 62044; P. R. Gorham, PhD, Department of Botany, University of Alberta, Edmonton, Alberta, Canada T6G 2E9

Journal of the AVMA 82:413-414 (1983)

In mid-August of 1981, 10 of 65 sows died over a 2-day period after ingestion of water containing a wind-concentrated bloom of blue-green algae in a 2-ha farm pond in southwestern Illinois. Large accumulations of blue-green algae and green algae with blue-green flecks were at the eroded south end of the pond, where the sows had the greatest access. This pond received surface runoff from adjacent fields, as well as from the large lot in which the sows were kept, and was their sole source of drinking water. A bluish coating on the soil was apparent on the edges of the pond where water had splashed onto the soil.

Approximately 20 to 30 m from the pond was a large blue stain that comprised the dehydrated vomitus of 1 of the sows. Of the sows that died, this 1 was most closely observed by the referring veterinarian as the clinical syndrome progressed. The sow vomited approximately 4 L of dark green liquid. Trembling progressed to violent shaking, and the sow died within one-half hour of the onset of illness.

Postmortem examinations of this and another sow revealed generalized cyanosis, flaccid myocardium, mild emphysema, and ecchymotic hemorrhages of the gastric fundus mucosa and the serosal and mucosal surfaces of the jejunum. The sow that was observed antemortem had blue discoloration of the ingesta of the stomach, duodenum, and jejunum. It also had a somewhat orange-colored liver, with more obvious than normal lobular patterns and with some lobules being dark red. Both sows had blue-green staining on the skin of the legs and around the mouth. Definitive histologic lesions were not detected; however, specimens for histologic examination were not taken until 4 hours after necropsy, and autolytic changes may have obscured tissue alterations.

Swine in other groups receiving the same feed but different water supplies were unaffected. Upon removal of the sows to a lot away from the pond, the death losses immediately stopped.

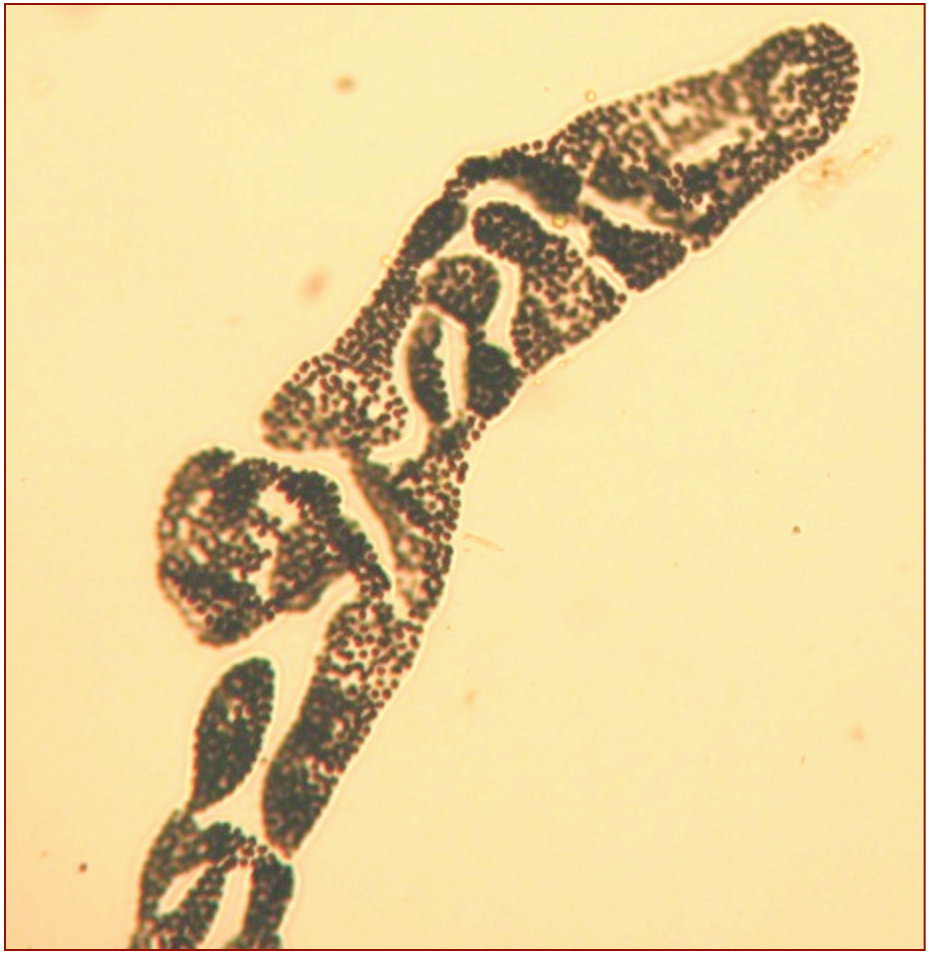
Water samples containing the algae were transported on ice to the laboratory. On the same day as the losses, microscopic examination of these samples revealed an essentially pure culture of *Anabaena*. Specimens of the algae were fixed by adding an equal volume of neutral-buffered 10% formalin to the water-algae sample and sent to one of us (PRG) for specific identification. On the basis of microscopic measurements and structural characteristics (Fig 1 and 2), the organism was identified as *Anabaena spiroides* var *contracta* Klehbahn.[1] The only other noteworthy organisms in the water sample were bacterial rods (1 × 6 μm) singly or in pairs, end to end. These may have contributed to the poisoning syndrome in these sows inasmuch as bacterial toxins have sometimes been implicated in conjunction with blue-green algae consumption.[2]

Another member of the genus *Anabaena*, *A flos-aquae* is 1 of the cyanophytes (blue-green algae) most commonly implicated in poisoning of cattle. Dogs, geese, human beings, mice, rats, ducks, and goldfish are susceptible to poisoning by strains of this organism.[3-6] Previous studies in rats and calves demonstrated that some death losses attributable to *A flos-aquae* ingestion resulted from respiratory paralysis.[7] Artificial respiration, even for periods of up to 24 hours, was of insufficient duration to allow for detoxification.

Unialgal colony isolates of *A flos-aquae* from various sources have been cultivated, lyophilized, and tested for toxicity by intraperitoneal injection into mice, rats, and young chickens.[3,6] One of the toxins of *A flos-aquae*, anatoxin-*a* (previously called "very fast death factor"), is an alkaloid that causes pre- and postsynaptic neuromuscular blockade, which is not reversed by edrophonium or neostigmine. This toxin apparently causes respiratory paralysis in mammals and opisthotonus in ducks, pheasants, and young chickens.[5] Whether the cyanosis and rapid death associated with the bloom dominated by *A spiroides* that these sows consumed was attributable to algal and/or bacterial toxins was not established.

Cyanobacterial Peptide Hepatotoxins

Microcystins were structurally characterized in mid-80s.



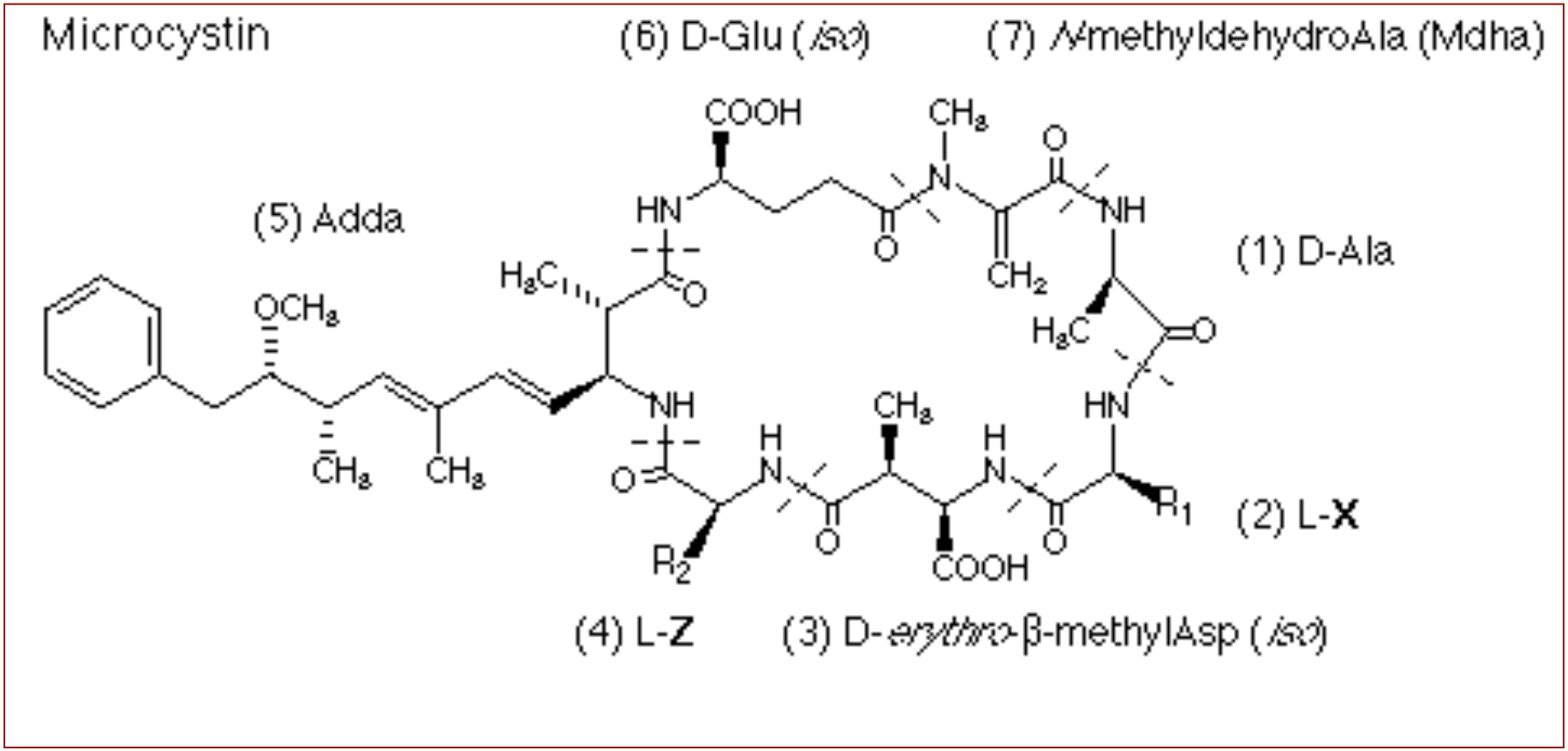
Microcystis aeruginosa from catfish pond in Mississippi, USA.

Often involved in deaths of multiple cattle in a herd.

Implicated in many other animal species as well.

Cyanobacterial Peptide Hepatotoxins

Microcystins: One of the most often encountered – Microcystin-LR



For microcystin-LR, R_1 is leucine and R_2 is arginine.

Field case of microcystin toxicosis in dairy cattle in southern Wisconsin; calf dosed with bloom material for confirmation & toxicologic pathology.

- **20 of 60 Holstein cows affected & 9 died.**
- **Dosed calf** developed classical lesions (few other syndromes cause such lesions).
- **Dosed mice (bioassay)** had equivalent lesions.
- **HPLC/UV detection of derivatized toxin matched with two microcystin standards.**

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Blue-green algae (*Microcystis aeruginosa*) hepatotoxicosis in dairy cows

F. D. Galey, DVM; V. R. Beasley, DVM, PhD; W. W. Carmichael, PhD; G. Kleppe, DVM; S. B. Hooser, DVM; W. M. Haschek, BVSc, PhD

SUMMARY

Twenty cows from a dairy herd consisting of 60 healthy, lactating Holsteins developed clinical signs of anorexia, mental derangement, dehydration, recumbency, and ruminal atony after ingesting water containing blue-green algae. Of the 20 cows, 9 died. The algal bloom, which developed in a stagnant pond during hot, dry weather, was identified as the cyanobacterium *Microcystis aeruginosa*, a potentially hepatotoxic algae. One week after the onset of toxicosis, affected cows seemed healthy, although liver-associated enzyme activities (alkaline phosphatase, γ-glutamyl transferase, aspartate transaminase, and lactate dehydrogenase) were increased.

Intraruminal administration of the intact wet bloom to a healthy 125-kg Angus heifer was followed by hepatic necrosis and death. The liver was large, friable, and gunmetal blue, with microscopically evident hepatocyte dissociation, degeneration, and necrosis. The ingesta of the heifer contained typical clumps of cells that were identified as *M aeruginosa*. The intraperitoneal administration of lyophilized cell material from that bloom to 18 mice caused marked hepatic enlargement. The intraperitoneal median lethal dose of the dried bloom was estimated to be 10 mg/kg of body weight. A cyclic peptide toxin purified from the algae seems to be similar structurally to toxins from other characterized hepatotoxic blooms of *M aeruginosa*.

Poisoning as a result of the blue-green alga (cyanobacterium) *Microcystis aeruginosa* has been reported in livestock.[1-3] A cyclic peptide produced by toxic blooms of this alga causes massive hepatotoxicosis in mice, rats, sheep, and cattle when sufficient amounts are ingested.[1-3] Potentially toxic blooms of *Microcystis* tend to form in water when dry weather and high nutrient concentrations are coupled with slow water movement.[1] Poisoning is more likely when animals have access to an area where wind or water flow have concentrated the bloom near the edge of a lake, pond, or stream.

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From the National Animal Poison Control Center, Departments of Veterinary Biosciences (Galey, Beasley) and Veterinary Pathobiology (Hooser, Haschek), College of Veterinary Medicine, University of Illinois, Urbana, IL 61801; the Department of Biological Sciences, Wright State University, Dayton, OH 45435 (Carmichael); and the Monroe Veterinary Service, 1317-31st Ave, Monroe, WI 53566 (Kleppe).
Supported in part by the US Army Medical Research Development Command contract No. DAMD 17-85-C-5241 and DAMD 17-83-C-4156.

The authors thank Mr. Andrew M. Dahlem and Mr. Jeff Eschedor for technical assistance and A. S. Dabholkar for electron microscopy.

Purposes of the present report were to describe (i) environmental, clinical, and serum chemical findings associated with an epizootic of *M aeruginosa* toxicosis in dairy cattle; (ii) clinical, serum chemical, and pathologic findings in a heifer given the suspected algal blooms; (iii) isolation and characterization of the toxin; and (iv) a field diagnostic technique for confirming exposure to a *Microcystis* bloom.

Materials and Methods

Exposure history and clinical findings—In September 1985, a blue-green algae toxicosis of 1 weeks' duration was suspected in a dairy herd (n = 60) near Monroe, located in Green County of southern Wisconsin. The weather had been hot and dry for several months, and a thin, grainy, green film had formed on a stagnant, spring-fed pond (Fig 1) before the onset of the clinical signs of toxicosis and death losses. On Sept 17, 1985, wind had concentrated the bloom near the pond overflow pipe, and it began to rain. By the next day, 2.5 cm of rain had fallen, filling the pond, which then began to drain. A large amount of the thick, concentrated algal bloom was carried out of the pond and deposited along a shallow ditch in a pasture where lactating dairy cows were often kept.

On the evening of Sept 18, after the rain had subsided, 60 healthy adult lactating Holstein dairy cows (in all stages of lactation) were turned into the pasture. Twelve hours later (Sept 19), 20 cows were anorectic, and 3 of the 20 were recumbent. Affected cows had the green, grainy algal material on their backs, which, when examined by light microscopy, consisted of the same clumps of cellular material as in the pond and outflow. Cows had clinical signs resembling milk fever (anorexia, unresponsiveness, reluctance to move, and occasional mental derangement). Recumbent cows rapidly became dehydrated. Ruminal atony and mild bloat developed.

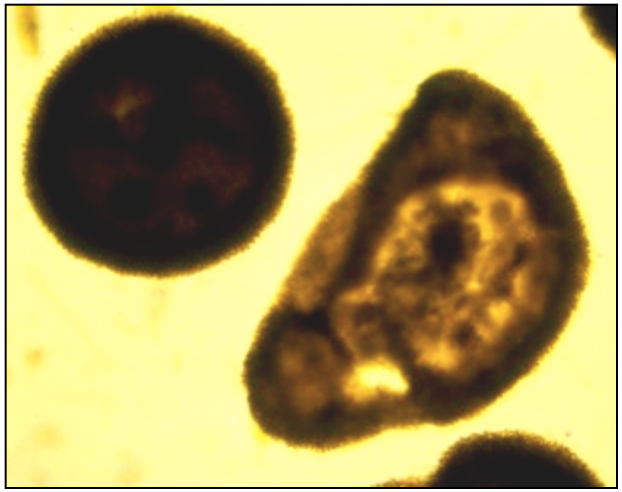
All cattle were removed from the pasture. The 3 recumbent cows had low blood calcium concentrations of 6.4, 7.5, and 7.4 mg/dl and initially responded by getting up when given 1 L of calcium* IV that morning. Later in the morning, 10 additional severely affected cows from the original group of 20 also were given calcium IV along with 1 g of activated charcoal/kg of body weight PO in a 1:5 slurry with water. Of the 3 cows initially recumbent, but that had responded to calcium treatment, 2 became recumbent again and died late that day. Of the 20 cows, 5 became anorectic on Sept 19 and were dead the next morning (36 hours after the original exposure). Two additional cows died the following day (60 hours after the original exposure). Therefore, 9 of 20 cows anorectic 12 hours after exposure to the algal bloom died within 60 hours of that exposure. Postmortem examinations were not conducted on cows that died. One week after exposure, the remaining 11 of the 20 affected cows seemed healthy and were returning to their normal feed consumption and lactation rates.

* Cal Dextro No. 2, Fort Dodge Laboratories, Fort Dodge, Iowa.

Dairy Cows Poisoned, Monroe, WI

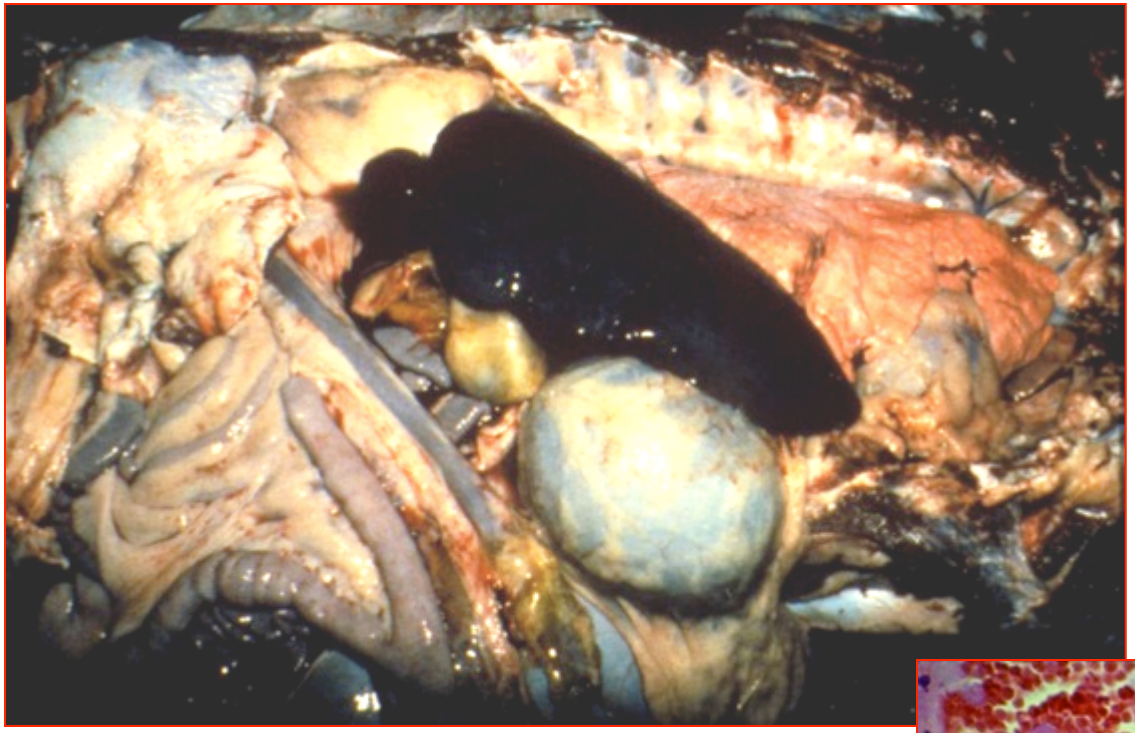
Microcystis aeruginosa in the farm pond.

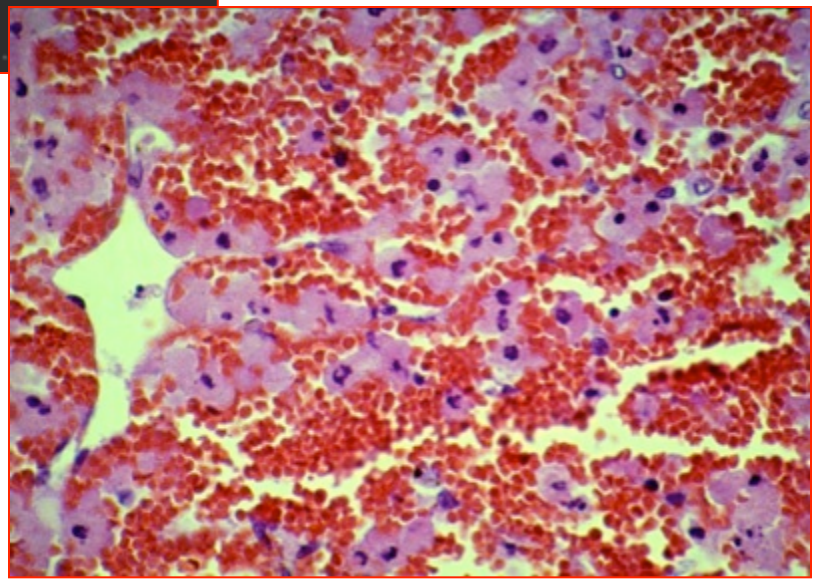
M. aeruginosa from pasture.



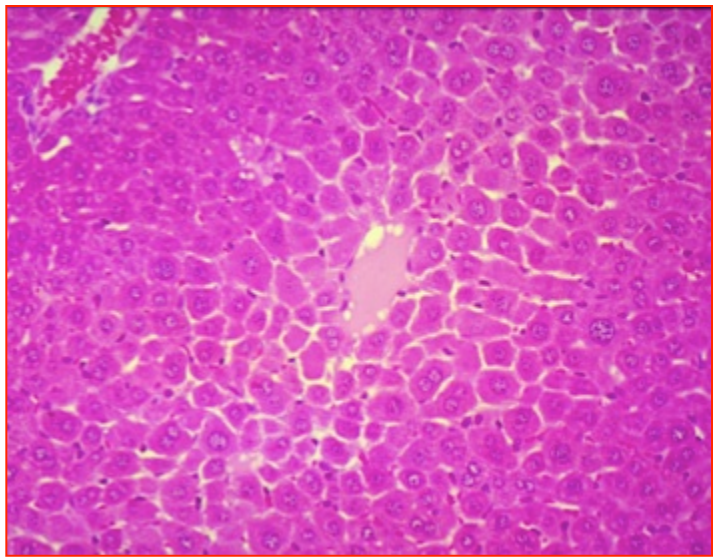
M. aeruginosa from rumen contents.

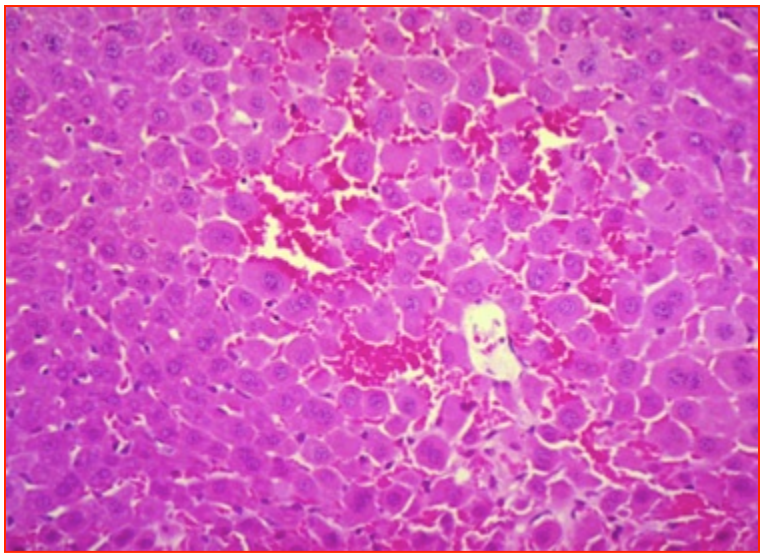
Liver Lesions Microcystins: Calf

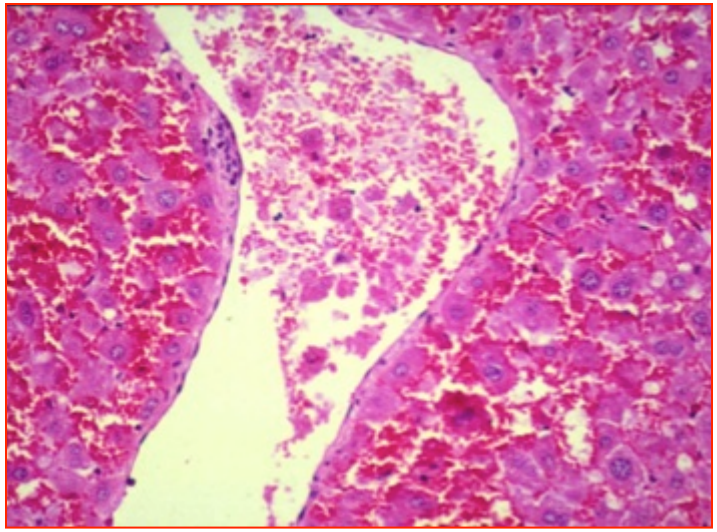


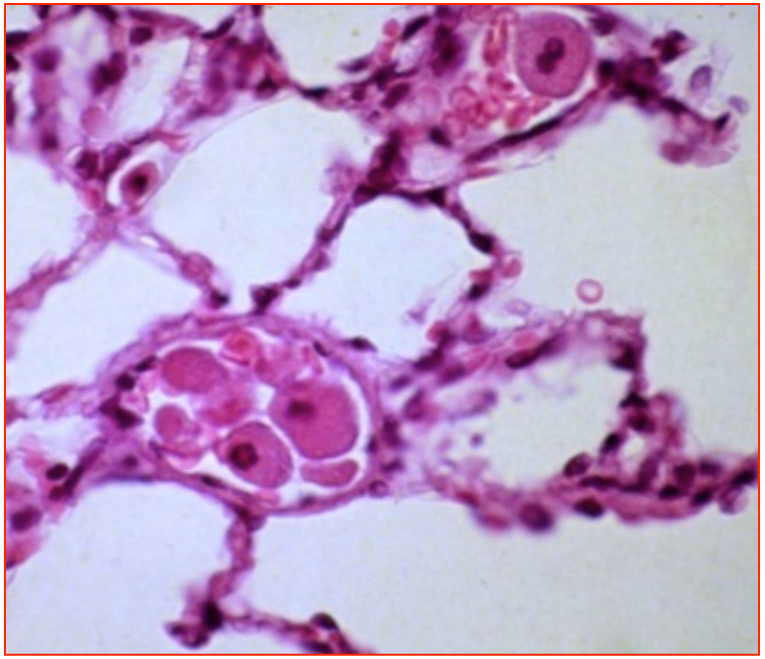


Liver Lesions Microcystins: Time Course Rodents

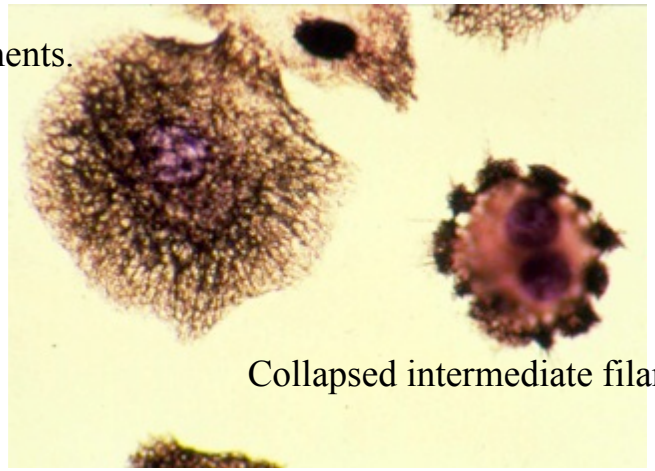








Normal appearing intermediate filaments.

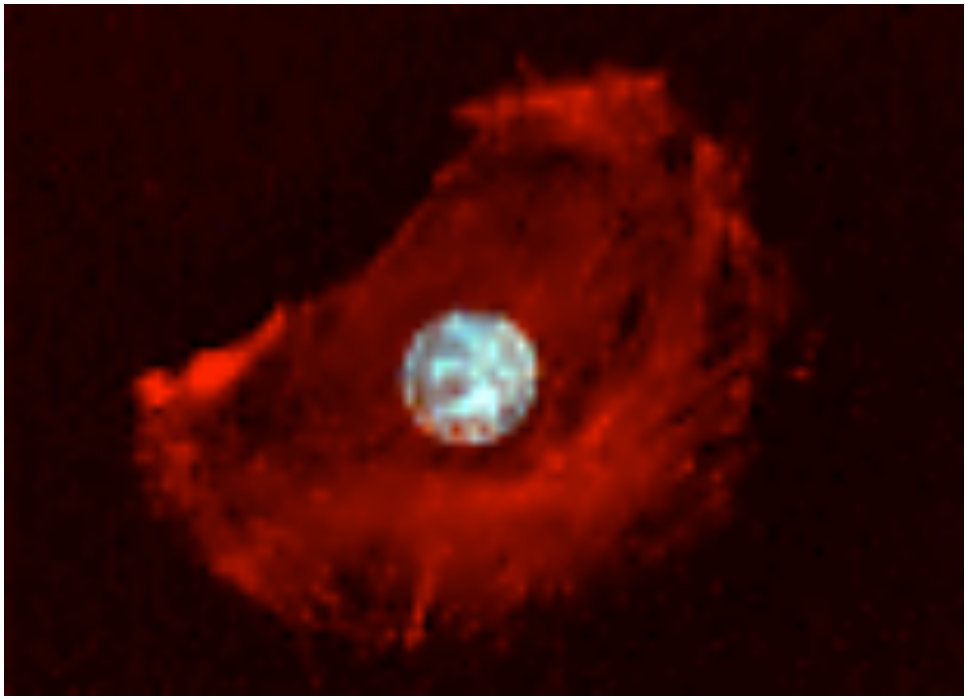


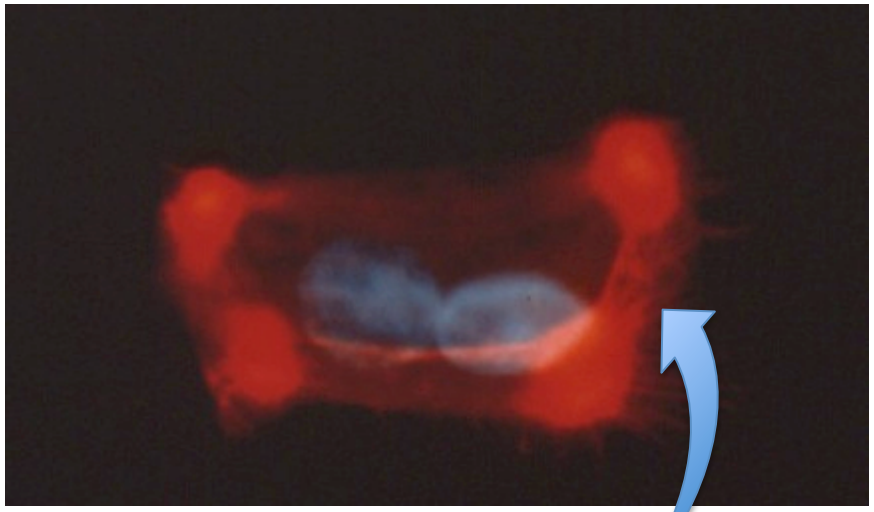
Collapsed intermediate filaments.

Microcystins

- Hepatic uptake via bile acid carriers:
  - Liver-specific effect.
- Inhibits protein phosphatases 1 & 2 A:
  - Protein phosphatases cleave phosphate groups from proteins.
  - Inhibition of PP-ases → excessive phosphorylation.
  - Protein kinases add phosphate groups.
  - Microcystin & nodularin: Similar to net effect of protein kinase induction.
  - Disrupts cytoskeleton (intermediate filaments).
  - Causes apoptosis, necrosis.
  - Activates tumor necrosis factor.
  - Hepatocytes round up, separate, & sinusoids are disrupted.
  - Intrahepatic hemorrhage → often lethal.

Microcystins





- Microcystin-induced cytoskeletal (actin filaments) disruption:
- Rhodamine-phalloidin & fluorescent antibody labeling.

Homer Lake, Champaign County, Illinois

for permission to collect and export plant material. Financial support from the Herman T. Frasch Foundation is gratefully acknowledged.

Supplementary Material Available: ^1H and ^{13}C NMR spectra for compounds **1** and **2** and crystal data for compound **2** (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Identification of 12 Hepatotoxins from a Homer Lake Bloom of the Cyanobacteria *Microcystis aeruginosa*, *Microcystis viridis*, and *Microcystis wesenbergii*: Nine New Microcystins

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Richard R. Stotts, Andrew M. Dahlem,† and Val R. Beasley

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Wayne W. Carmichael and William R. Evans

Department of Biological Sciences, Wright State University, Dayton, Ohio 45435

Received June 25, 1991

Eleven minor components were isolated, together with microcystin-LR (LR, **1**, Scheme I) as the principal toxin (ca. 90% of the toxic components), from *Microcystis* cyanobacteria (blue-green algae) collected from Homer Lake (Illinois) in the summer of 1988. The components were characterized by amino acid analysis and HRFABMS, FABMS/MS, ^1H NMR, and UV spectroscopic methods as microcystins-RR (**2**) and -YR (**3**) (Scheme I) and nine new microcystins. The structures of seven new microcystins were assigned as [DMAdda^5]microcystin-LR (**4**), [Dha^7]microcystin-LR (**5**), microcystin-FR (**6**), microcystin-AR (**7**), microcystin-M(O)R (**8**), [Mser^7]microcystin-LR (**9**), and microcystin-WR (**12**). Compound **4** is the first microcystin containing 9-*O*-demethyl-Adda, while phenylalanine, *N*-methylserine, and tryptophan are also new variations in amino acid components of microcystins. Compound **11** was deduced to be a (C_3H_7O) monoester of the α-carboxyl on the Glu unit of LR (**1**). New microcystin **11** caused no apparent toxic effects in mice dosed ip at 1 mg/kg, while the others had LD_{50}'s of 90–800 μg/kg.

Seven More Microcystins from Homer Lake Cells: Application of the General Method for Structure Assignment of Peptides Containing α,β-Dehydroamino Acid Unit(s)

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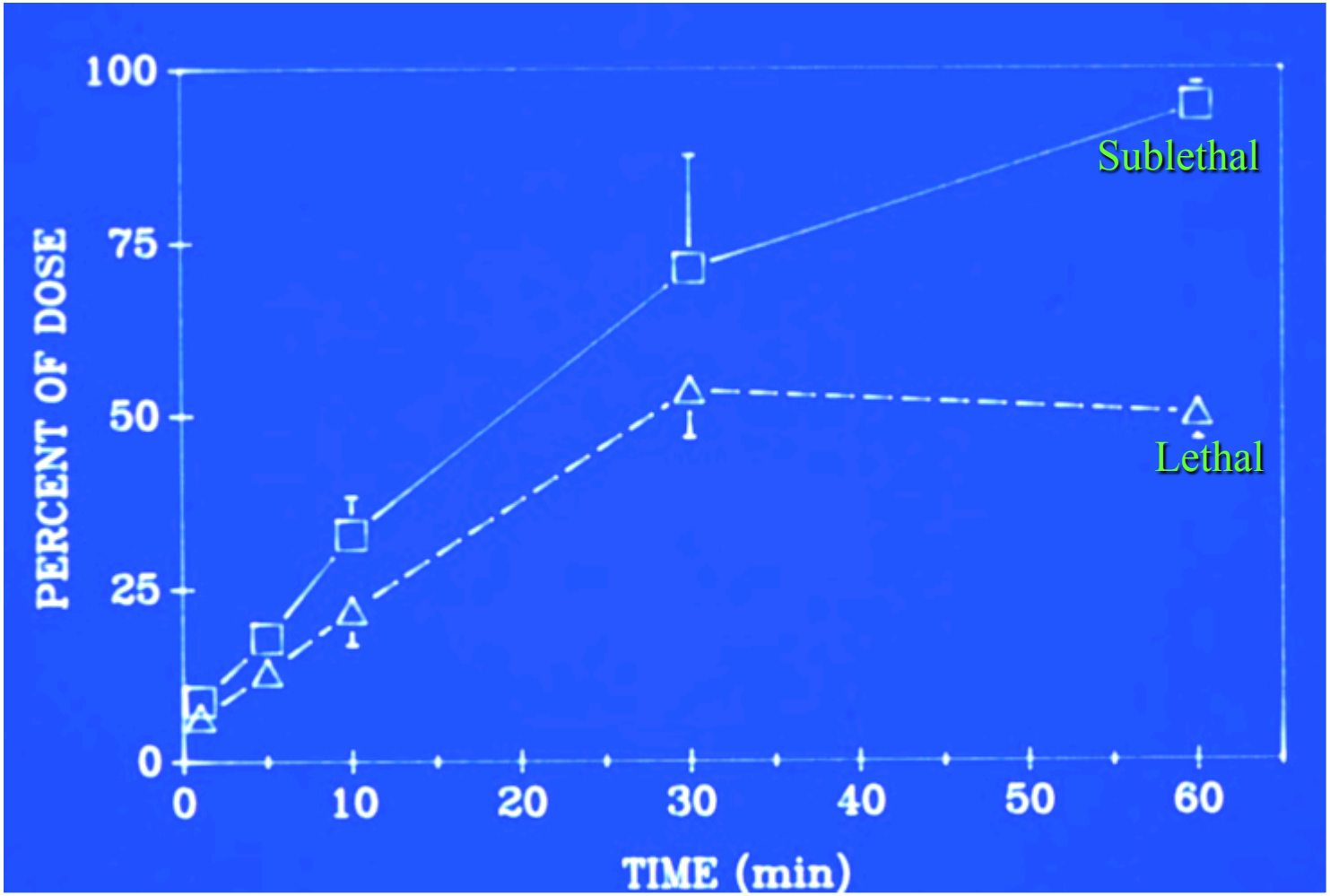
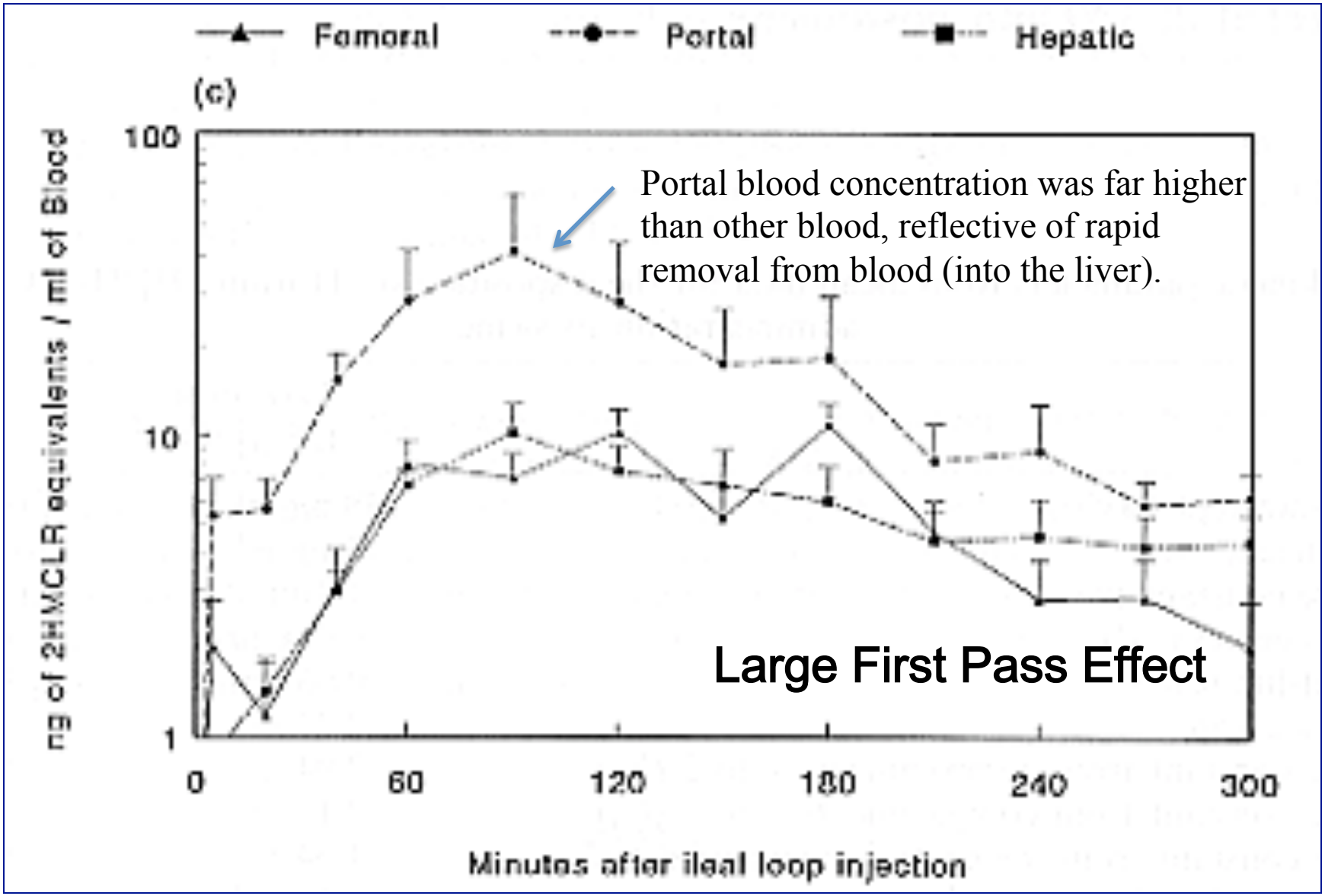
Received October 13, 1994 (Revised Manuscript Received April 7, 1995®)

Larger scale isolation of microcystins, cyclic heptapeptide hepatotoxins, from a water bloom of *Microcystis* spp. collected from Homer Lake (Illinois) gave the previously reported **1**–**5**, additional quantities of [L-MeSer^7]microcystin-LR (**6**), and microcystin-(H_4)YR [**8**, (H_4)Y = 1′,2′,3′,4′-tetrahydrotyrosine], which were previously isolated in insufficient amounts to complete the structure assignment, and seven more microcystins, **9**–**15**. A general method for assigning the structures of cyclic peptides containing α,β-unsaturated amino acid unit(s) developed with nodularin, a cyclic pentapeptide hepatotoxin, was applied to confirm the previously assigned structures of **1**–**5** and to assign the structures of [D-Asp^3]microcystin-LR (**9**) and the new microcystin-HilR (**10**, Hil = homoisoleucine). The method consists of linearization of a cyclic molecule by a one-pot reaction sequence (ozonolysis followed by $NaBH_4$ reduction) and tandem FABMS (FABMS/CID/MS) analysis of the product (linear peptide). A new microcystin, **11**, was assigned the structure [L-MeLan^7]-microcystin-LR (MeLan = *N*-methyllanthionine) and synthesized from **1** and L-Cys. Four linear peptides **12**–**15**, which are reasonable biogenetic precursors of the cyclic compounds, were also assigned structures based on their FABMS/CID/MS data.

Cyanobacterial Peptide Hepatotoxins
Microcystin Uptake – Mouse Liver: Sublethal compared to Lethal Doses



Tritiated Dihydro-Microcystin: Fate in Anesthetized Swine

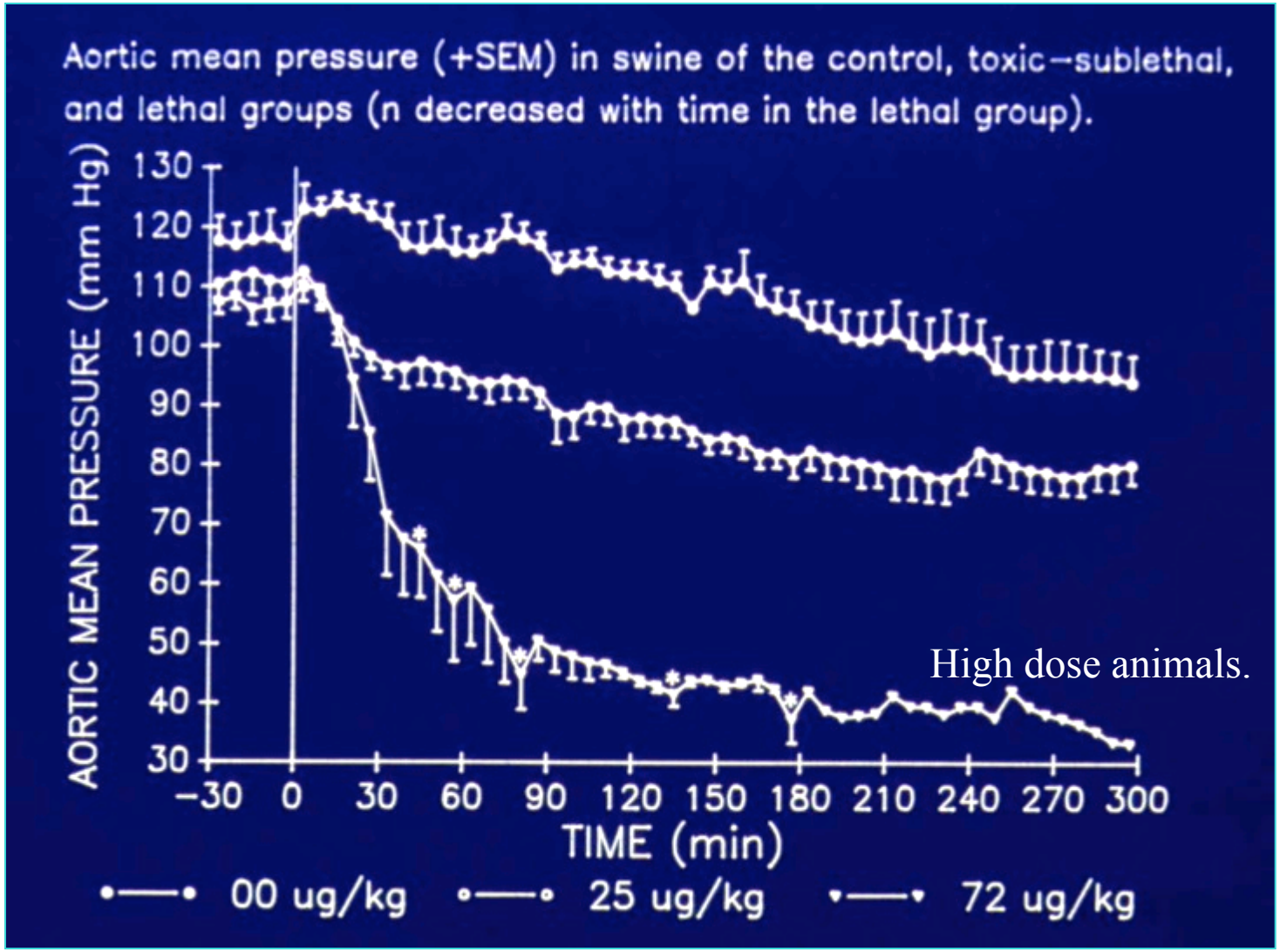


MICROCYSTIN-LR DECREASES HEPATIC & RENAL PERFUSION, & CAUSES CIRCULATORY SHOCK, SEVERE HYPOGLYCEMIA, & TERMINAL HYPERKALEMIA IN INTRAVASCULARLY DOSED SWINE

Journal of Toxicology and Environmental Health, Part A, 61:281±303, 2000

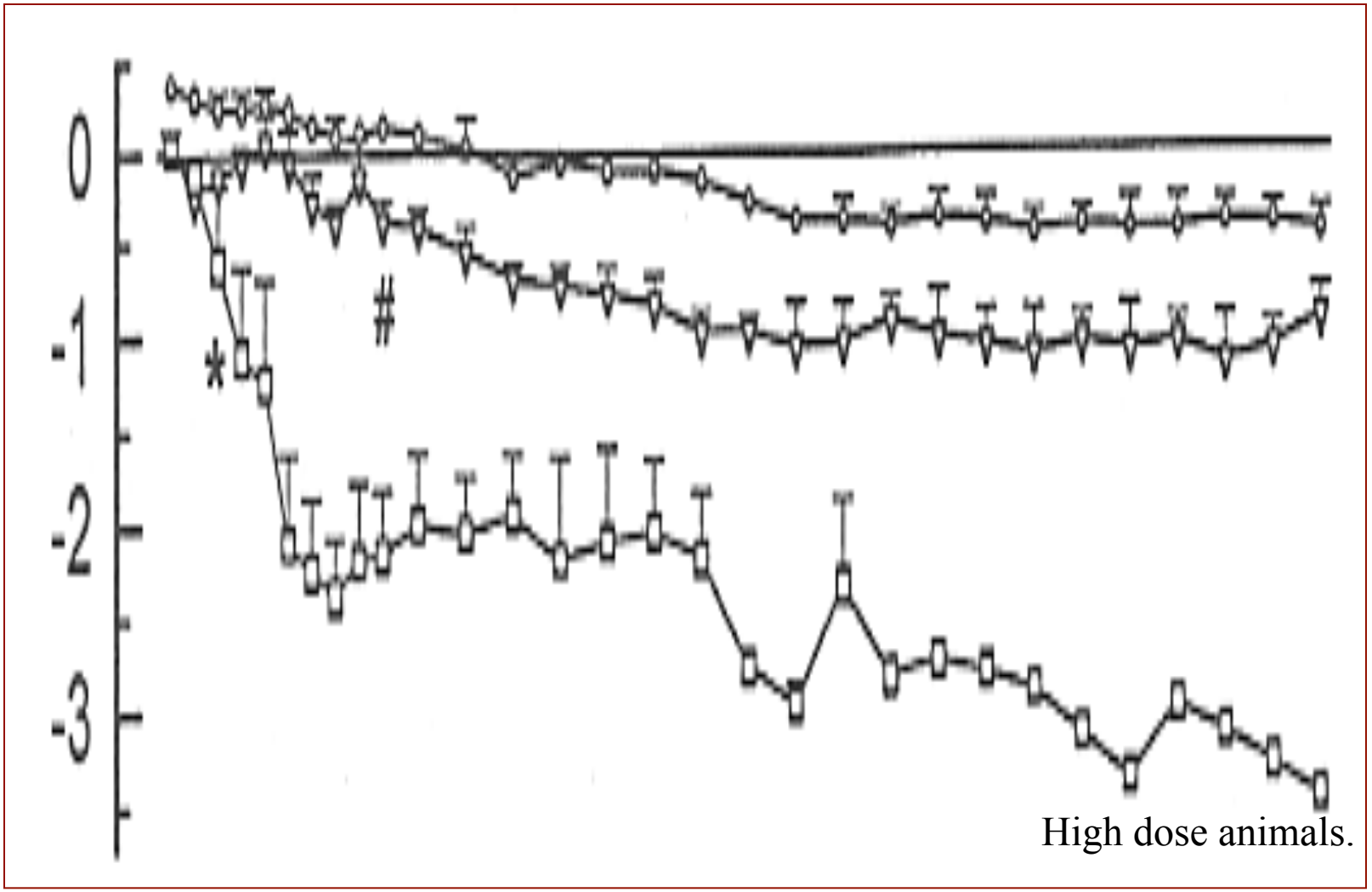
- Val R. Beasley, Randall A. Lovell, Kenneth R. Holmes, Horace E. Walcott, David J. Schaeffer, Walter E. Hoffmann, Wayne W. Carmichael
- Cross-bred, anesthetized female swine were given intravascularly a lethal (72 µg/ kg; n = 6) or toxic-sublethal (25 µg/ kg; n = 6) dose of microcystin-LR (MCLR), from *Microcystis aeruginosa*, or the vehicle (n = 4) .
- At the high dose, from 12 to 18 min after administration, central venous pressure & hepatic perfusion were significantly lower….

Microcystin: Pathophysiology of Shock Syndrome in Anesthetized Pigs



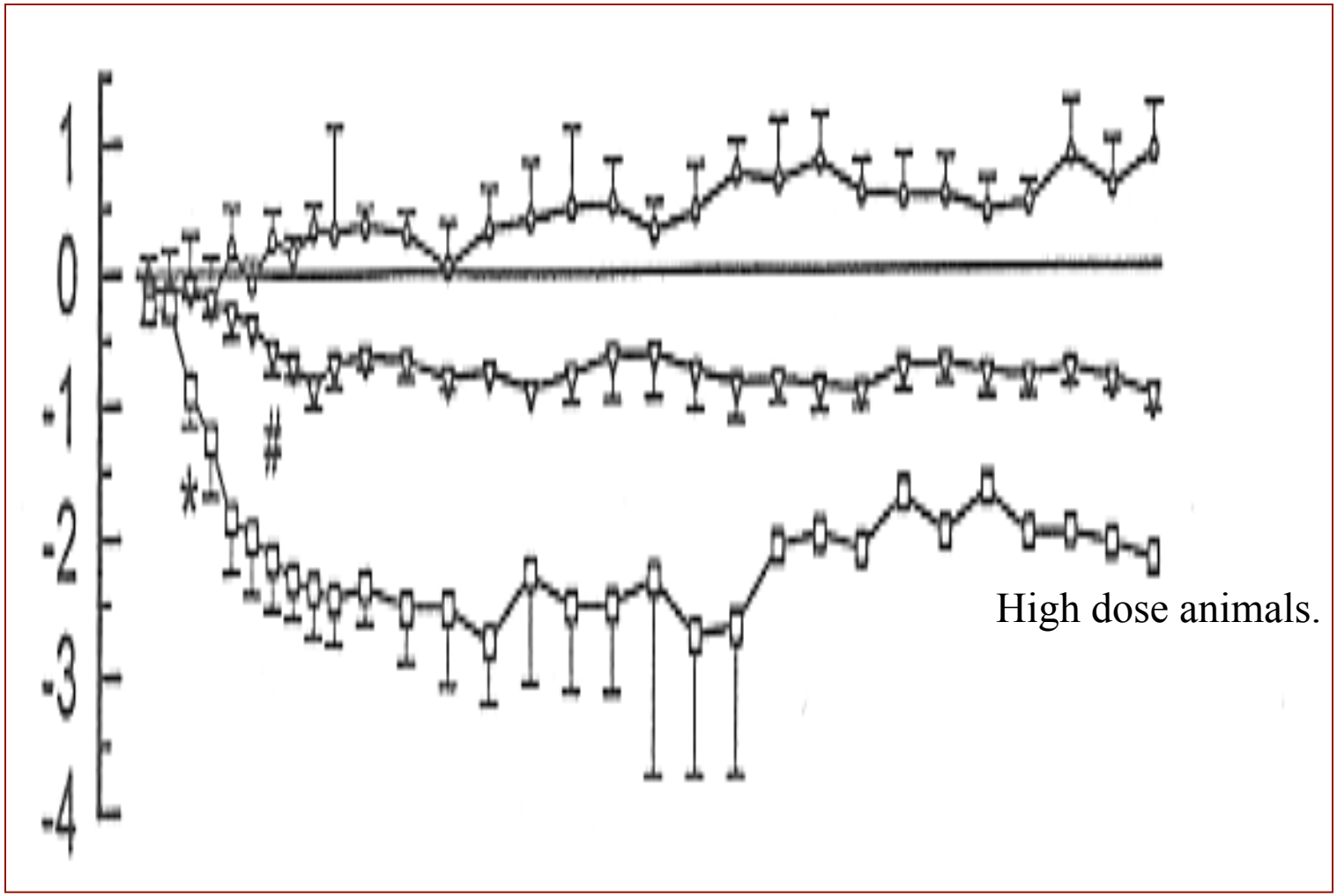
- **Microcystin: Pathophysiology of Shock Syndrome in Anesthetized Pigs**

Central Venous Pressure (mm Hg from predose mean)

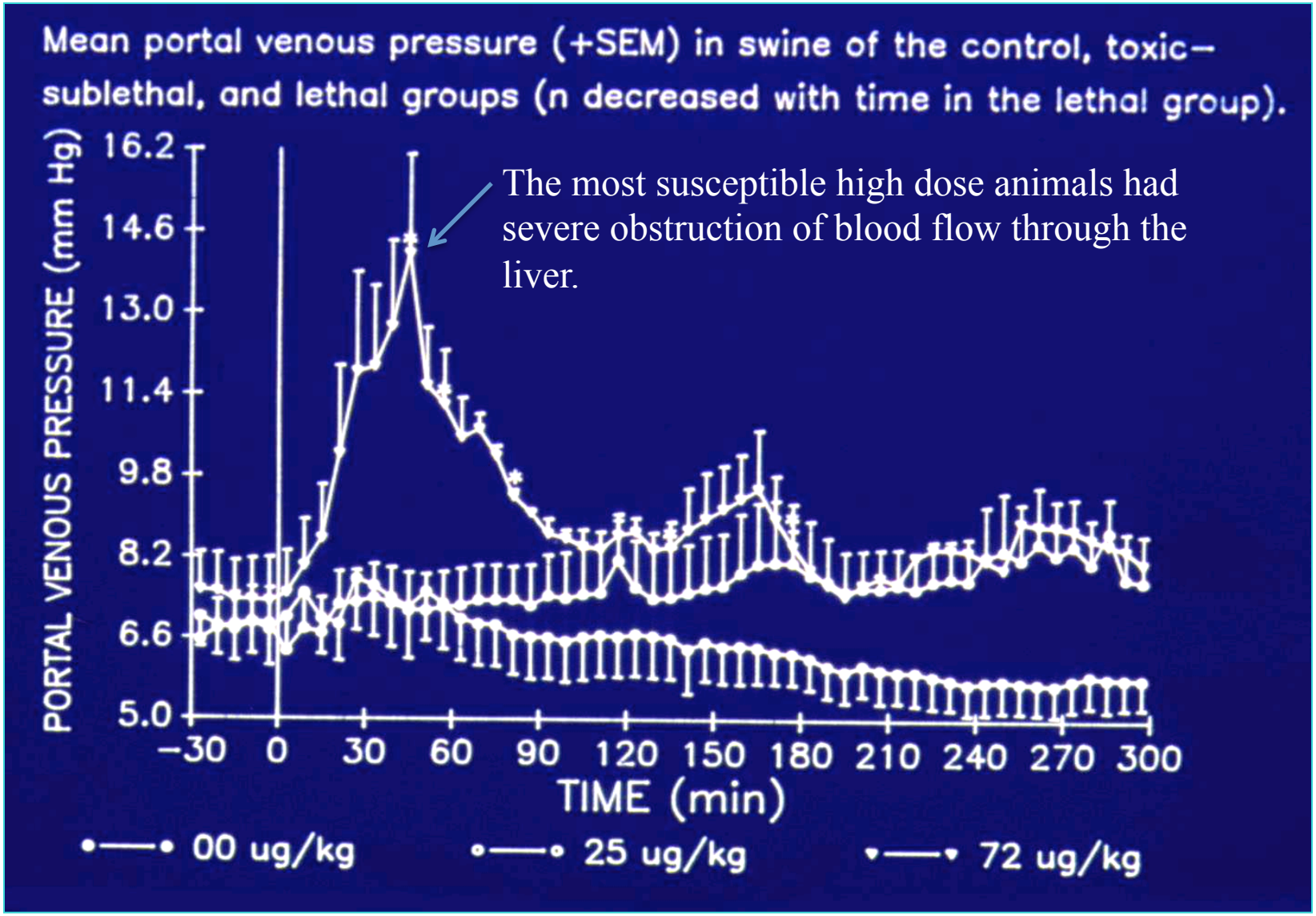


Microcystin Pathophysiology: Organ Perfusion in Anesthetized Pigs

Hepatic Perfusion (ml/min/g from predose mean)

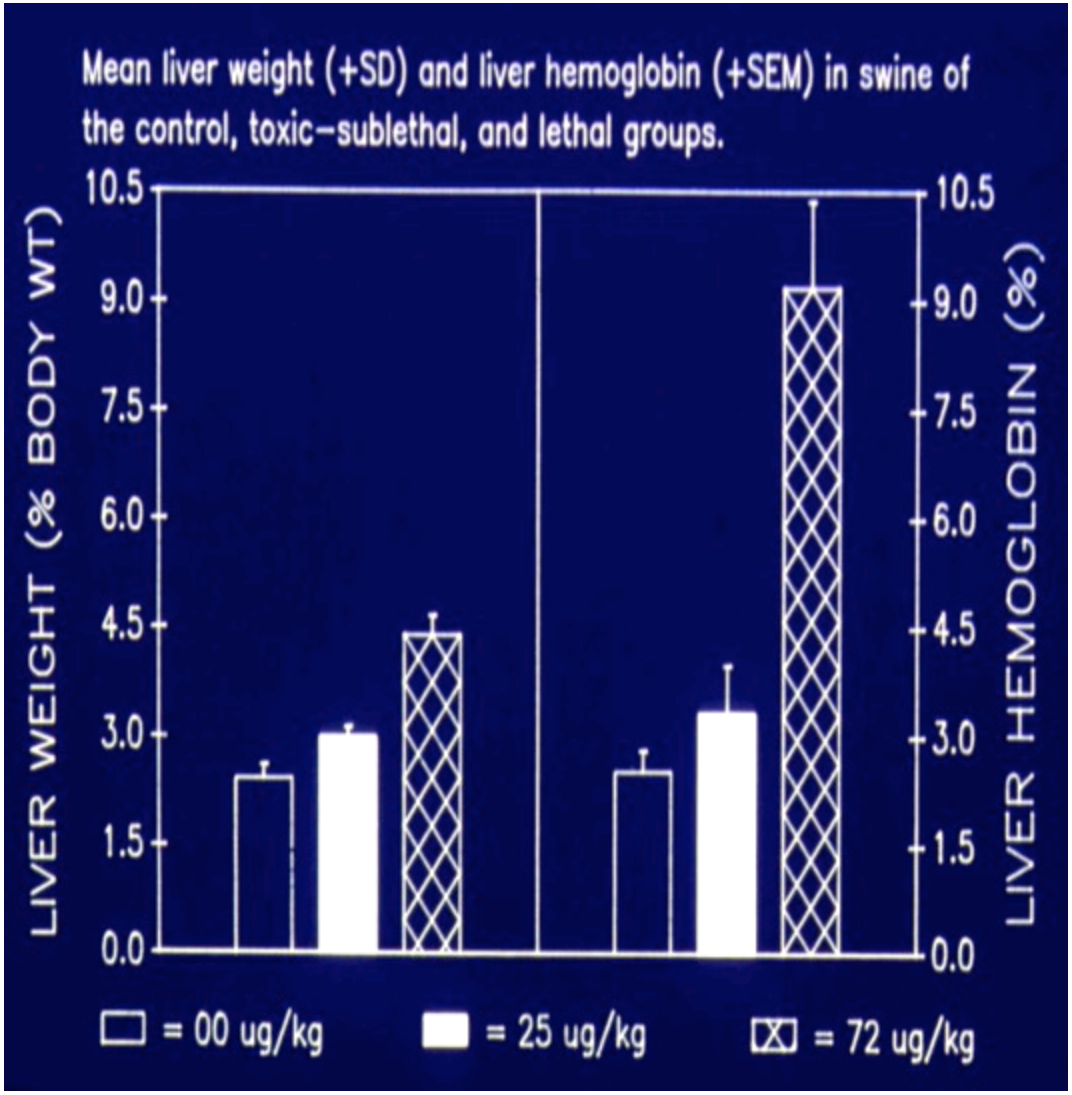


Microcystin Pathophysiology: Portal Venous Pressure in Anesthetized Pigs

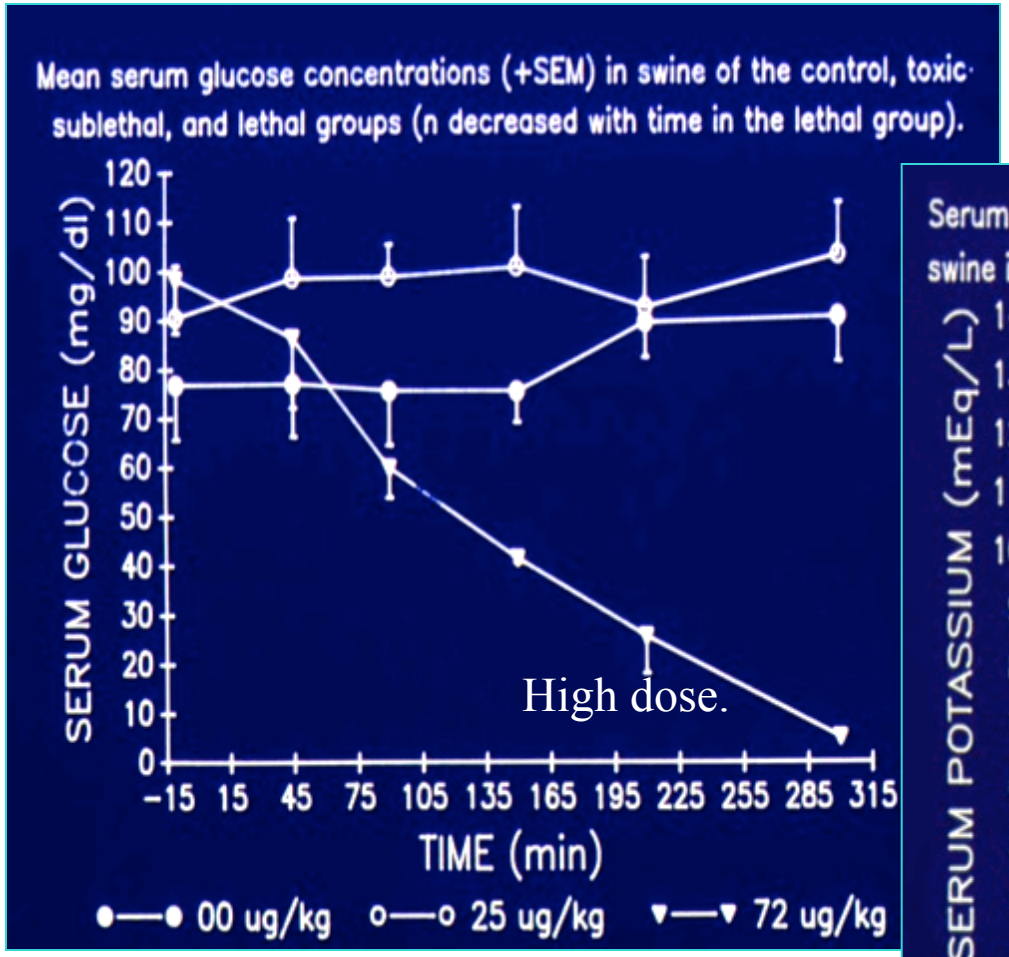


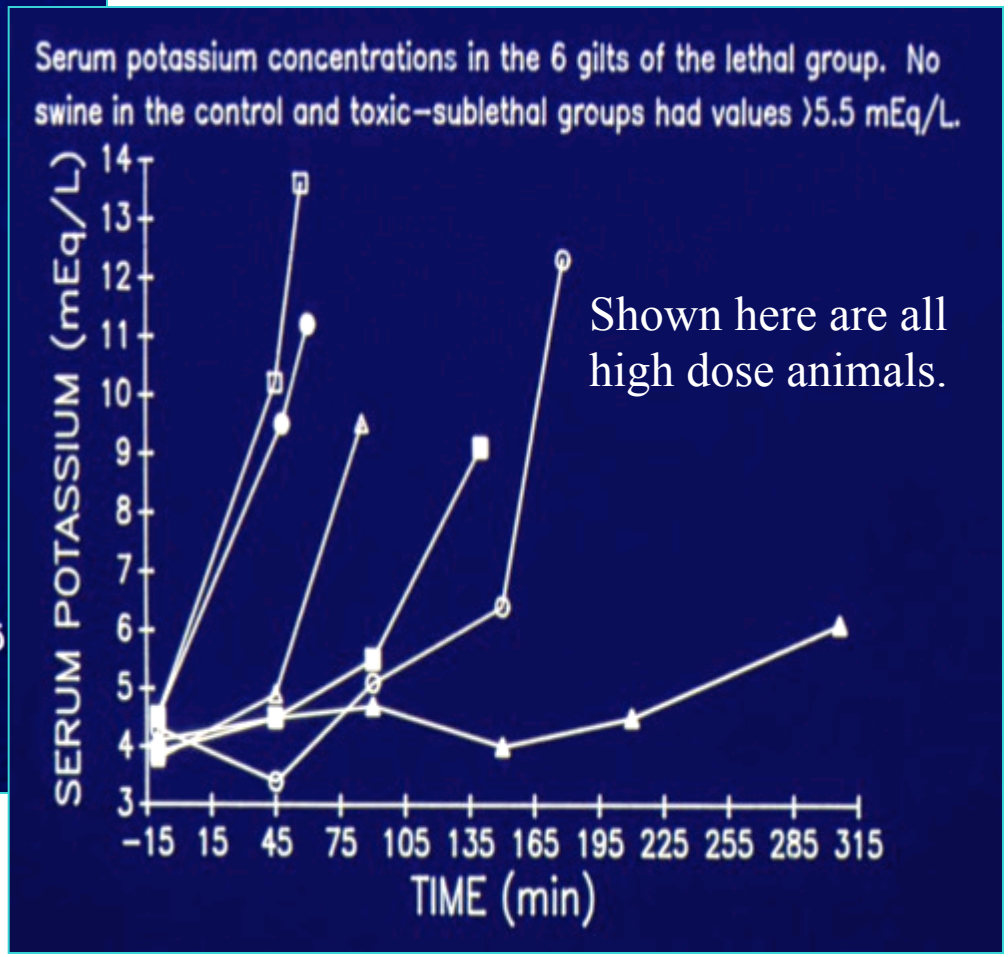
Microcystin-LR in Anesthetized Pigs

- Assuming blood volume of pig = 8.23% of BW,
- & increase in liver weight after dosing was due to hemorrhage…..
- then pigs in the lethal dose group lost 27.3% of total blood volume into the liver.
- *Rapid loss of this much blood was consistently lethal in early studies of hemorrhagic shock.*



Microcystin-LR Toxicosis in Anesthetized Pigs: Serum Glucose & Potassium





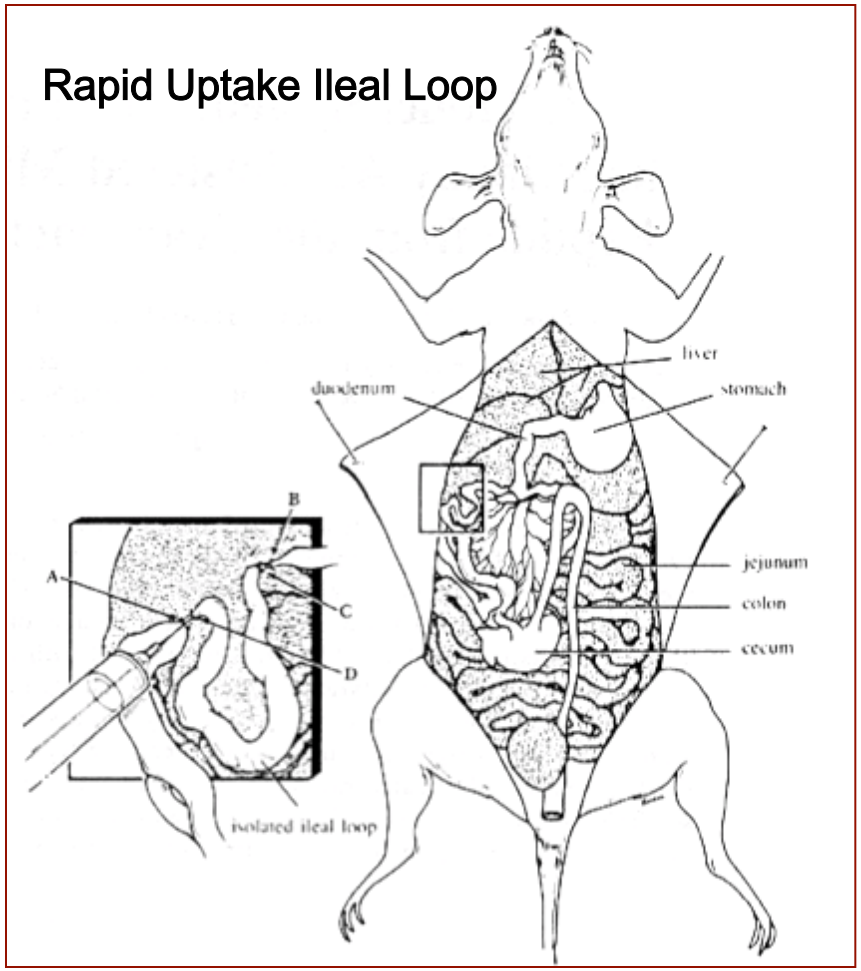
Cyanobacterial Hepatotoxins Microcystins & Nodularin

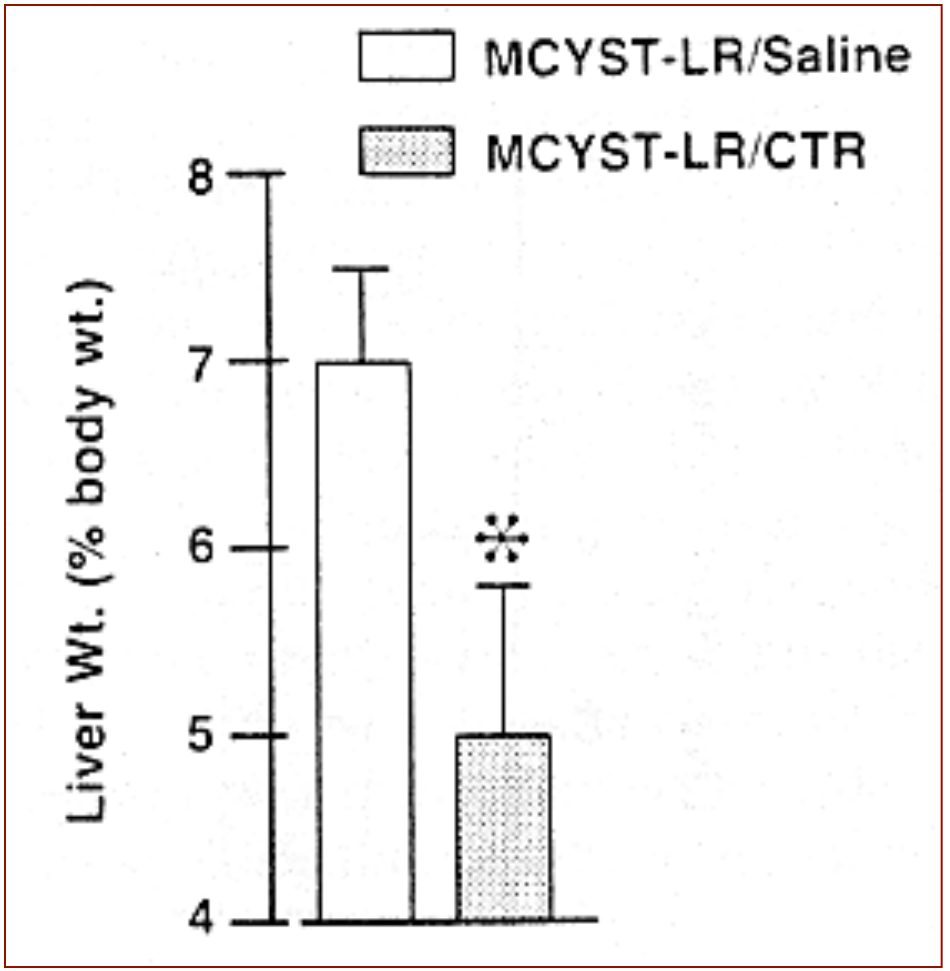
Lethal Mechanisms……

- Animals die from:
  - Hemorrhagic shock
  - Hypoglycemia (blood glucose too low to support life).
  - Hyperkalemia (high blood potassium lethal).

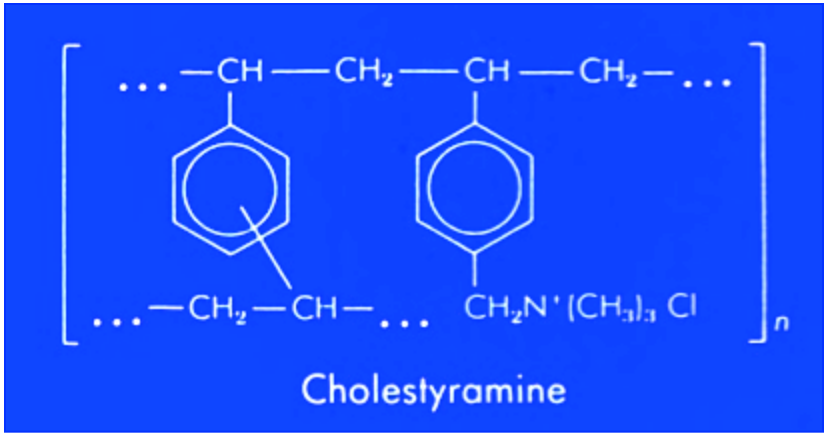
Microcystin Toxicosis:
Consider Fate in Therapeutic Management

- 99% bound to cholestyramine (CTR), a drug used to lower cholesterol.
- Activated charcoal also binds to microcystin & so it too can be used to reduce absorption from the digestive tract.



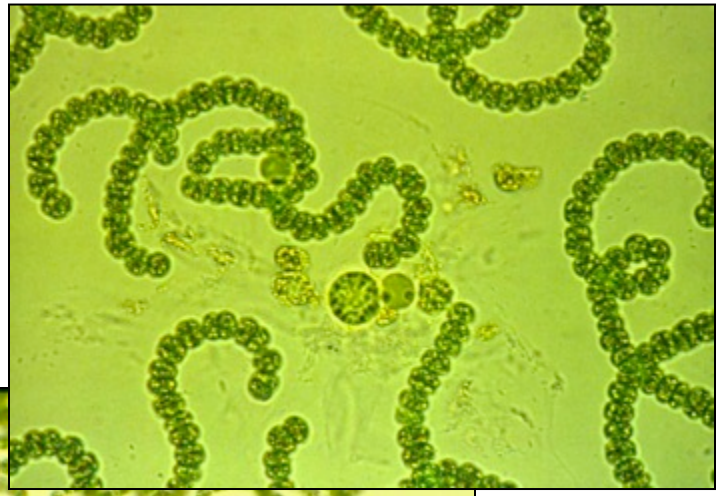


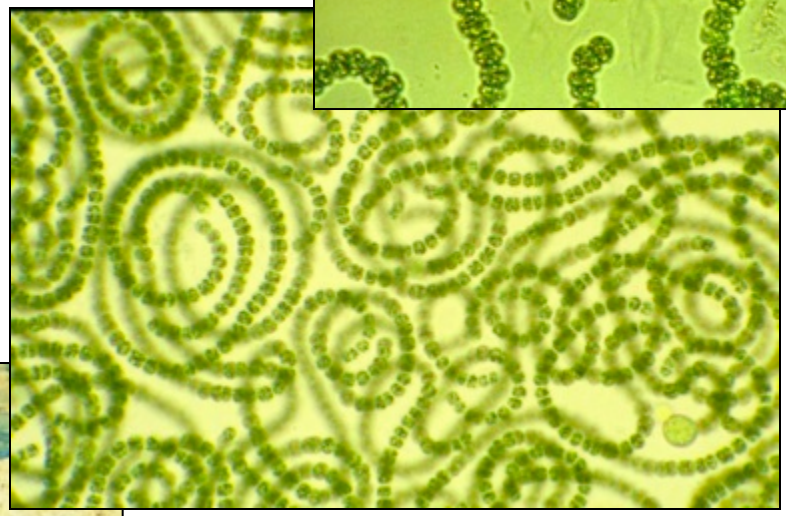
Treatment for Microcystin Toxicosis



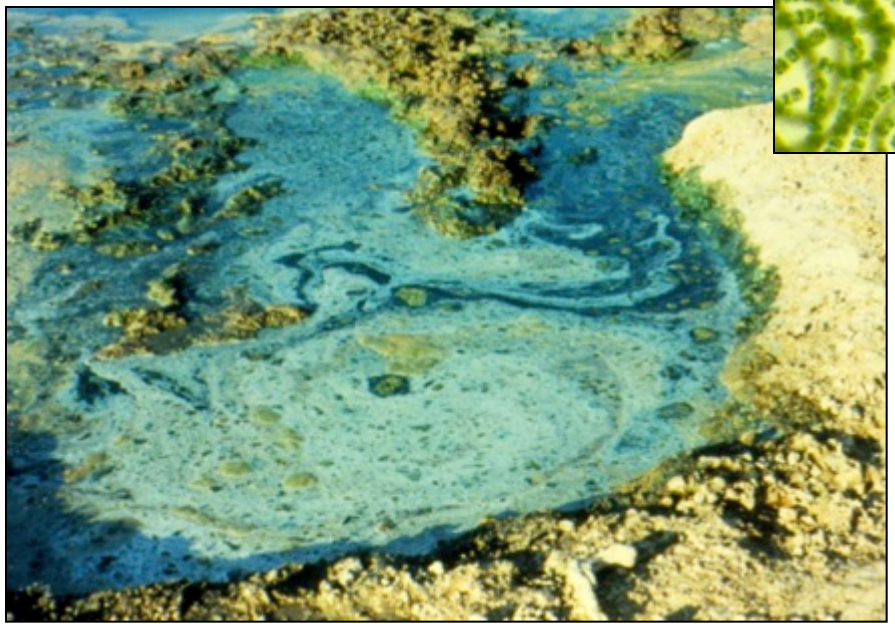
- Remove from source of algae.
- Lavage if feasible (depending on time, species involved, number of animals exposed).
- Bind microcystin:
  - Cholestyramine binds organic anions.
  - Second choice to bind = activated charcoal.
- Hemorrhagic shock: blood, fluids, corticosteroids.
- Hypoglycemia: glucose.
- Hyperkalemia: saline, glucose, insulin.
- **Keep herbivores out of sunlight – potentially photosensitized.**
- **Supportive care for several days: fluids, low fat diet, rest.**

Anabaena flos-aquae & other *Anabaena* spp.





- Produce neurotoxins.
- Produce hepatotoxins.



Learning about/teaching about cyanotoxins.

Potent Blue-Green Algal Neurotoxins: *Mechanisms*

- Anatoxin-A(s)
  - Cholinesterase inhibitor
- Anatoxin-A
  - Nicotinic agonist
- Saxitoxin & neosaxitoxin
  - Sodium channel blockers

Bird found dead with predator/scavenger impact adjacent to an *Anabaena* bloom that had killed swine in Illinois.

How Algal Neurotoxins Kill

- Cyanobacterial neurotoxins disrupt signaling between neurons & muscles in several ways.
- All of them lead to death by causing paralysis of respiratory muscles → suffocation!

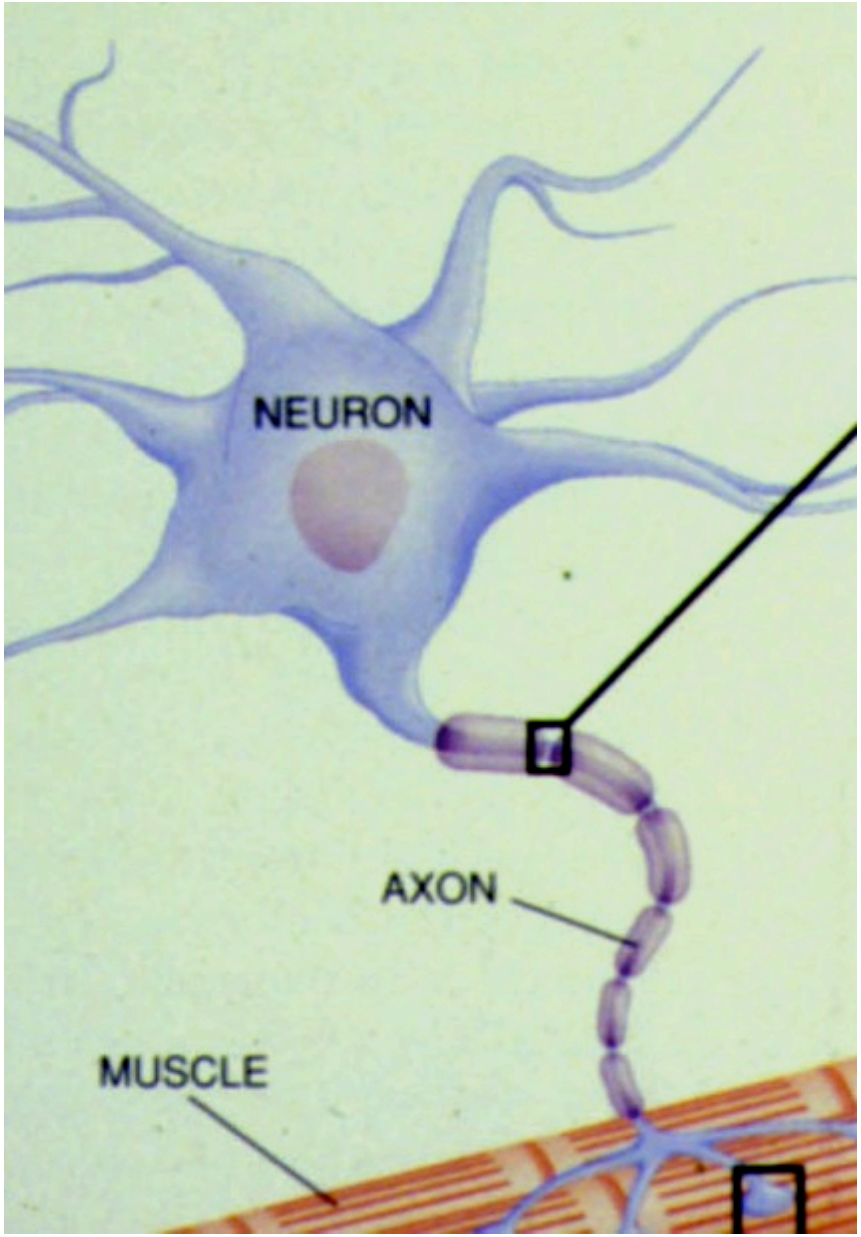
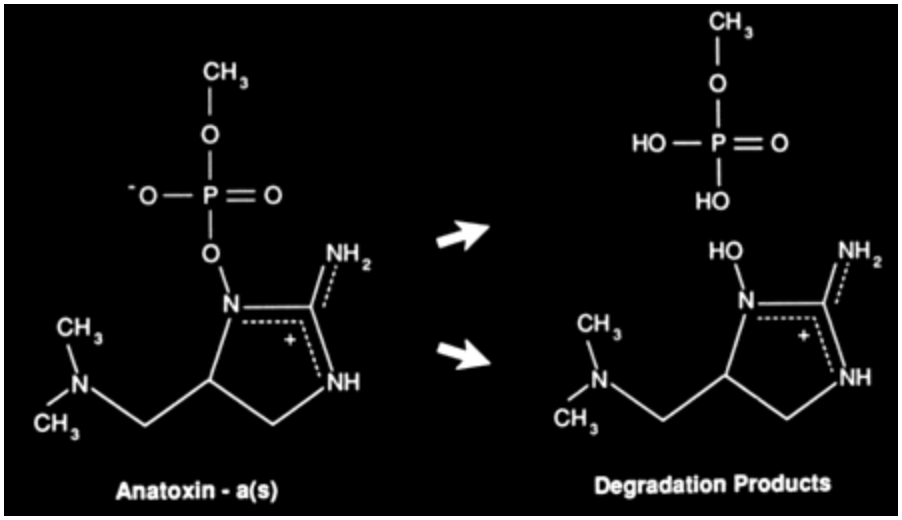


Photo from http://bufozemylu.github.com/chocolate-labrador-retrievers-california.html

Example Field Cases: Anatoxin-A(s) Toxicosis

- Dogs, Illinois & South Dakota
- Swine, Illinois
- Muscovy Ducks, Illinois

Anatoxin-A(s)
from
Anabaena flos-aquae & A. lemmermannii



- Only known naturally-occurring organophosphorous cholinesterase inhibitor.
- Polar, water soluble toxin.
- Extremely labile → hydrolyzes to "non-toxic" products, especially in more alkaline solutions.
  - Ruminants appear resistant to oral exposure.
- Somewhat more stable under acid conditions.
  - Monogastric animals highly susceptible.

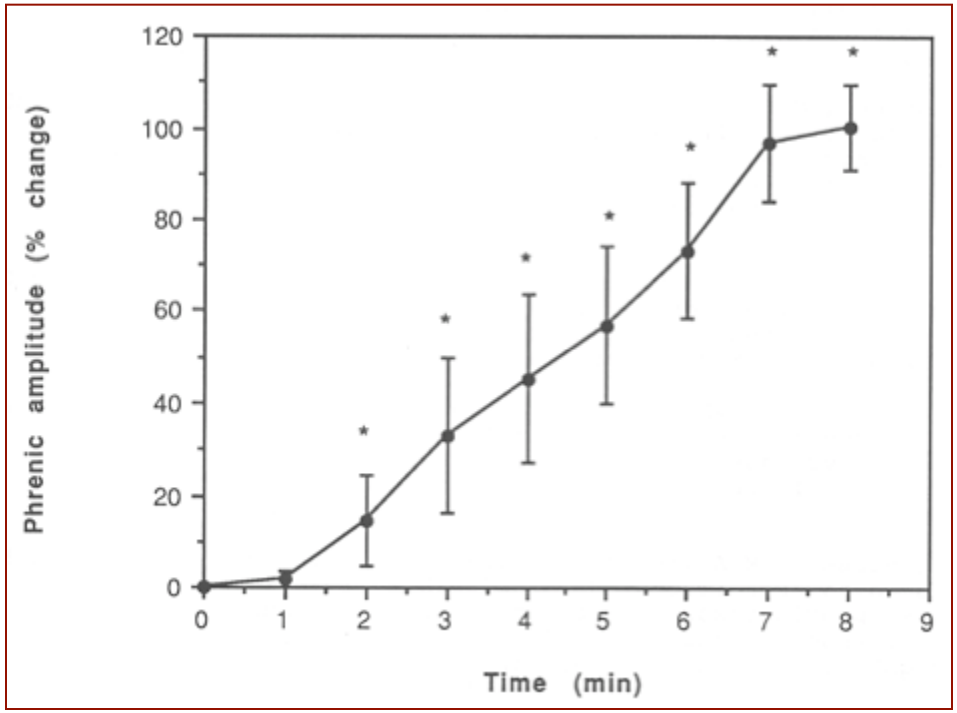
Clinical Manifestations of Anatoxin-A(s) Toxicosis

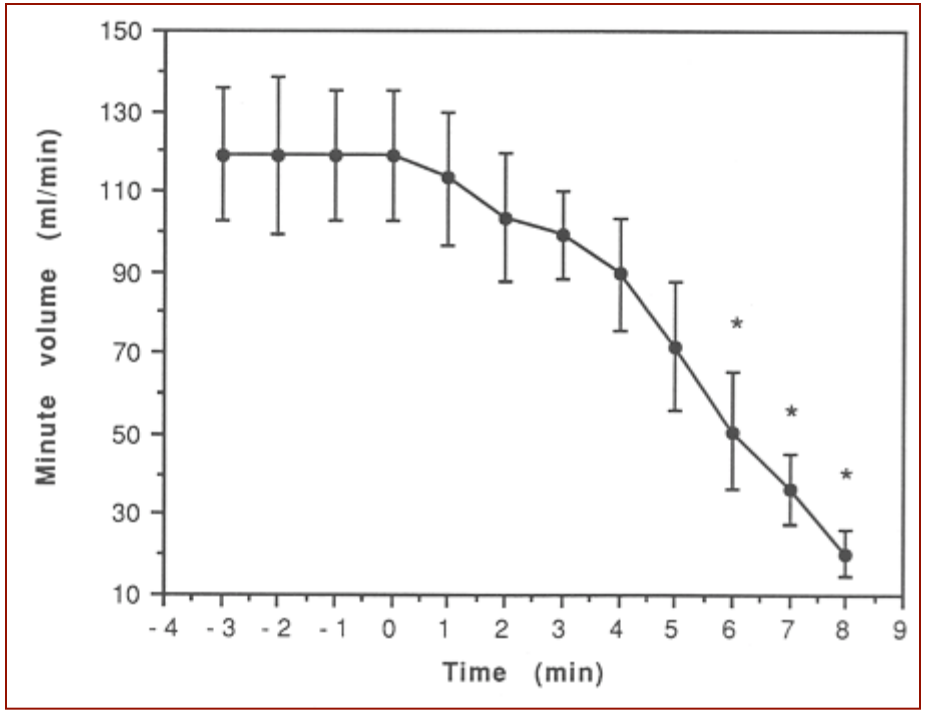
- Muscarinic effects – excess acetylcholine
  - Hypersalivation [“(s)” stands for salivation].
  - Vomiting.
  - Defecation, diarrhea.
  - Urination.
  - Vagal stimulation → possible bradycardia → hypotension (experimentally non-lethal).
- Nicotinic effects – excess acetylcholine
  - Ganglionic stimulation probably aggravates GI upset.
  - Tremors → Paralysis → Death.

Anatoxin-A(s): Studies with anesthetized rats

- Phrenic nerve amplitudes increased.
- But diaphragm EMG activity declined.
- Respiratory paralysis → the proximate cause of death.

Anatoxin-A(s): Anesthetized Rats





Cholinesterase Activities of Muscovy Ducks Given *Anabaena* with Anatoxin-A(s)

•Specimen	Plasma (mmol/L/min)		Tissue (micromol/g/min)			
	Pre-dose	Death/Euth	Brain	Retina	Lung	Muscle
•Control	0.27	0.26	12.7	8.5	0.78	0.27
•Control	0.29	0.27	12.9	8.9	0.78	0.36
•*Euthanasia*	0.29	0.05	13.0	10.4	0.37	0.20
•*Euthanasia*	0.22	0.03	12.7	10.9	0.48	0.10
•*Death*	0.27	BDL*	12.8	8.9	0.20	0.09
•*Death*	0.23	BDL	13.0	9.1	0.14	0.08
•*Death*	0.26	BDL	12.1	7.0	0.25	0.09

- *BDL = below detection limit

Low cholinesterase in plasma, lung, & muscle only indicates inability of the toxin to cross the blood-brain and blood-retinal barriers.

Swine - Anatoxin-A(s) Toxicosis

- Anesthetized swine dosed orally with *Anabaena flos-aquae* from a field outbreak:
  - Brain & retinal cholinesterase values remained in the normal range.
  - Blood, plasma & RBC cholinesterase fell to <15% of control.

Anatoxin-A(s) Toxicosis: Diagnosis

- Expect to encounter this toxicosis.
- Sporadic, under-diagnosed = missed syndrome.
  - → Other animals (including human beings) unnecessarily left at risk of toxicosis.
- Evidence of ingestion.
- Change in algal species in bloom can occur rapidly.
  - → Investigate & sample right away.
- Clinical signs.
- Rapid death, often no lesions.

Anatoxin-A(s) Toxicosis: Diagnosis

- No easy, widely available detection method for toxin.
- Inhibits blood but not brain acetylcholinesterase (ACh).
- If no animals from field are available for workup → mix algal lysate with blood or ACh standard → check ACh activity → reduced activity is consistent with toxicosis.
- Less esthetically desirable, but more definitive:

  Intact mouse bioassay → excessive salivation, urination, diarrhea, tremors, death → necropsy → lab:

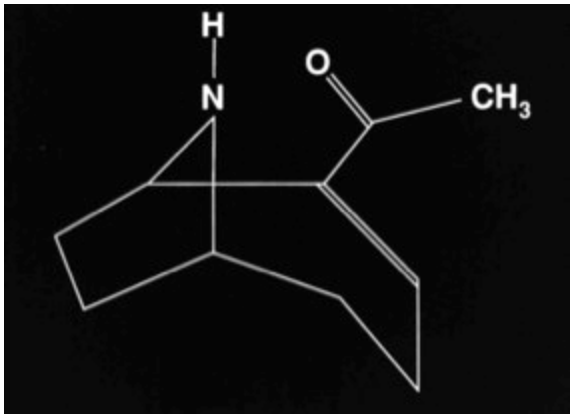
  – Normal brain & retinal ACh.

  – Inhibited blood & muscle ACh.

Anatoxin-A(s) - Treatment

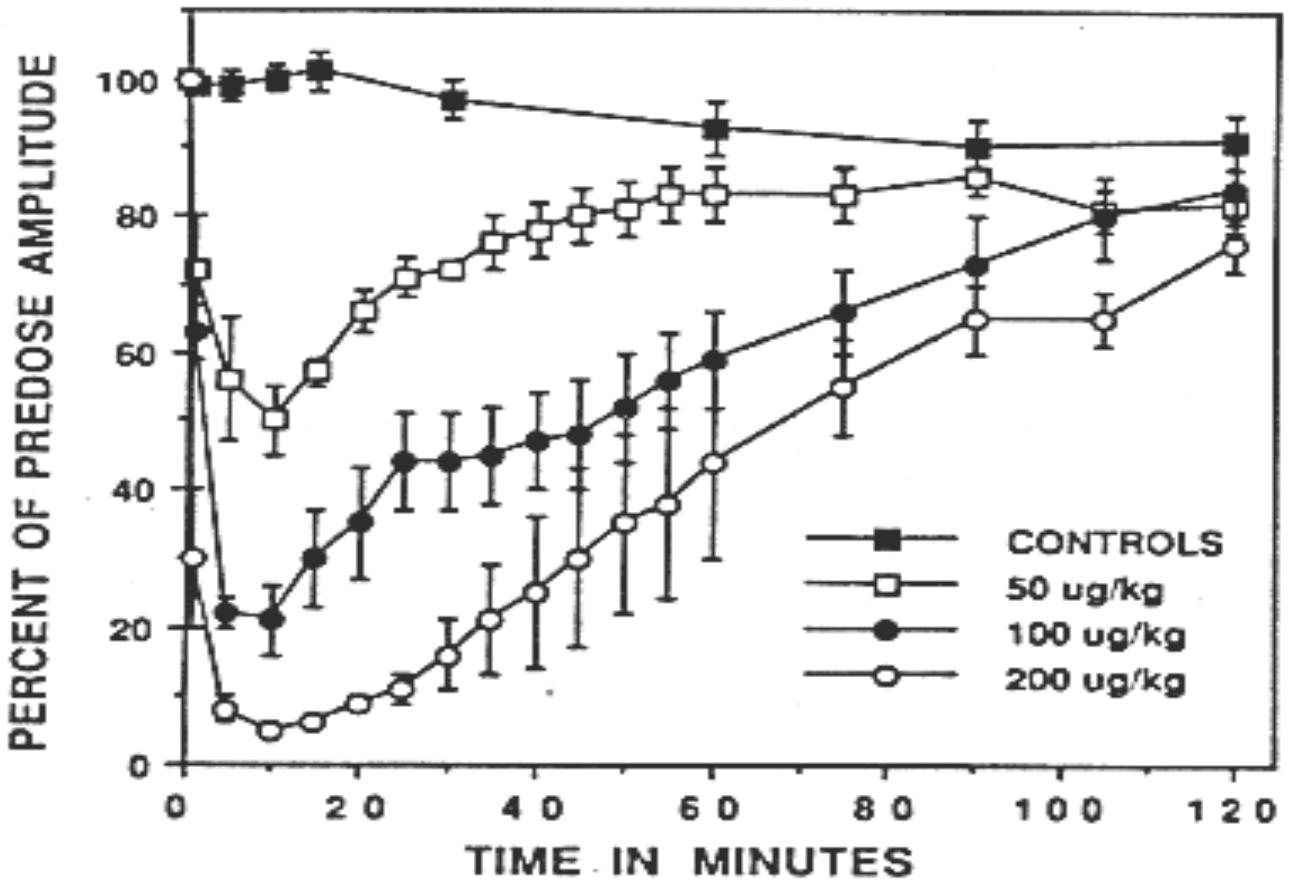
- Bathe off skin, enterogastric lavage, activated charcoal.
- Atropine may improve cardiac function, but animals die from respiratory paralysis. → Artificial respiration.
- To counteract muscarinic effects without CNS effects of high doses of atropine, rather than atropine sulfate, we recommend cholinergic-blockers unable to cross BBB:
  - Methyl atropine nitrate (Metropine)
  - Glycopyrrolate (Robinul-V)
  - Methscopalamine
- **Recent study indicates 2-PAM not likely to be of value.**

Anatoxin-A



- Bicyclic semi-rigid secondary amine
- Produced by *Anabaena*, *Oscillatoria, Aphanizomenon, Cylindrospermum,* and *Microcystis*.

Anatoxin-A Study Using Anesthetized Rats



Mean changes in amplitude of evoked compound action potentials for muscles (+/- SE) over time after an IV dose of anatoxin-A hydrochloride (n=4). Recovery of nervous transmission within two hours suggests that detoxification of the gut & artificial respiration could save lives or poisoned animals (including people).

Diagnosis of anatoxin-a poisoning in dogs from North America.

Birgit Puschner, Brent Hoff, Elizabeth R Tor

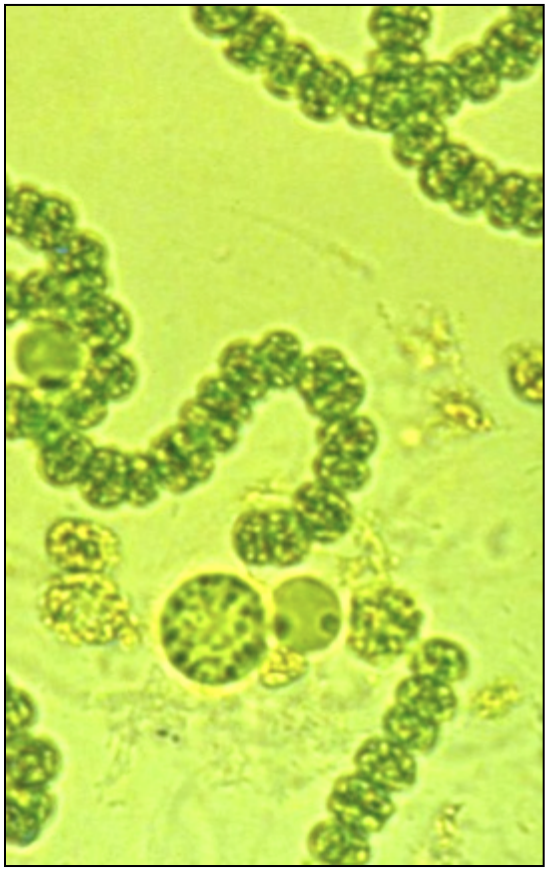
J Vet Diagn Invest. 2008 Jan ;20 (1):89-92 18182518

- Anatoxin-a, a toxin produced by several genera of blue-green algae, is considered a potent neurotoxin. Ingestion of water contaminated with the toxin results in acute neurological signs and often death. **This report describes fatal cases of anatoxin-a ingestion in 6 dogs, with confirmation of anatoxin-a exposure by liquid chromatography/tandem mass spectrometry (LC-MS/MS/MS). In 1 outbreak, 3 dogs developed seizures and died within an hour after swimming in a river in California, while the other outbreak involved 3 dogs that died within 1 hour after swimming in a pond in Ontario.** Anatoxin-a poisoning is rarely reported in dogs as a cause of acute neurological signs and death. However, increased occurrences of blue-green algae blooms in North America make this neurotoxin an important consideration in the diagnosis of sudden death associated with environmental water exposure. This brief communication reports on the isolation and detection of anatoxin-a from environmental water sources and the stomach contents of North American dogs dying of acute neurotoxicosis. **This demonstrates the first documented cases of anatoxin-a poisoning in dogs in North America and the importance of LC-MS/MS/MS in identifying neurotoxins responsible for sudden death in cases of suspected blue-green algae toxicosis; especially those cases showing no gross or histological lesions.**

Anatoxin-A: Mechanism of Action
Nicotinic Agonist → Depolarizing Blockade at Neuromuscular Junctions

- Recognized as cause of toxic syndromes more often than is anatoxin-a(s).
- Poisonings occur sporadically.
- Respiratory paralysis.
- Rapid death in a range of species including ruminants.

Anatoxin-A: Diagnosis



- Evidence of exposure.
- Clinical signs.
- Absence of lesions except related to anoxia, agonal death.
- Toxin is relatively stable:
  - Analyses can be done.
  - Send algae & stomach content to UC-Davis diagnostic lab (call first).
- Whole animal bioassay (mouse given algal lysate IP):
  - Neurologic signs.
  - No specific lesions.
  - Normal AChE.
  - Humane considerations – avoid if possible.

In addition to studies of toxins, mechanisms of action, pathophysiology, & fate in animals, we worked to help veterinarians diagnose, manage, & prevent additional cyanotoxin poisonings..

- Presentations.
- Book chapters.
- Journal articles.
- Lectures & test questions given to veterinary students.

J Vet Diagn Invest 1:359–365 (1989)

REVIEW ARTICLE

Diagnostic and clinically important aspects of cyanobacterial (blue-green algae) toxicoses

Val Richard Beasley, Andrew M. Dahlem, William O. Cook, William M. Valentine, Randall A. Lovell, Stephen B. Hooser, Ken-Ichi Harada, Makoto Suzuki, Wayne W. Carmichael

Previous efforts have been made to provide concise summaries on the hazards of toxicoses from exposures of domestic animals to blue-green algae.[14] It is clear, however, that veterinarians need improved access to information currently emerging with regard to blue-green algae toxicoses. Recent investigations are shedding light on the identity of the potent toxins responsible and the pathophysiology of the syndromes produced. Reviews from the past few years provide an idea of the reported occurrence of cyanobacterial toxicoses and the toxins detected,[12,13] but the diagnosis of blue-green algae toxicosis has remained difficult because of a lack of concise information on appropriate diagnostic procedures. One must recognize that many algal blooms are not hazardous; therefore, a diagnosis of toxicosis following ingestion of an algal bloom, even when it is dominated by organisms known to have produced toxins in the past, should be confirmed. At the present time, demonstration of toxins in the algae and documentation of appropriate responses in the animal form the basis for many diagnoses.

Identification of blue-green algae

When examined with the unaided eye, blue-green algae are nonfilamentous and often have the appearance of blue or green "scum" or paint on or under the surface of the water. In some instances, the death of the algae results in a "skin" on the surface of the water. The appearance of *Microcystis aeruginosa* frequently can be described as floating grains of green sand.[20] The blue pigment of cyanobacteria becomes apparent as the cells begin to deteriorate; thus, a blue color is often seen on the edges of the pond where algae has dried, or it may predominate as the color of the free floating algae. Animals are most often exposed to toxic amounts of algae as a result of drinking from an area where prevailing winds have concentrated the bloom. Usually the animals have evidence of green or blue colored algae on their hair coats.

In order to establish a diagnosis of blue-green algae poisoning, it is essential to obtain a suitable specimen of the algal bloom material. As soon after ingestion of the toxic algae as possible, a specimen of the implicated cyanobacteria should be obtained. It is suggested that healthy algal cells be gathered from the surface of the water or just underneath it in such a way as to concentrate the cells maximally. An efficient approach to substantiation of the diagnosis of selected blue-green algae toxicoses is shown in Fig. 1. Material to be freeze-dried can be frozen immediately; however, cells for microscopic examination and identification must be kept refrigerated and not frozen. In addition to submission to the laboratory of approximately 50 ml of refrigerated cells, a 10- to 20-ml portion of the bloom material can be fixed so that a sample displaying the typical physical characteristics of the algae will be available indefinitely. Selected organisms that have been shown to produce cyanobacterial toxins are shown in Fig. 2a–c. The assistance of a biologist skilled in the characterization of cyanobacteria is recommended for confirmation of taxonomic designations.

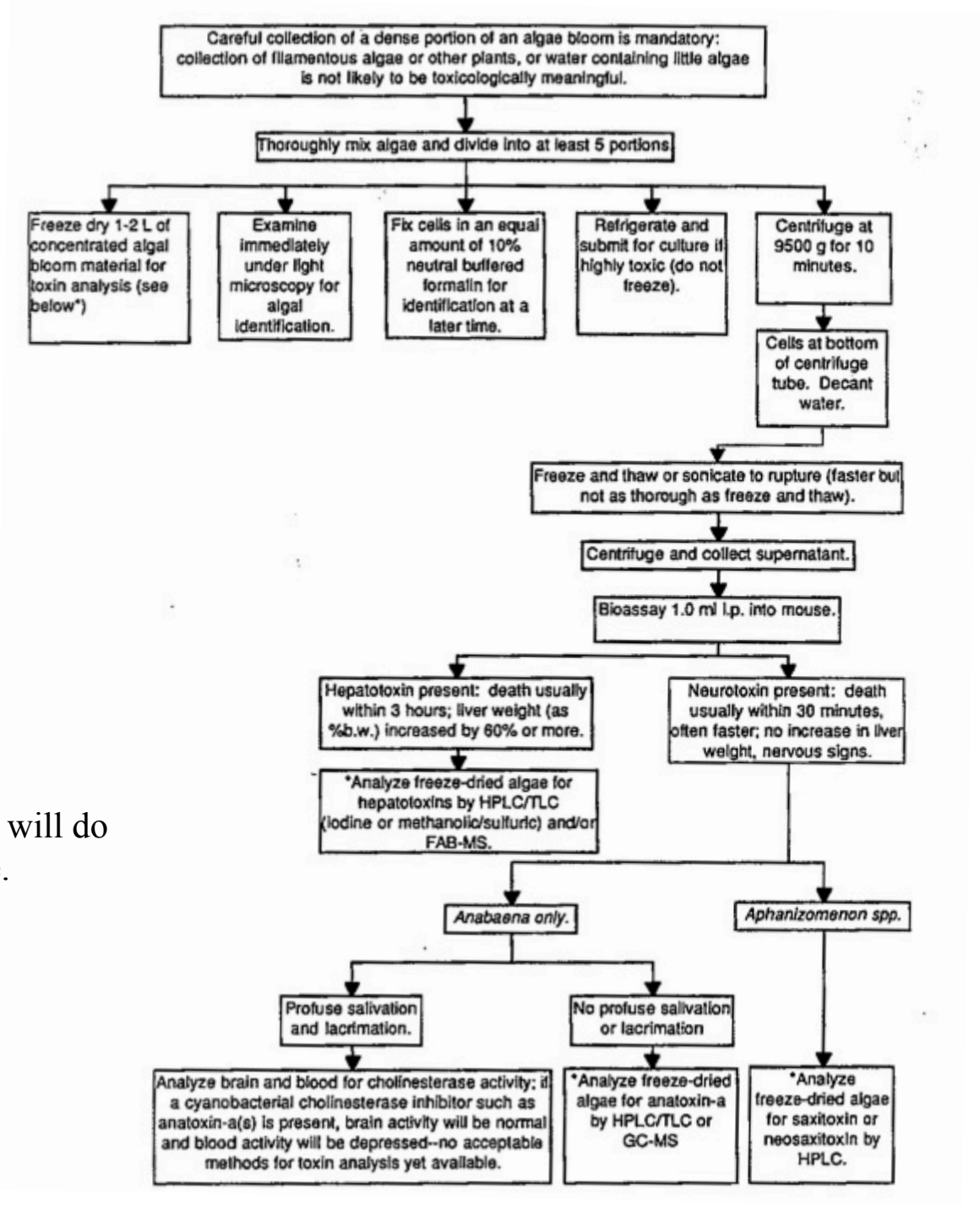
Principal toxicoses

The primary types of blue-green algae toxicoses documented to date include acute hepatotoxicoses, peracute neurotoxicoses, and gastrointestinal disturbances. Lipopolysaccharide endotoxins are believed to have caused gastroenteritis among humans.[42] Other effects have been reviewed and include cytotoxicity and cutaneous or respiratory irritation similar to hypersensitivity; the latter has been documented only in man.[5,24,25]

From the Departments of Veterinary Biosciences and Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, IL 61801 (Beasley, Dahlem, Cook, Valentine, Lovell, Hooser), and the Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, Japan (Harada, Suzuki), and the Department of Biological Sciences, Wright State University, Dayton, OH 45435 (Carmichael).

Received for publication March 16, 1989.

This flow chart
needs to be updated.
Hopefully colleagues will do
that in the near future.



Review Paper

http://www.egynattox.com/Volumes/Volume_8/7%20Val%20_Final_.pdf

Mycotoxin poisonings also tend to increase as a result of drought.

Egyptian Journal of Natural Toxins, Vol. 8(1,2): 104-133, June 2011

Review Article

PATHOPHYSIOLOGY AND CLINICAL MANIFESTATIONS OF MYCOTOXIN AND PHYCOTOXIN POISONINGS

Val Richard Beasley*

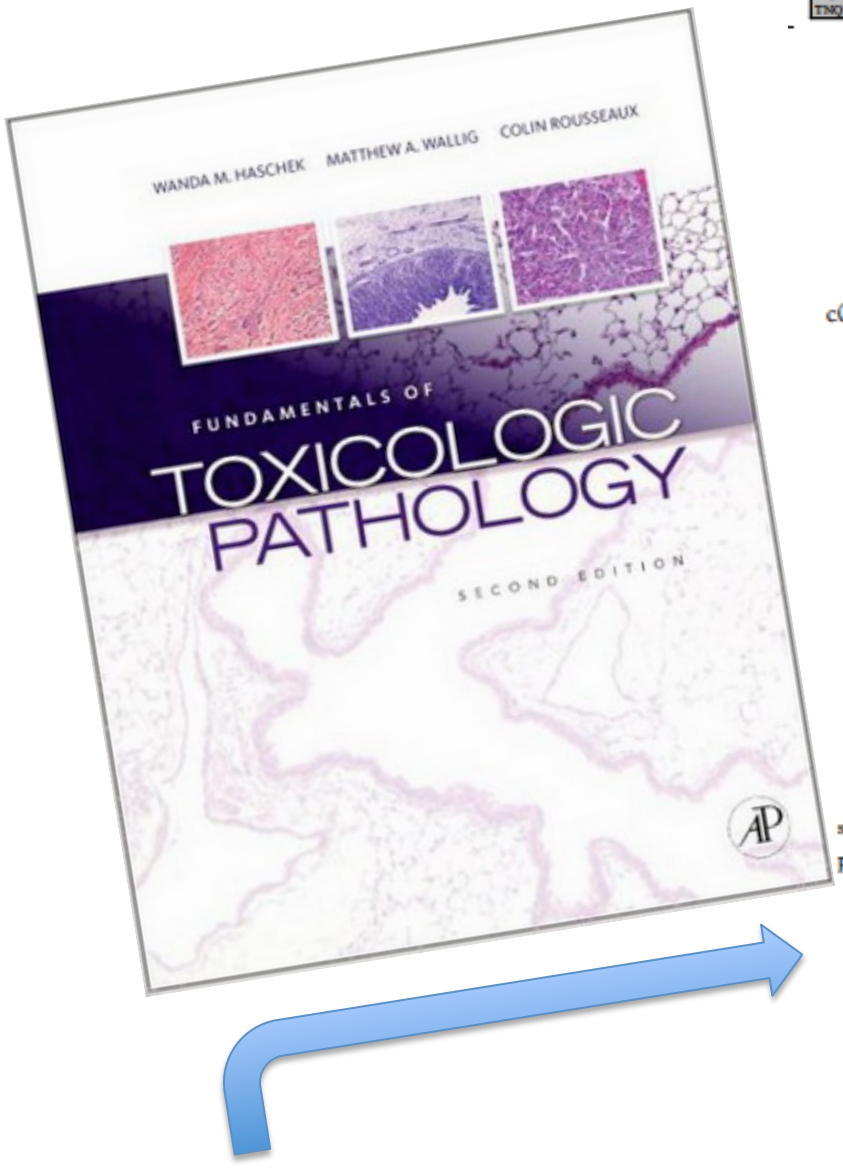
Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, 2001 South Lincoln Avenue, Urbana, IL, 61802, USA

Received: 20/11/2010 Accepted: 7/6/2011

ABSTRACT

Monocultures, nutrients and climate change increase mycotoxicoses and phycotoxicoses. Among mycotoxins, ergot alkaloids are dopaminergic, serotonergic and adrenergic agonists and antagonists, reducing prolactin and causing reproductive problems, neurologic dysfunction and gangrene. Tremorgens cause excitation, seizures, ataxia and paralysis. Penitrem-A damages cerebellar neurons, while lolitrem-B inhibits potassium channels. Zearalenone and analogs are estrogenic and impair pig fertility. Aflatoxins metabolized to the 8-9-epoxide bind macromolecules, and cause immunosuppression, hepatopathy and cancer. Trichothecenes recruit MAP kinases, causing ribotoxicity, inhibition of protein synthesis and radiomimetic injury. Fumonisins inhibit sphingosine N-acyltransferase and cause liver damage, leukoencephalomalacia, edema and esophageal damage. Ochratoxin A inhibits protein synthesis, causing nephropathy, tumors, embryotoxicity and immunosuppression. Among phycotoxins, microcystins and nodularin inhibit hepatic protein phosphatases and cause tumor promotion, cytoskeletal collapse, apoptosis, shock and death. Cylindrospermopsin damages DNA, depletes glutathione and causes centrilobular hepatic necrosis, enteritis and pup mortality. Nicotinic anatoxin-A and peripheral acting anti-cholinesterase anatoxin-A(s) cause respiratory failure. Debromoaplysiatoxin, aplysiatoxin and lyngbyatoxin A from *Lyngbya* activate protein kinase-C, irritate epithelia and promote tumors. Domoic acid activates N-methyl-D-aspartate-mediated calcium channels and causes amnesia, seizures and coma. Marine

* Corresponding author: Prof. Val R. Beasley. e-mail: val@illinois.edu



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CHAPTER

38

Phycotoxins

c0038

Philip F. Solter, Val R. Beasley
University of Illinois at Urbana-Champaign, Urbana, IL, USA

OUTLINE

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s0010 p0010

1. INTRODUCTION

Phycotoxins are potent organic compounds produced by dinoflagellates, other flagellated phytoplankton, and cyanobacteria that inhabit marine, brackish, or freshwater bodies or soils. Among the most important cyanobacterial phycotoxins are microcystins, nodularin, cylindrospermopsin, *Lyngbya* toxins, anatoxins, and saxitoxins; the most important marine phycotoxins from diatoms or dinoflagellates include saxitoxins, domoic acid, brevetoxins, and ciguatoxins. At least 100 different species of organisms may harbor toxins. Toxicoses from marine phycotoxin producers in man, other mammals, and birds can occur from direct contact via skin, inhalation, or ingestion of toxigenic organisms or lysates, or by ingestion after passage of the toxins through food chains to shellfish or finfish. Large die-offs of fish may also occur. Concentrated harmful algal blooms (HABs) can color the waters, and some of the marine organisms cause what are known as "red tides". However, clear water may also harbor toxic levels of phycotoxins. Exposure to cyanobacterial toxins in freshwater environments is most frequently through direct consumption of contaminated water, but inhalation or wound absorption is also possible. Eutrophication of fresh, estuarine, or brackish marine waters can result in massive blooms of toxic cyanobacteria (Figure 38.1). Excessive nutrient runoff can result from burning, overgrazing, herbicide- or tillage-related absence of plant cover, and urban/suburban development. Nutrient loading also frequently arises from additions of animal and human excreta, and atmospheric deposition from fossil fuel burning. Droughts, storms, and/or

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10038-HASCHEK-9780124157590

This chapter in the third edition should be out later this year (2013).

Investigation of a *Microcystis aeruginosa* cyanobacterial freshwater harmful algal bloom associated with acute microcystin toxicosis in a dog J Vet Diag Invest 24:679-687 (2012)

- **Kansas State Veterinary Diagnostic Laboratory,** Manhattan, KS (**van der Merwe**, Nietfeld)
- Department of Clinical Sciences and College of Veterinary Medicine, Kansas State University, Manhattan, KS (Sebbag)
- GreenWater Laboratories, Palatka, FL (Aubel, Foss)
- Kansas Department of Health and Environment, Topeka, KS (Carney)

- **Microcystin poisoning diagnosed in summer, 2011.**
  - Lake water microcystin concentrations determined at intervals during the summer, using competitive enzyme-linked immunosorbent assays, & indicated extremely high, localized microcystin concentrations of up to 126,000 ng/ml.
  - Multiple extraction and analysis techniques were used in the determination **of free and total microcystins in vomitus and liver samples from the poisoned dog.**
  - Vomitus and liver contained microcystins, as determined by enzyme-linked immunosorbent assays, and the presence of microcystin-LR was confirmed in vomitus and liver samples using liquid chromatography coupled with tandem mass spectrometry.
  - Major toxic effects in a dog presented for treatment on the day following exposure included fulminant liver failure and coagulopathy.
  - The patient deteriorated rapidly despite aggressive treatment and was euthanized.
  - Lesions included diffuse, acute, massive hepatic necrosis and hemorrhage, as well as acute necrosis of the renal tubular epithelium.
  - **Diagnosis of microcystin poisoning based on demonstration of *M. aeruginosa* & microcystin-LR in the lake water & vomitus produced early on; microcystin-LR in liver tissue; & a typical clinical course including gastroenteritis & fulminant liver failure.**

Follow up Conversation with Dr. Deon van der Merwe in January, 2013

In 2011:

- **Five dogs died around different parts of the lake during the summer after ingesting cyanobacterial blooms.**
- **Birds also died.**
- **Milford Lake (>6,300 hectares) is a few miles from Manhattan, KS (site of K-State).**
- **Monitoring of Milford Lake ahead of the incident was serendipitous.**
- **Water taken from same beach area the day before the incident had no detectable *Microcystis* or microcystin.**
- **Monitoring cyanobacterial blooms with remote sensing from small aircraft: Conclusion: Blooms vary, & a few feet can make an immense difference in density.**

Follow up Conversation with Dr. Deon van der Merwe in January, 2013

In 2012:

- **2 incidents in which cattle died** after exposure to apparent cyanobacterial blooms:
  - **First case: 26 cattle died.**
  - **Second case: 24 cattle died.**
  - **Water examined from one of the sites had *Anabaena* present & clinical signs were mainly neurologic, so anatoxin-A might have been involved.**

ASPCA Animal Poison Control in Urbana

Summary Data from Last 5 Years: Dr. Mike Knight

- **36 unique cases referenced cyanobacterial toxin concerns (2 from Illinois**, others from 18 other states, plus one from Quebec.
  - MA (5), WI (4), MD (3), several states (2 or 1).
- **17 of the 36 were from 2012! (1 of these was from Illinois**).
- **Every call pertained to dogs.**
  - Most calls to center pertain to small animals.
  - Far more dog owners than livestock owners.
  - Far less controlled lives.
- **30% of cases classified as high suspicion of toxicosis.**
  - 15% as medium suspicion.
  - 15% as low suspicion (unsure).
  - 25% classified as unrelated causes.
- **Clinical signs:** vomiting 9%, diarrhea 7%, lethargy 7%, ataxia 5%, seizures 4%.
  - One death.
  - One euthanasia.
  - Only 10% had no follow up outcome data.

UI College of Veterinary Medicine Veterinary Diagnostic Laboratory in Urbana

Summary Data from 2005-January 2013: Dr. Rick Fredrickson

Numbers of cases by species of animal in which potential involvement of cyanobacterial (blue-green algal) toxins was mentioned in the history. **Note: no analytical tests were performed. In most cases, this was believed to be related to the costs of the assays.**

- 6 dog
- 2 cat
- 2 cattle
- 1 frog
- 1 squirrel
- 1 mute swan
- 1 sheep
- 1 otter

University of California-Davis, College of Veterinary Medicine: California Animal Health and Food Safety Laboratory

Drs. Robert Poppenga & Birgit Puschner, & Chemist Elizabeth Tor

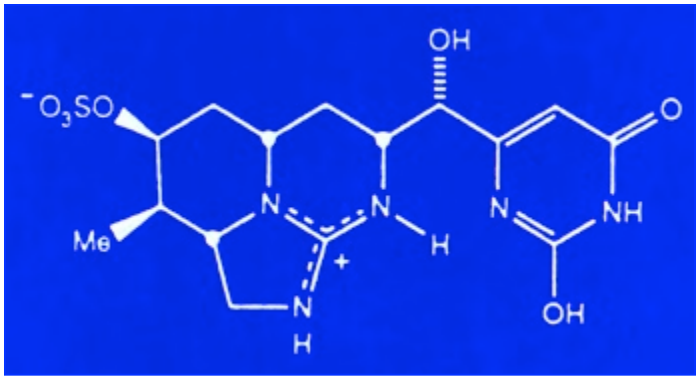
	Out of State Costs
Combination: of Microcystins, Anatoxin-A, Saxitoxin, & Cylindrospermopsin.	1-3 samples: $600 4 or more each: $500
Individual Toxins:	
Microcystins	1 sample: $175, then $150 ea.
Anatoxin-A	1 sample: $175, then $150 ea.
Saxitoxin	1-4 samples: $150, then $175/4 samples
Cylindrospermposin	1-4 samples: $150, then $175/4 samples

Abstract of Presentation by Dr. Keith Loftin of USGS in Lawrence, KS on Sep 5, 2012 at EPA in RTP, NC

"Reassessment of cyanotoxin mixtures in the 2007 U.S. E.P.A. National Lakes Assessment"

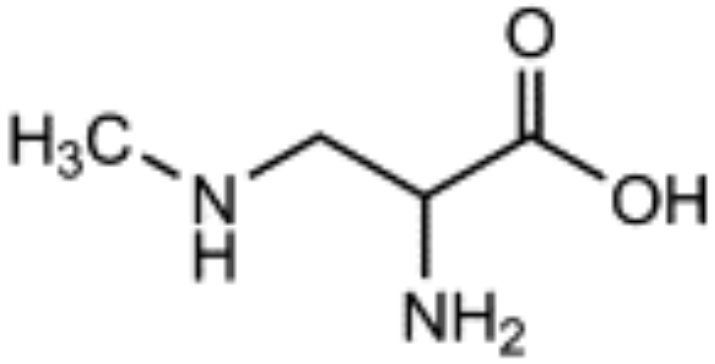
- Microcystins have historically been the most frequently reported class of cyanotoxins. In the 2007 US EPA National Lake Assessment (2007 NLA), **the USGS found that microcystins were detected in integrated photic zone samples from approximately 30% of sampled lakes (n= 1028).** Based on World Health Organization (WHO) microcystin recreational guidelines, 1 % of lakes were categorized as either moderate (> 10 µg/L) or high (> 20 µg/L) probable health risks based on total microcystin concentrations. However, health risk assessment by concurrently sampled chlorophyll and cyanobacterial abundance, two other metrics that can be used according to WHO recreational guidelines, resulted in approximately 42% and 27% of lakes being categorized as either moderate (chlorophyll > 10 µg/L or cyanobacterial abundance > 20,000 cells/mL) or high (chlorophyll > 50 µg/L or cyanobacterial abundance > 100,000 cells/mL) probable health risk, respectively. This demonstrates the possibility of a disconnect between the WHO metrics used to assess potential health risk because of microcystin exposure in the United States. Review of the cyanobacteria data also demonstrated that cyanobacteria were present in the 2007 NLA samples capable of producing other classes of cyanotoxins. Frozen, archived 2007 NLA samples were reanalyzed for cylindrospermopsins and saxitoxins by enzyme-linked immunosorbent assay. Preliminary results from this subset show that **cylindrospermopsins (n= 659) and saxitoxins (n=678) had a detection frequency of 5% and 8%, respectively. Maximum concentrations for cylindrospermopsins and saxitoxins were 3.5 and 0.38 µg/L, respectively. Results indicate that co-occurrence of multiple toxin classes and variants do occur in US water bodies. Therefore, routine monitoring for microcystins alone may be insufficient to adequately assess exposure to cyanotoxins.**

Cylindrospermopsin



- Small cyclic guanidine derivative produced by *Cylindrospermopsis*.
- Dominant cyanobacteria in much of the world, including Florida.
- Possibly spreading into Midwest; easily confused with related cyanobacteria.
- No dense blooms.
- Implicated in human "Palm Island liver disease."
- May contribute to the incidence of chronic liver disease.
- Need to look for it, at least until we are confident that it is not causing problems.

Beta-methyl-amino-alanine (BMAA)



- Alanine derivative produced by *Nostoc* & several other cyanobacterial genera.
- Seems to bioconcentrate in foodwebs.
  - Possibly of concern in carp that eat algae & predatory fishes.
- Implicated in the literature in amyotrophic lateral sclerosis (Lou Gehrig's disease), Parkinsonism, & Alzheimer's disease.
- Recent paper on mechanisms:
  - Okle et al., 2013. L-BMAA Induced ER Stress and Enhanced Caspase 12 Cleavage in Human Neuroblastoma SH-SY5Y Cells at Low Nonexcitotoxic Concentrations. Toxicol. Sci. 131:217–224.
- More research is needed to determine if this toxin poses a significant risk from ingestion of water and fish.

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- Preparing a grant application for studies related to mitigation of risks from HABs in relation to climate change.
- Relying on the established phycology capabilities of Dr. John Downing of Iowa State.
- Developing analytical capabilities to assay for:
  - Microcystins
  - Anatoxin-A
  - Saxitoxins
  - Cylindrospermopsin
  - BMAA
- Perhaps a group in Illinois should do something similar.

Workshop on Cyanobacteria Harmful Algae Blooms (CyanoHABs)

January 5-6, 2013

Day One	
8:00-8:30 a.m.	Introduction-Goals of Workshop
8:30-9:00 a.m.	History, Biology and Health Risk from CyanoHABs
9:00-10:015 a.m.	Cyanobacteria Taxonomy and Identification. Includes PCR methods for taxonomy
10:15 – 10:30 a.m.	Coffee Break
10: 30 – 12 noon	Sampling, Handling, Storage and Shipment of CyanoHABs. Includes guidance on their classification as hazardous substances **Cell/filament/colony enumeration and biovolume methods-Action levels for postings and closures**
Noon– 1:15 p.m.	Lunch
1:15 p.m. – 4:45 p.m.	**Lab – Identification of CyanoHABs-discussion of taxonomy keys plus some discussion/demonstration of sampling, handling and enumeration methods for cell/filaments/colonies**
4:45-5:00 p.m.	**Lab - Discussion/summary of algal identification**
Day Two	
8:00 -8:30 a.m.	Review-Discussion from Day 1
8:30-10:30 a.m.	Chemistry, toxicology, detection of Cyanotoxins (2 hr)
10:30-10:45 a.m.	Coffee Break
10:45 a.m.– 12:00 noon	Water Treatment for Removal and Inactivation of Cyanotoxins Management and Mitigation of Waterblooms
12:00-1:00 p.m.	Lunch
1:00 p.m.-2:00 p.m.	**Review of chemistry, detection of cyanotoxins, removal and inactivation of cyanotoxins and mitigation of water blooms Plus other CyanoHAB topics of interest**
2:00 – 5:00 p.m.	**Laboratory – Cyanotoxin detection, analysis (ELISA)**
5:00 p.m.	Wrap up-Discussion

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Room locations:
Lectures VPTH2768
Labs VPTH2780

Take Home Messages

- Most veterinary practitioners are familiar with blue-green algal poisoning as an acute threat to the health of dogs, cattle, swine & perhaps other species.
- Many know that blue-green algae toxins are hepatoxic or neurotoxic.
- Histopathology is extremely helpful in ruling in/our acute microcystin poisoning as well as infectious diseases. Also, not finding such abnormalities is consistent with cyanobacterial neurotoxin poisoning.
- The expense of analytical chemistry assays for cyanobacterial toxins is high relative to the value of most animals & the budgets of most owners.
- The delay in sending samples to the laboratory & waiting for results limits the value of diagnostic assessments.
- Veterinarians treat nearly all of their animal patients that have suspected cyanobacterial poisonings without phycological or chemical analytical confirmations.
- The management for cyanotoxin poisonings generally consists of detoxification with activated carbon, bathing cyanobacterial cells from the body, & supportive care. Such care is similar to that used for many other toxicoses.

Take Home Messages

- There is no reporting requirement for animal poisonings by cyanotoxins.
- Although there are regular meetings of veterinary toxicologists (American Board of Veterinary Toxicology, American Academy of Veterinary and Comparative Toxicology, American Association of Veterinary Laboratory Diagnosticians, & the Veterinary & Comparative Toxicology of the Society of Toxicology, there is no central collection point for data on cyanobacterial toxicoses.
- The ASPCA Animal Poison Control Centers has immense data collection & storage on case calls of animal poisoning, searchable by species, toxicant group, & organ/system effects. They are often open to collaborations!
- Domestic & wild animals can be early sentinels for cyanotoxin risks, but to optimize animal health data as a warning system, veterinary practitioners must be alerted that the Animal Poison Control Center &/or diagnostic laboratories, &/or researchers want to hear about cases, sample submissions must be straightforward, & costs of consultation & analysis should be covered by a grant or other funding.

The Usual Circumstances

- Drought is a risk factor.
- Wind blowing bloom toward the animal access point is major concern.
  - Visible dense bloom material is involved in vast majority of cases.

- Affected animals were typically in, or drinking from, eutrophic water bodies with green or blue-green discoloration of the water.
- Like sources:
  - Animal waste runoff → drinking from farm ponds → food animal illnesses & potential death losses (usually cattle or pigs, & possibly domestic ducks, geese, or other species).
  - Fertilizer & septic system pollution of ponds/lakes → dogs in the water, drinking the water, &/or licking cyanobacteria from hair coats → illnesses & potential deaths.

Mitigation & Prevention (All are essential)

- Keeping animal wastes & other nutrients out of water bodies.
- Fencing to keep food-producing animals at a distance from water bodies to avoid runoff & drinking from water.
- Astute waste management (composting & application on land so that significant runoff will be prevented.
- Cessation of fertilizer applications near ditches, streams, ponds or lakes.
- Generous-sized well-vegetated buffers.
- Providing clean drinking H2O to animals.

Avoiding Cyanotoxin Poisoning while Building up the Economic Value of Agriculture to Illinois

- The economy of Illinois needs to improve & partnering Ag with IL EPA could help.
- In my opinion, Illinois agriculture focuses excessively on raw commodities(corn/soy) & far too little on value-added, in-state production, processing & sales of eggs, milk, meat, fish, & products from them.
- As long as demand for such foods is strong, stewardship to prevent cyanobacterial blooms should rely on best management practices for increased production of milk, meat, and eggs.
- Closing the nutrient loop with more in-state animal agriculture could reduce use of mineral fertilizers as well as pollution from transport of grain, animals, & products.
- **Done well, substantial increases in animal agriculture can accompanied by substantial reductions in toxic blooms of cyanobacteria.**
- Data on animal agriculture - production trends: http://web.extension.illinois.edu/state/newsdetail.cfm?NewsID=7085
  - 1994-2004: overall livestock numbers decreased significantly & nominal gross receipts from livestock declined by 4%.
  - 1994-2004: Growth in Illinois animal industry was mainly in swine production.
  - Since 1999: There has been a 30% increase in nominal receipts—mostly from swine production.

More Recent Data - Illinois Livestock Developing Group (http://illinoislivestock.org/Research/)

- The production sector of the livestock industry contributes $3.5 billion of total impact and over 25,000 jobs to the state's economy.
- When combined with meat & dairy processing, the entire complex produces more than $27 billion of total impact, equal to 5% of the state's economy & 99,000 jobs.
  - Increase of about $6 billion since 2004.
- Since 2000, the trend in Illinois livestock output shows modest growth in the real value of products sold.
  - Pork & poultry lead with positive real growth.
  - Dairy is nominally flat & has declined in real terms.
  - Beef, sheep, & lamb income declined both nominally & in real terms.
- **Of the 40,070 operations, 15,000 were cow-calf enterprises; 5,000 cattle feeding; 1,500 dairy cattle; 3,400 hogs; 1,900 sheep; 1,344 goats; 8,980 horse farms; & 64 aquaculture units.**
- Clinton County, in south central Illinois, produces the most livestock products of any county in Illinois.
  - It produces $122 million in direct output and $168 million in total output.
  - The industry generates annual taxes of $13 million & total employment of 1,443 full time workers.
- In Jasper County of east central Illinois, the livestock industry generates the greatest share of the county's economic activity = 9.9% of all personal income.
- **Illinois could produce more beef, pork, poultry, eggs, milk, & fish – responsibly.**