

Health Effects of Algal Toxins

James E. Klaunig, Ph.D.,

Robert B. Forney Professor of Toxicology

Director, Center for Environmental Health

Associate Director, IU Cancer Center

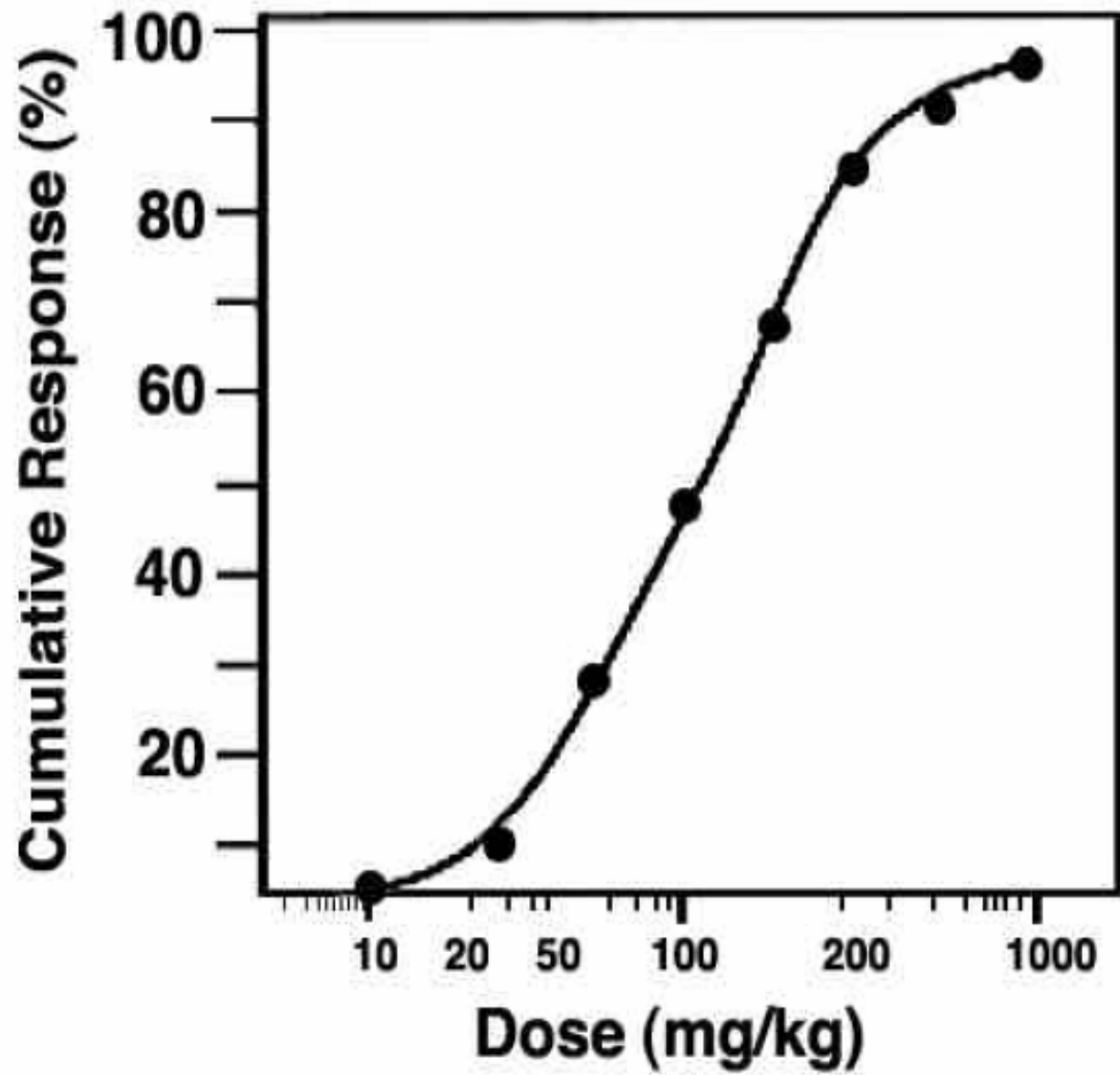
Indiana University School of Medicine

Toxicology

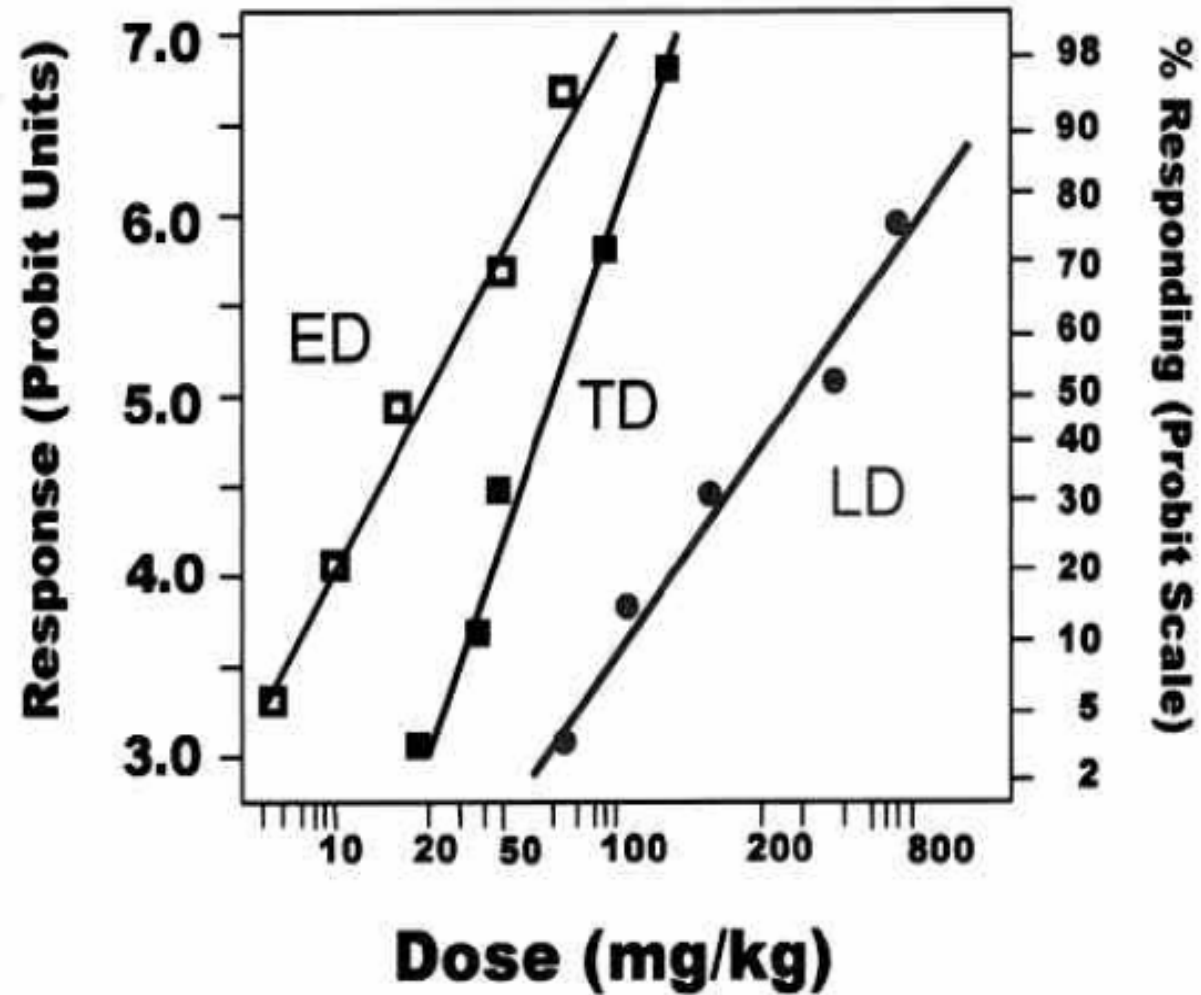
- **Paracelsus** (1493 – 1541)
- Alchemist, Physician,
- Father of Toxicology

- All things are poison and nothing (is) without poison; only the dose makes that a thing is no poison

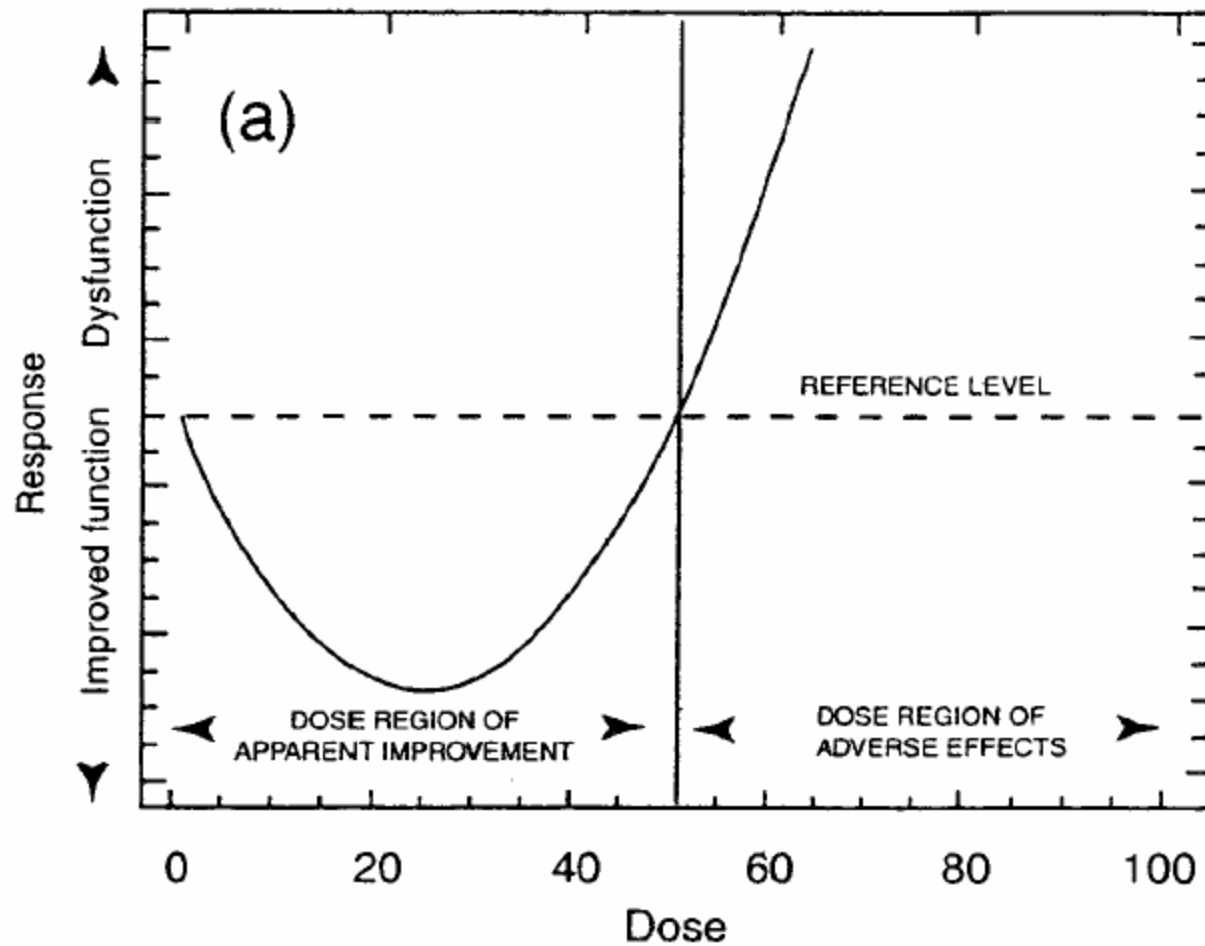




Comparison of effective, toxic and lethal dosages



CONCEPT OF DOSE RESPONSE



Cyanobacterial Toxins

- 1/3 Cyanobacteria studied produce toxins
- What is a toxin –
 - A toxic substance (usually protein based) produced by a living organism
- Toxins are classified as:
 - Hepatotoxic – can cause liver failure
 - Neurotoxic – disrupt nerve and brain function
 - Non-specific Toxins
 - Lipopolysaccharide (LPS) effects

Factors Affecting Toxin Production

- Toxins are not secreted but released from cell lysis (death)
- Older and senescing blooms tend to release toxins as well as those treated with copper sulfate
- Temperature (18-25C) and pH extremes increase toxin content
- High phosphorous stimulates toxin production in hepatotoxic strains
- Non nitrogen fixing species (Microcystis) produce more toxins in nitrogen rich conditions

Cyanobacterial toxins

- Cyanobacterial toxins are classified by how they affect the human body.
 - Hepatotoxins (which affect the liver)
 - Neurotoxins (which affect the nervous system)
 - Toxic alkaloids, causing gastrointestinal symptoms or kidney disease in humans.
 - Lipopolysaccharides
 - Not all cyanobacteria of these species form toxins

Toxins Produced From Cyanobacteria

- Many Cyanobacteria can produce multiple toxins

Toxin	Cyanobacteria
Microcystin	<i>Microcystis, Anabaena, Oscillatoria, others</i>
Anatoxin-a	<i>Anabaena, Aphanizomenon</i>
Cylindrospermopsin	<i>Cylindrospermopsis, Aphanizomenon</i>
Saxitoxins	<i>Anabaena, Cylindrospermopsis, Aphanizomenon, others</i>
Lipopolysaccharides	All

Cyanobacteria

- Two genera of cyanobacteria account for the majority of toxic blooms world-wide:
 - Microcystis and Anabaena.
- Microcystis
 - produce a family of toxins called "microcystins".
 - they are heptapeptides that primarily affect the liver in animals (hepatotoxins).
 - Poisoning symptoms may take 30 minutes to 24 hours to appear, depending upon the size of the animal affected and the amount of toxic bloom consumed.
 - Microcystin effects include jaundice, shock, abdominal pain/distention, weakness, nausea/vomiting, severe thirst, rapid/weak pulse and death.

Anabaena sp

- *Anabaena* sp. can produce several kinds of toxins. -
- **Neurotoxic-** Two different neurotoxins have been described.
- Anatoxin-a
 - a potent postsynaptic cholinergic nicotinic agonist, which causes a depolarizing neuromuscular blockade.
 - Produces staggering, paralysis, fasciculations (muscle twitching), gasping, convulsions and death in animals
 - (oral LD50 in mice = 5 ppm)
- Anatoxin-a(s)
 - chemically unrelated to the first, acts as an inhibitor of cholinesterase leading to a neuromuscular blockade.
 - Both cause a "tetanus- like" muscle paralysis. induced toxicosis in experimental animals may exhibit hypersalivation, tremors, fasciculations, involuntary muscle movement, diarrhea, cyanosis (tongue and mouth lining bluish) and death.

Lipopolysaccharide

- Lipopolysaccharides (LPS) are found in the outer cell wall of gram negative bacteria
- Consist of a sugar and fatty acid component
 - Fatty acid portion elicits an irritant or allergenic effect in humans
- LPS is a potent activator of macrophages
 - Can result in the production of cytokines, growth factors, and reactive oxygen species

Microcystins

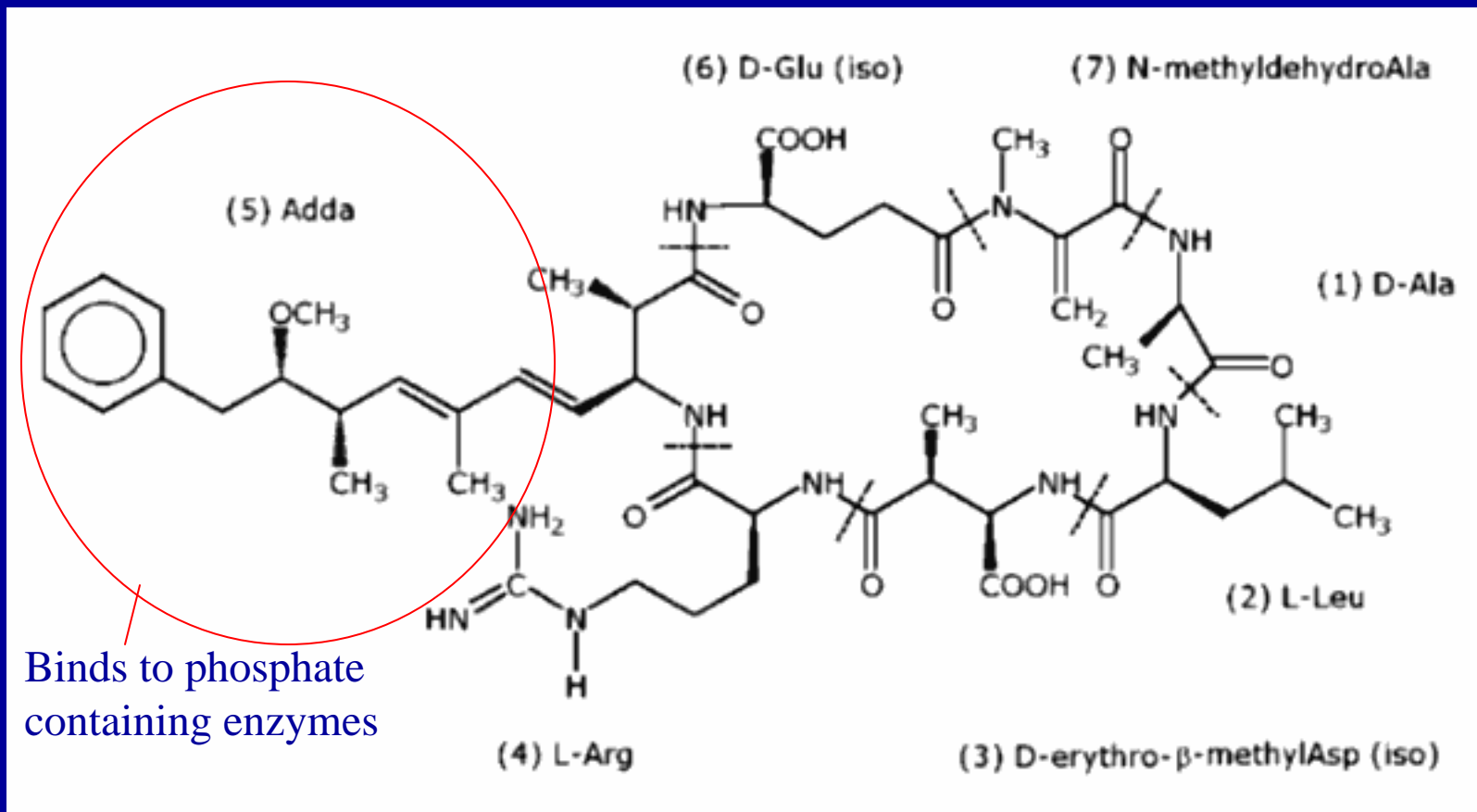
- Most commonly detected cyanobacterial toxin in fresh and brackish water
- Most well studied cyanobacteria for toxic effects
 - Water soluble, but does not readily cross cell membranes
 - Typically transported across cell membranes through special transporters present in the liver
 - Approximately 70% of administered dose distributes to the liver
- Highly toxic, low doses required for lethality, steep dose response curve
 - LD₅₀ (ip) = 25-150 ug/kg in mice
 - LD₅₀ (oral) = 5000 ug/kg in mice
- Liver is the target organ for toxic effects

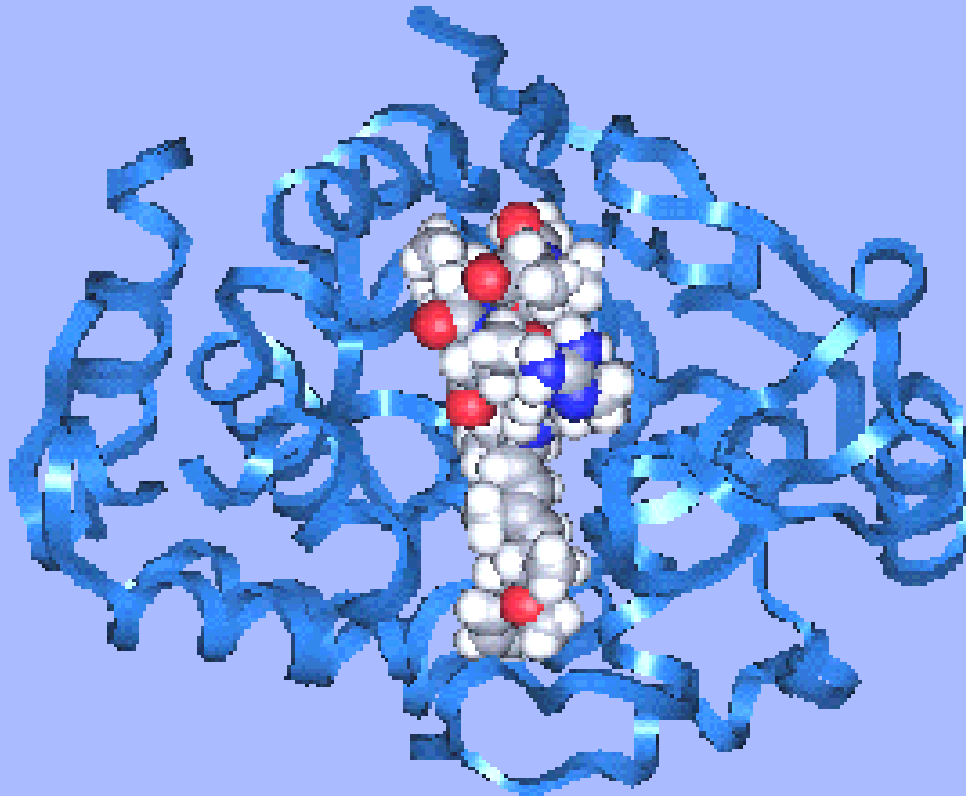
Microcystin Hepatotoxins

- These hepatotoxins inhibit the protein phosphatases inside hepatocytes
- Affecting the maintenance of the cytoskeleton by disruption the balance of phosphate groups on the cytoskeletal proteins
- Microcystin-LR does not readily cross cell membranes and does not enter most tissues.
- It crosses the ileum through the multispecific organic ion transport system and mainly enters hepatocytes, where it is covalently bound to a 40000-dalton protein (protein phosphatase 2A and possibly protein phosphatase 1)

Microcystin toxin:

- large, cyclic peptide with several structural variants
- the linear form (break-down or precursor products) is 100x less toxic compared with the cyclic toxin





- Binding of Microcystin with PP1 and PP2A

Microcystin Hepatic Toxicity : Mechanisms

- The Toxicity of Microcystin in mammals is characterized by
 - Fulminant intrahepatic hemorrhage,
 - Followed by hypovolemic shock or hepatic insufficiency
 - And death of the animals secondary to massive hepatocellular necrosis and collapse of hepatic parenchyma
 - Rounding of hepatocytes occurs concurrently with the loss of normal hepatic architecture
 - Interaction of microcystin-LR with serine/threonine phosphatases-1 and -2A
 - Inactivation of these protein phosphatases

Detoxification

- The liver plays a large role in the detoxification of microcystins (Brooks & Codd, 1987; Robinson et al., 1991b).
- Detoxification products were seen in urine, faeces, and liver cytosolic fractions (Robinson et al., 1991a)
- The detoxification products of microcystin-LR are more water soluble than the parent toxin.

Microcystin Toxicity

- Microcystin is an extremely acute toxin.
 - The LD50
 - by the intraperitoneal route is approximately 25–150 $\mu\text{g}/\text{kg}$ of body weight in mice;
 - the oral (by gavage) LD50 is 5000 $\mu\text{g}/\text{kg}$ of body weight in mice, and higher in rats (Fawell et al., 1994).

TOXICITY LD50

Table 1. Toxicity of cyanobacterial toxins.

Toxin	LD ₅₀ (µg/kg, ip, mouse)	Organism	Reference
Microcystin-LR	50	<i>M. aeruginosa</i> , <i>Aph. flos-aquae</i> , <i>M. viridis</i>	(31, 125)
Microcystin-LA	50	<i>M. aeruginosa</i> , <i>M. viridis</i>	(138)
Microcystin-YR	70	<i>M. aeruginosa</i> , <i>M. viridis</i>	(31)
Microcystin-RR	600	<i>M. aeruginosa</i> , <i>Anabaena</i> sp., <i>M. viridis</i>	(139–141)
[<i>D</i> -Asp ³]microcystin-LR	50–300	<i>M. aeruginosa</i> , <i>Aph. flos-aquae</i> , <i>M. viridis</i> , <i>O. agardhii</i>	(142, 143)
[<i>D</i> -Asp ³]microcystin-RR	250	<i>O. agardhii</i> , <i>M. aeruginosa</i> , <i>Anabaena</i> sp.	(19, 139)
[<i>D</i> ha ⁷]microcystin-LR	250	<i>M. aeruginosa</i> , <i>Anabaena</i> sp., <i>O. agardhii</i>	(139, 144)
[(6 <i>Z</i>)-Adda]microcystin-LR	> 1200	<i>M. viridis</i>	(143)
[(6 <i>Z</i>)-Adda]microcystin-RR	> 1200	<i>M. viridis</i>	(143)
Nodularin	50	<i>N. spumigena</i>	(145)
[<i>D</i> -Asp ¹]nodularin	75	<i>N. spumigena</i>	(146)
[(6 <i>Z</i>)-Adda ³]nodularin	> 2000	<i>N. spumigena</i>	(146)
Anatoxin-a	200–250	<i>Aph. flos-aquae</i> , <i>Anabaena</i> spp., <i>Oscillatoria</i> sp., <i>Aphanizomenon</i> sp., <i>Cylindrospermum</i> sp.	(145, 147)
Anatoxin-a(s)	20	<i>Aph. flos-aquae</i>	(148)
Saxitoxin	10	<i>Aph. flos-aquae</i> , <i>A. circinalis</i> , <i>Cylindrospermopsis</i> <i>raciborskii</i> , <i>Lyngbya wollei</i>	(42, 149)
Cylindrospermopsin	2000	<i>C. raciborskii</i> , <i>Umezakia natans</i> , <i>Aph. ovalisporum</i>	(150)

Table 2-1

Approximate Acute LD₅₀s of Some Representative Chemical Agents

AGENT	LD ₅₀ , mg/kg*
Ethyl alcohol	10,000
Sodium chloride	4,000
Ferrous sulfate	1,500
Morphine sulfate	900
Phenobarbital sodium	150
Picrotoxin	5
Strychnine sulfate	2
Nicotine	1
<i>d</i> -Tubocurarine	0.5
Hemicholinium-3	0.2
Tetrodotoxin	0.10
Dioxin (TCDD)	0.001
Botulinum toxin	0.00001

*LD₅₀ is the dosage (mg/kg body weight) causing death in 50 percent of exposed animals.

Short-Term Exposure

- Microcystin-LR was administered orally by gavage to groups of 15 male and 15 female mice at 0, 40, 200, or 1000 $\mu\text{g}/\text{kg}$ of body weight per day for 13 weeks.
- No definite treatment related changes were noted at the lowest dose,
- At 200 $\mu\text{g}/\text{kg}$ of body weight per day, there was slight liver pathology in some male and female mice.
- At the highest dose level, all male and most female mice showed liver changes, which included chronic inflammation, focal degeneration of hepatocytes, and haemosiderin deposits.
- The NOAEL for microcystin-LR was considered to be 40 $\mu\text{g}/\text{kg}$ of body weight per day (Fawell et al., 1994).

Long-Term Exposure

- An oral repeated-dose study was conducted with *M. aeruginosa* extract supplied to mice at five concentrations (equivalent to 750–12 000 µg of microcystin-YM per kg of body weight per day) in their drinking-water for up to 1 year.
- At the higher concentrations, increased mortality, increased incidences of bronchopneumonia, and chronic liver injury were noted.
- No liver cancer was seen, but the authors indicated that there may have been some evidence of tumor promotion.
- No clear NOAEL was established in this study (Falconer et al., 1988).

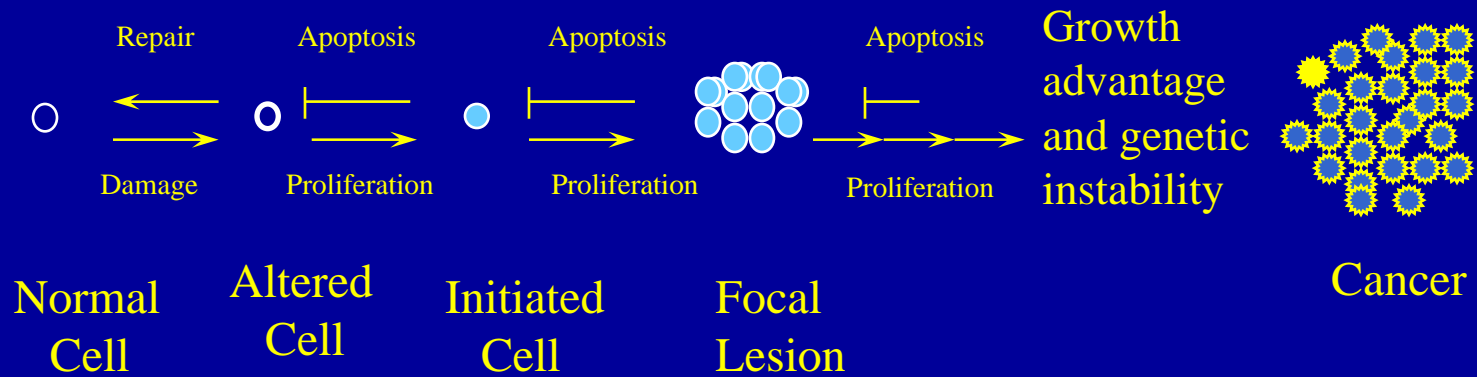
Microcystin

- Carcinogenicity
 - Not mutagenic
 - Some evidence that microcystins act as hepatic tumor promoters

Initiation

Promotion

Progression



Possible Human Exposure Pathways

- Ingestion
 - Chronic ingestion through contaminated drinking water
 - Ingestion of water through recreational contact
- Consumption of fish and shellfish from contaminated water
- Dermal
 - absorption of toxins from recreational activities

Cyanobacterial Toxins

- Several documented cases of human poisoning with various cyanobacteria have been identified
- Little dose-response information is available in humans
- In general, animal data has shown that **neurotoxic toxins** can produce severe acute effects but degrade rapidly while **hepatotoxic** compounds are more persistent and accumulate in human liver

Cyanobacteria and Human Health

Microcystis Poisoning

- One of the earliest reports of their toxic effects was in China 1000 years ago (Chorus and Bartram, 1999).
- 117 patients developed liver disease (> 50 deaths) following dialysis with microcystin contaminated water (Caruaru, Brazil, 1996)
 - Painful hepatomegaly, jaundice
 - Elevated transaminases, hyperbilirubinemia, prolonged prothrombin time
 - Disruption of liver plates, cell deformity, necrosis, cholestasis, leukocyte infiltration
- Microcystin levels
 - Up to 10 ng/ml (serum)
 - 0.1 – 0.5 ng/mg (liver)

Cyanobacteria and Human Health

Microcystis Poisonings

- Flooded dam resulted in severe gastroenteritis in more than 2000 people, death in 88 (mainly children) over a 42 day period (Bahia, Brazil, 1988)
 - Cases were restricted to areas supplied with drinking water from the dam
 - *Anabaena* and *Microcystis* genera were quantitated in water at 1,104 and 9,755 units/ml
- Water supply treated with copper sulfate to control algal bloom (*microcystis*) resulted in hepatotoxicity (Armidale Australia, 1981)
 - Liver function tests in exposed persons demonstrated increase transaminase levels during the bloom and post copper sulfate treatment

Cyanobacteria and Human Health

Microcystin Poisonings

- **Cancer**

- An epidemiological survey in Haimen city (Jian-Su province) and Fusui county (Guangxi province) in China found a close relationship between the incidence of primary liver cancer and the use of drinking-water from ponds and ditches (Ueno et al., 1996).
- In 1993 and 1994, microcystin concentrations ranged from 0.058 to 0.460 µg/litre; the highest concentrations occurred from June to September. Microcystin was not detected in deep wellwater.
- According to Ueno et al. (1996), the combined effect of microcystin toxin from the drinking-water of ponds/ditches and rivers or both and other carcinogens such as aflatoxin B1 found in food may be the cause of the high incidence of primary liver cancer in Haimen city and other areas in China.

Cyanobacteria and Human Health

anatoxin-a and anatoxin-a(S) Poisoning

- Dane County, WI, 2002
 - 5 teenagers swam in a golf course pond characterized as “dirty” and “scummy”
 - One of 2 that went underwater died of acute heart failure within 48 hours, the other became ill with acute diarrhea and abdominal pain.
 - Blood tests confirmed the presence of *anatoxin-a and anatoxin-a(S)*



“The problem with toxicology is not the practicing toxicologists, but chemists who can detect, precisely, toxicologically insignificant amounts of chemicals”

Rene Truhaut, University of Paris (1909-1994)



Thank you
Merci
Danke
Gracias
Xie Xie