HUMAN INTOXICATION EVENT BY CYANOTOXINS DURING RENAL DIALYSIS TREATMENT IN CARUARU – BRAZIL

Sandra M. F. O. Azevedo

UFRJ - Brazil

Co-authors: W.W. Carmichael, E.M. Jochimsen, K.L. Rinehart, S.Lau, G.R. Shaw and G.K. Eaglesham

Increased eutrophication process on aquatic environments

Decrease of water quality

Favor cyanobacterial blooms occurrence

South America

12 countries

369 million people

Fourth largest continent

12% of Earth's land surface

According PAHO

1990' situation in LA & Caribbean:

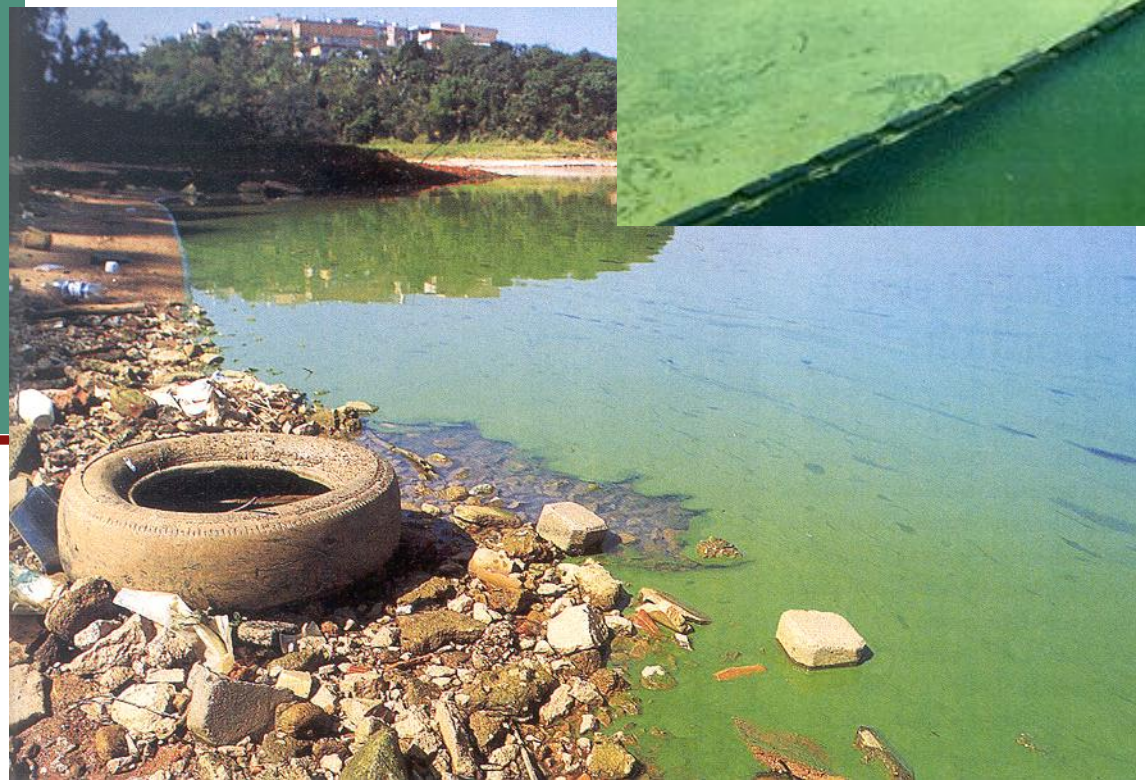
41% of population didn't receive safety water

Diarrhea - Children < 5 years: 25% of deaths

Cholera - 1991-1995: 1,300,000 cases 11,000 deaths

Cyanobacterial blooms in drinking water supplies

Oral exposure



Events in:

Argentina;

Brazil;

Chile;

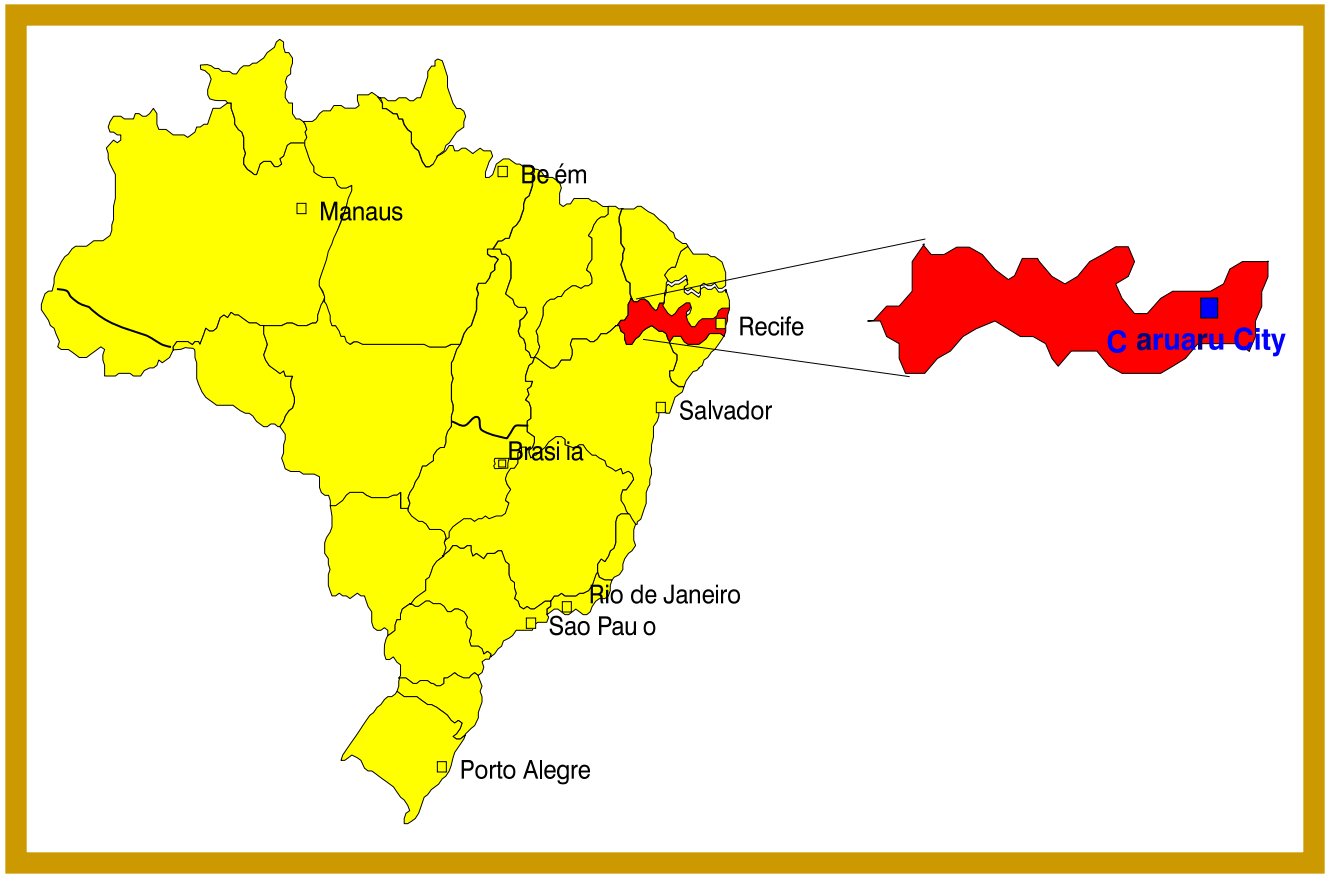
Honduras;

Mexico;

Uruguay;

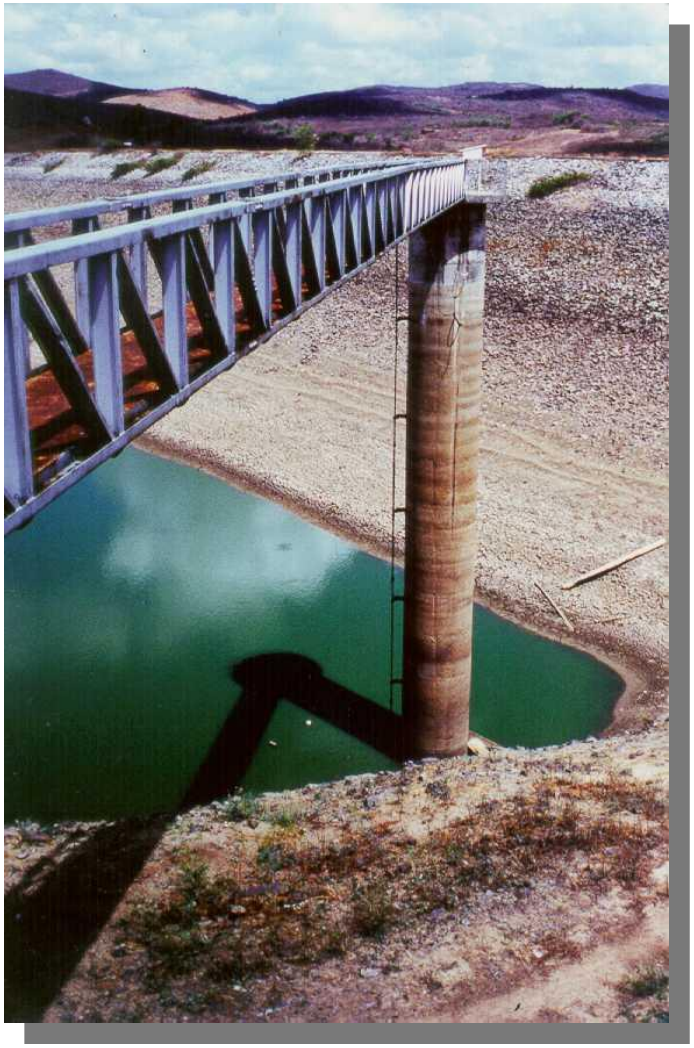
Venezuela

Pernambuco State and Caruaru city location



Tabocas reservoir

Recurrent Severe Drought



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Tabocas Reservoir - PE : 97-98-99

Cyanobacteria bloom

in a reservoir from Pernambuco State

VARIOUS CYANOBACTERIA GENERA AND SPECIES

Different toxic compounds → **CYANOTOXINS**

- Neurotoxins
- Hepatotoxins
- Cytotoxins
- Endotoxins -LPS

Cyanotoxins

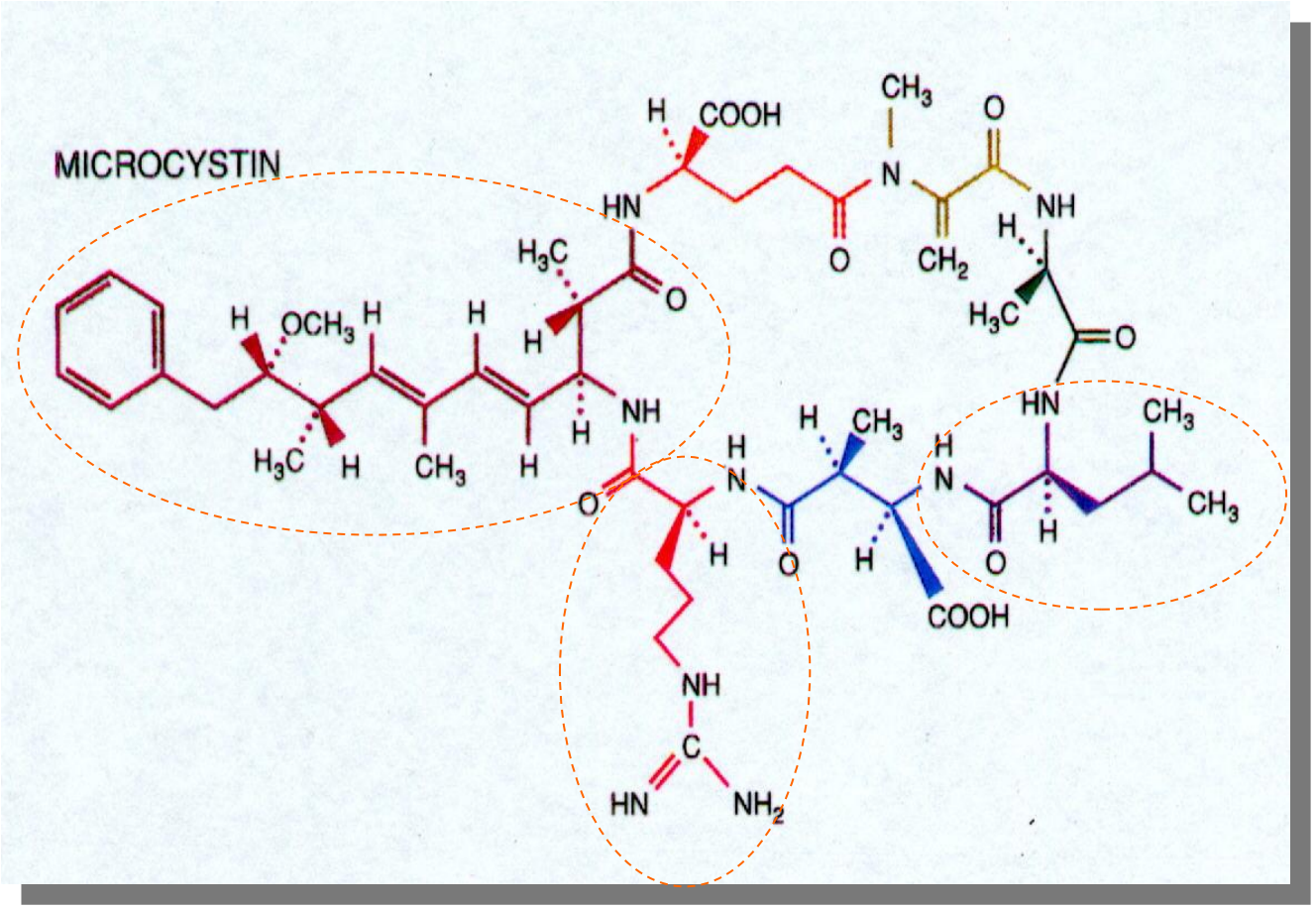
Neurotoxins

- Anatoxin-a
- Anatoxin-a(s)
- Saxitoxins

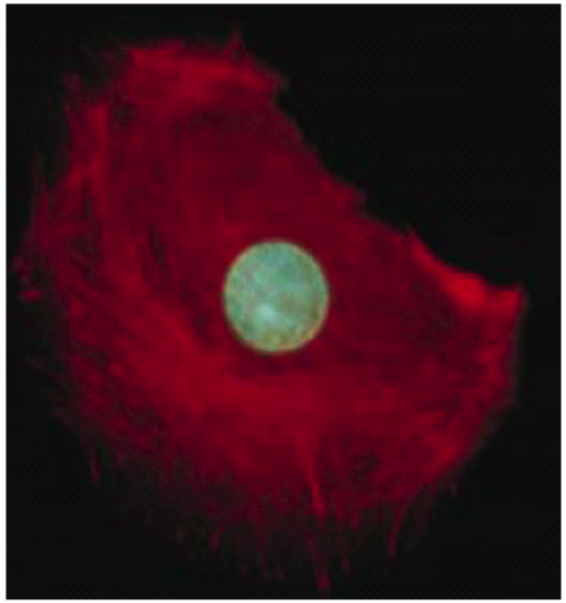
Hepatotoxins

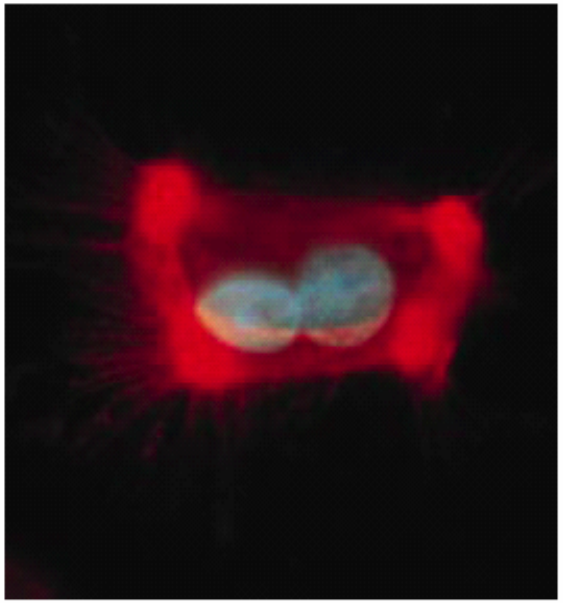
- Microcystins
- Nodularin
- Cylindrospermopsin

Microcystin-LR

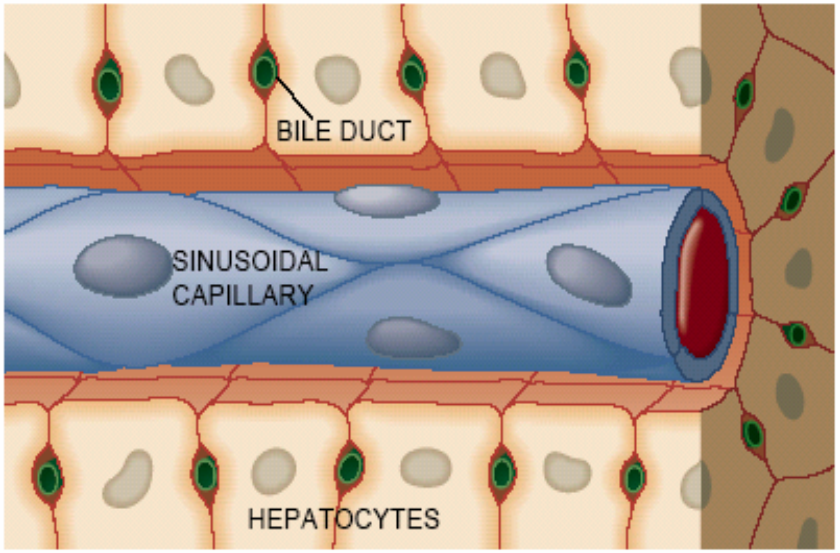


MICROFILAMENTS (*red threads in micrographs*), structural components of cells, are usually quite long, as in the rat hepatocyte at the left. But after exposure to microcystins (*right*), microfilaments collapse toward the nucleus (*blue*). (This cell, like many healthy hepatocytes, happens to have two nuclei.) Such collapse helps to shrink hepatocytes—which normally touch one another and touch sinusoidal capillaries (*left drawing*). Then the shrunken cells separate from one another and from the sinusoids (*right drawing*). The cells of the sinusoids separate as well, causing blood to spill into liver tissue. This bleeding can lead swiftly to death.

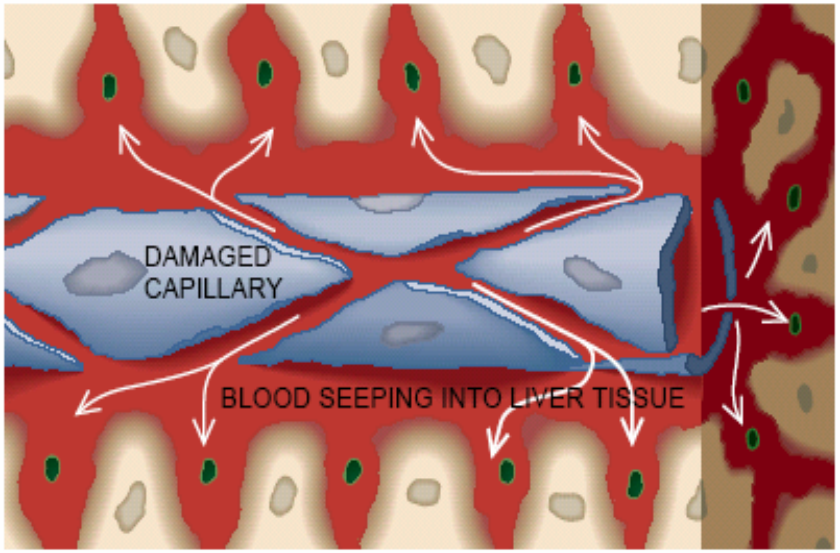




NORMAL LIVER

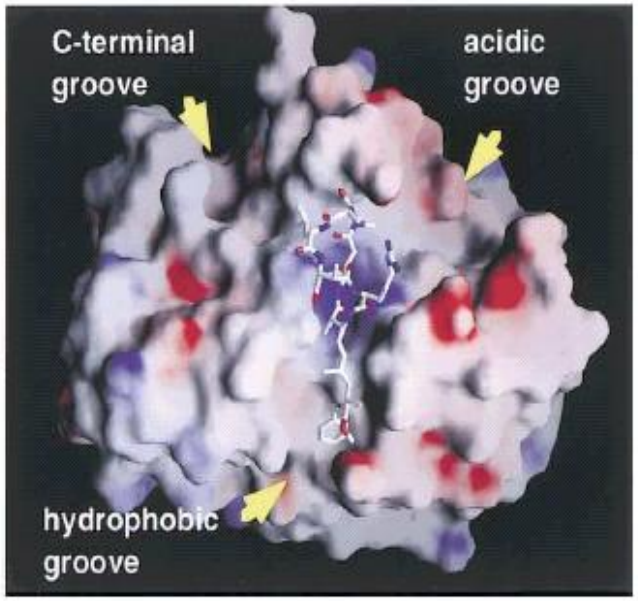


LIVER AFTER TOXINS ACT



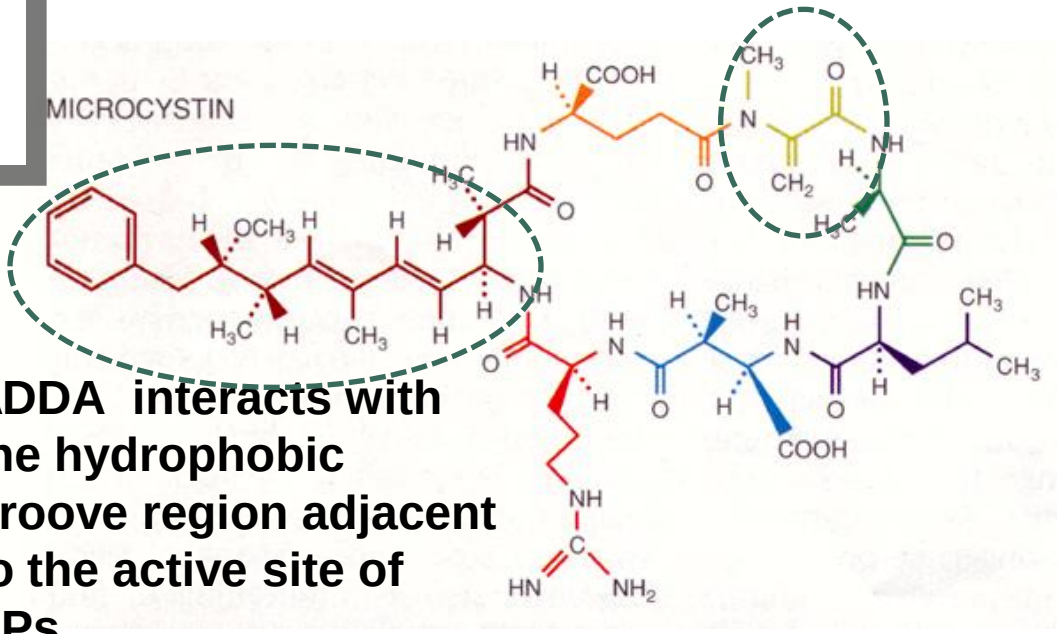
W.W. Carmichael, Scientific American, 1994

Molecular level of MCYST-LR effect: Inhibition of Protein Phosphatase 1 and 2A



Gupta et al., (1997)

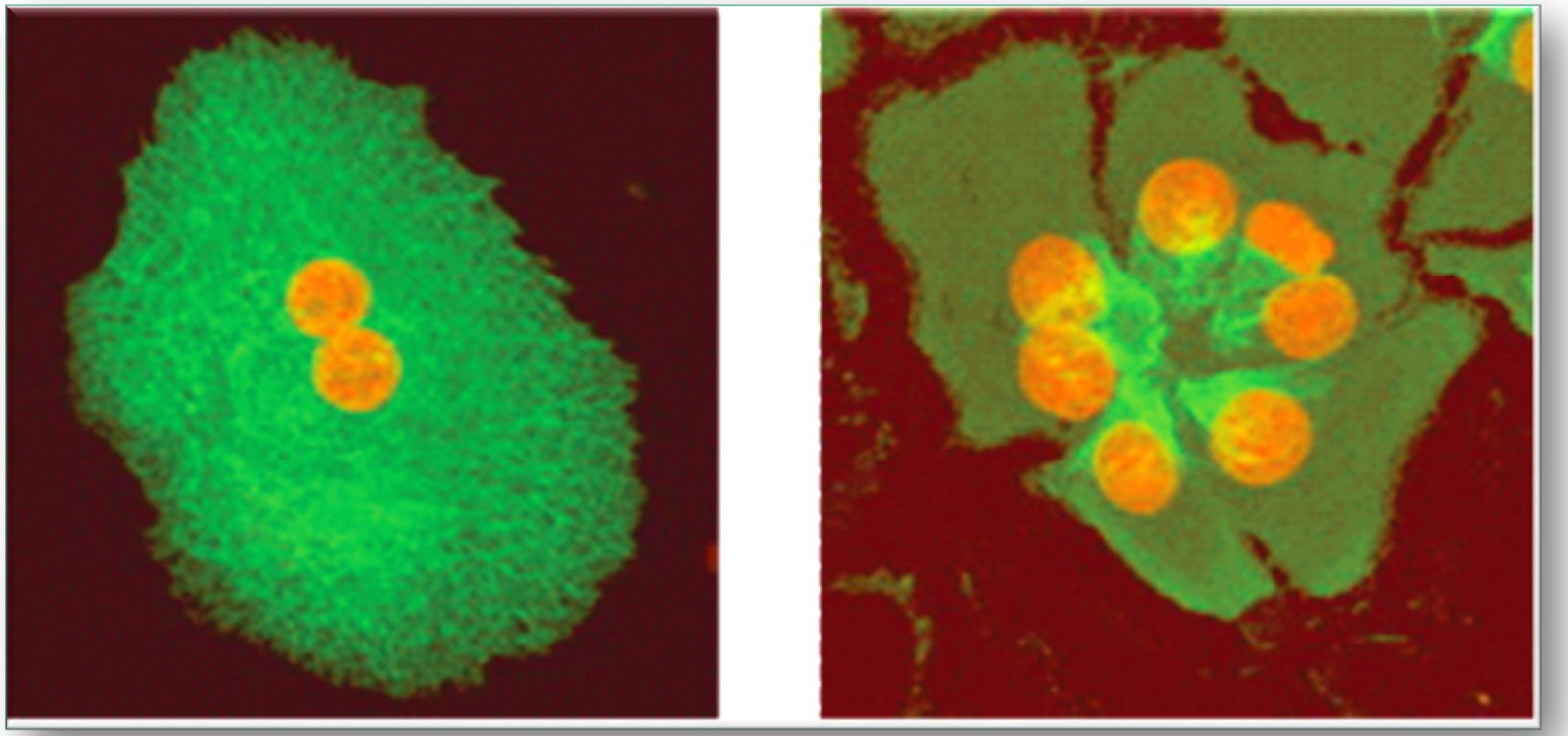
Mdha is covalently binding to Cys-273 from PPs



Recently it was demonstrated that MCYST binds to thiol groups of various proteins

ADDA interacts with the hydrophobic groove region adjacent to the active site of PPs

… increasing in the phosphorylation of various subcellular targets, including cytoskeletal proteins



Photomicrography of hepatocytes before (left) and after (right) exposure to microcystins. Note disruption of the cytoskeleton architecture. *Source: John Eriksson, University of Turku, Finland, from website (2005, http://www.btk.fi/Research_Groups/Protein_Phosphorylation_Group/protein_phosphorylation_group.htm*

Azevedo et al. (2002)

1996 -Caruaru Syndrome

117 of 136 patients with chronic renal failure (86%) had the following symptoms:

Symptoms: painful large hepatomegaly, jaundice and a bleeding diathesis manifested by ecchymosis, epistaxis and methrorrhagia;

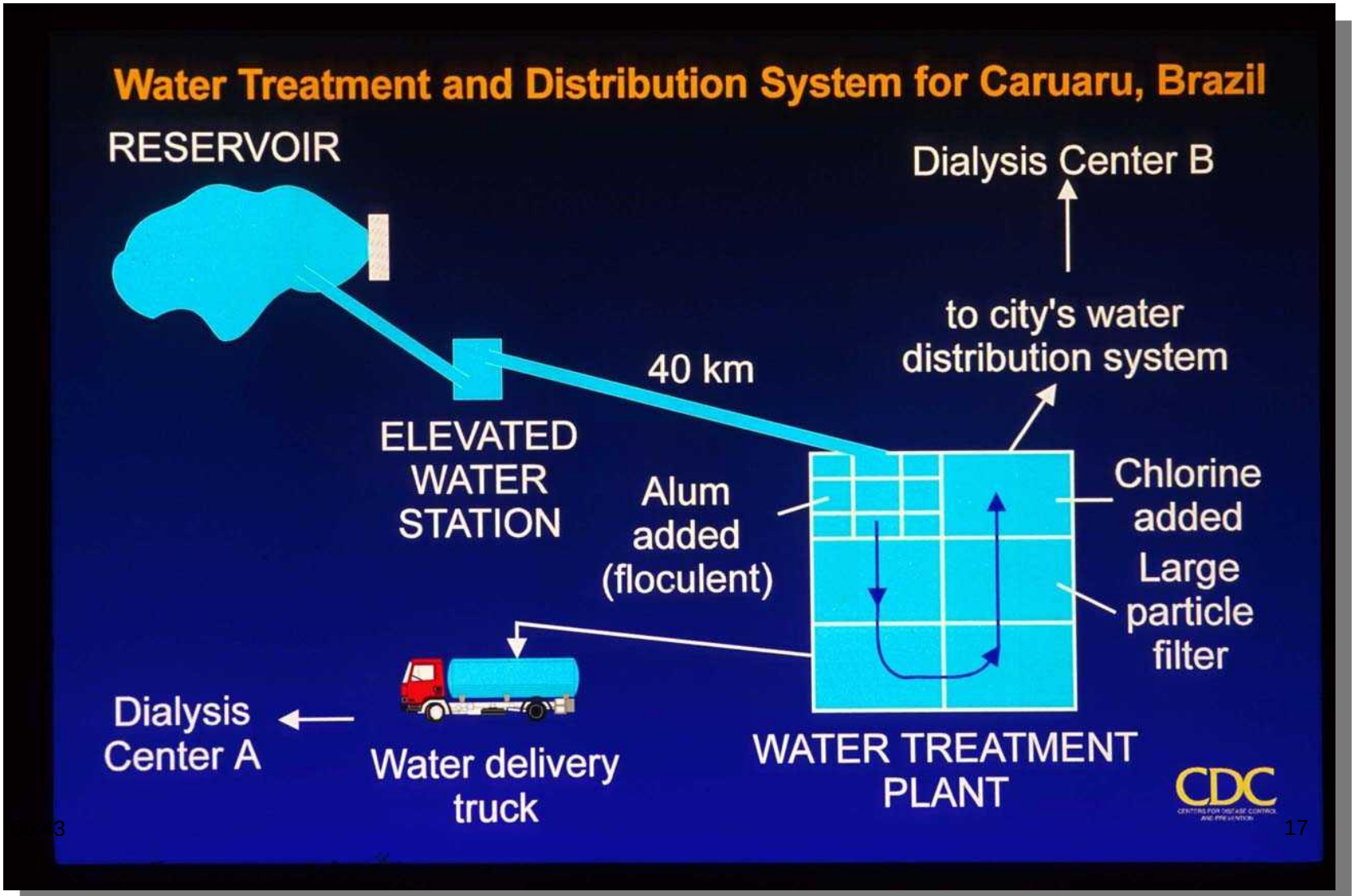
Laboratory profile: elevated transaminases, hyperbilirubinemia, prolonged prothrombin time and severe hypertrigliceridemia;

60 deaths occurred until October 1996 could be attributed to same syndrome

Chronology of Events

- ✓ February 17-20, 1996: IDR patients experience symptoms of headache, eye pain, blurred vision, nausea and vomiting associated with dialysis section;
- ✓ February 20 to March 6: 12 patients die from seizure and/or acute hemorrage associate with visual disturbance, nausea and vomiting;
- ✓ March 7: Secretary of Health – Pernambuco State notified of the cluster of deaths associated with dialysis, epidemiologic investigation begun;
- ✓ March 11: IDR dialysis center closed;
- ✓ March 7-24: IDR patients develop cholestatic liver failure with painful hepatomegaly, elevated transaminases, elevated direct and total bilirubins, elevated triglycerides and elevated prothrombin times. 14 patients die of liver failure;
- ✓ March 23: S.Azevedo. visit for the first time Pernambuco State and presents hypothesis of cyanotoxins as main cause of chronic renal patient's intoxication;
- ✓ March 24: CDC-Atlanta notified of outbreak;
- ✓ March 27: W. Carmichael notified of outbreak;
- ✓ April 1-13: CDC team in Brazil
- ✓ Mid-April: 32 patient death from complication of liver failure;
- ✓ Mid-September: 56 patient deaths – 48 having a common syndrome associated with liver failure

What happened:



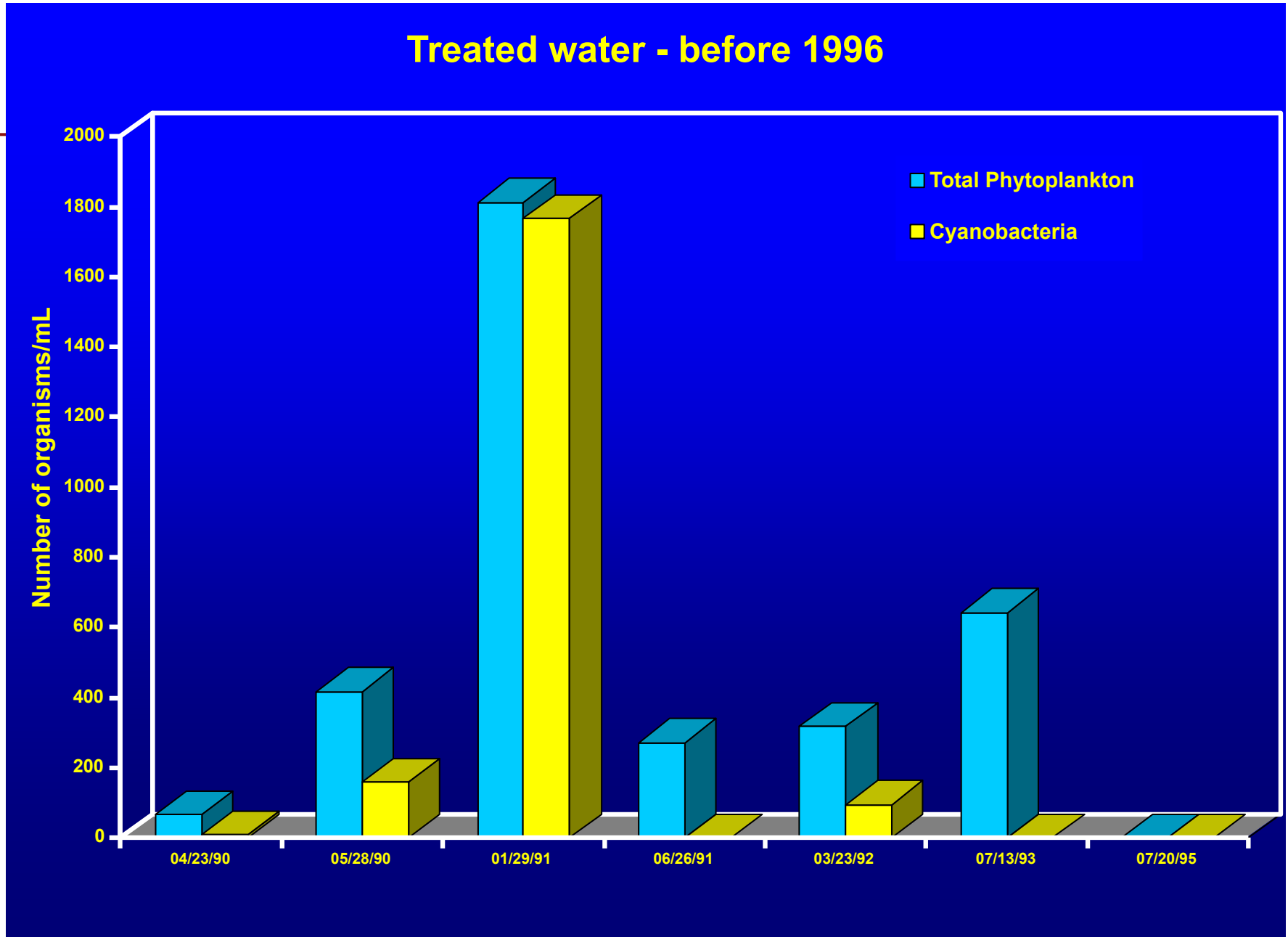
What happened:

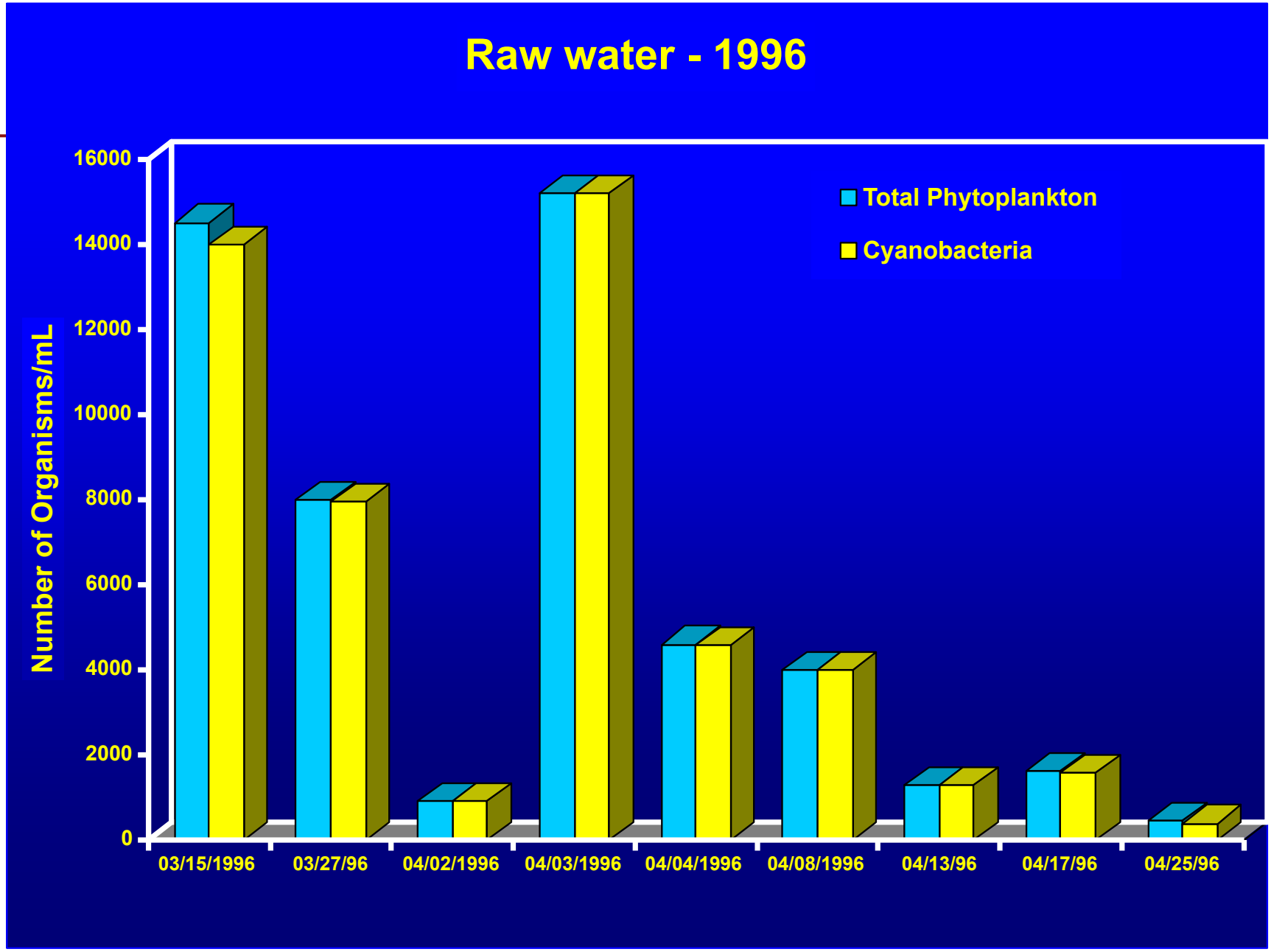
Water treatment system in dialysis clinic

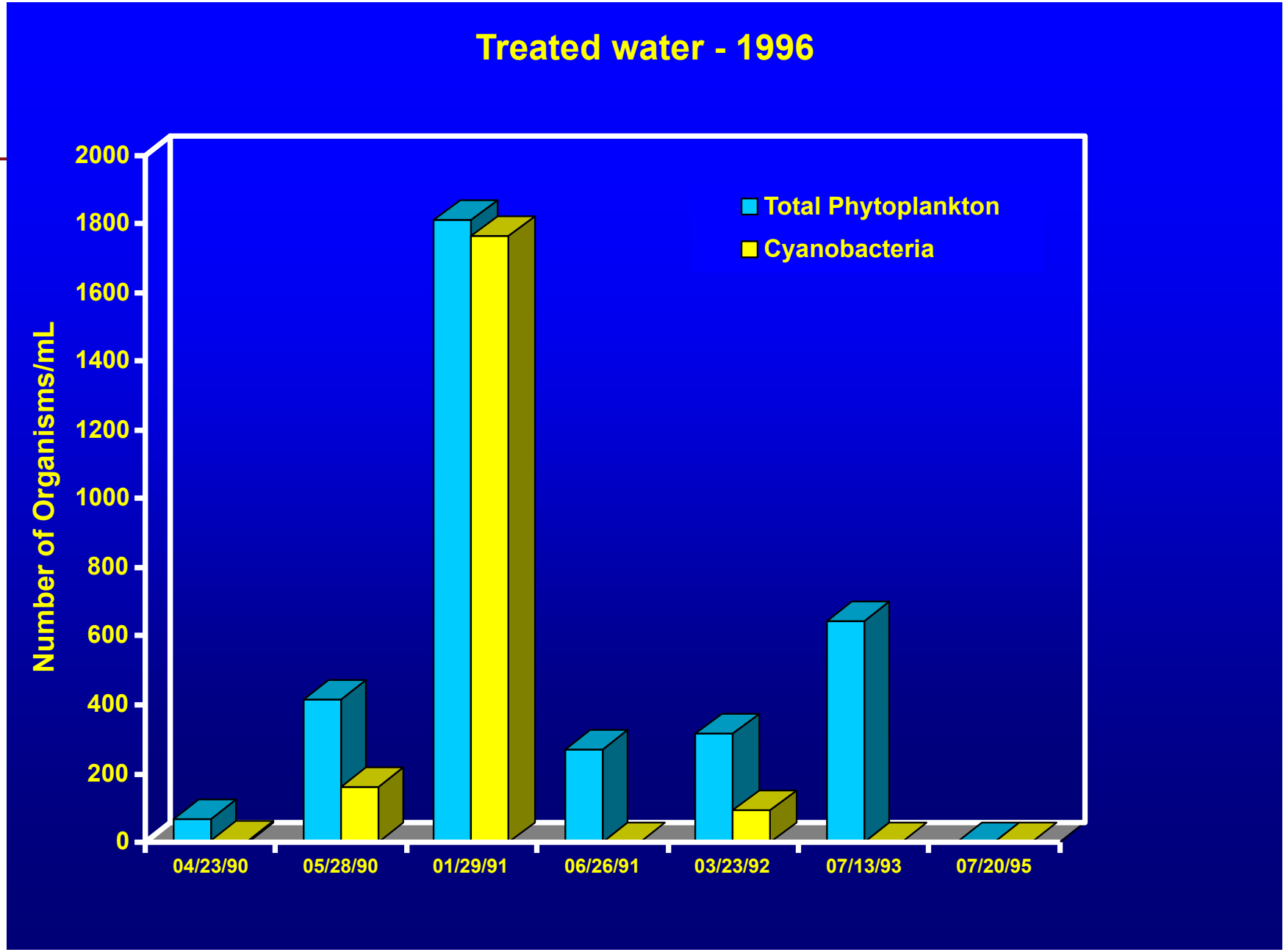
Heamodialysis room

Questions ??

1) Source of water to dialysis center
2) Overuse of dialyzer filters


Treated water - before 1996
Number of organisms/mL
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Total Phytoplankton
Cyanobacteria
04/23/90
05/28/90
01/29/91
06/26/91
03/23/92
07/13/93
07/20/95


Raw water - 1996
Number of Organisms/mL
16000
14000
12000
10000
8000
6000
4000
2000
0
Total Phytoplankton
Cyanobacteria
03/15/1996
03/27/96
04/02/1996
04/03/1996
04/04/1996
04/08/1996
04/13/96
04/17/96
04/25/96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Total Phytoplankton
Cyanobacteria
04/23/90
05/28/90
01/29/91
06/26/91
03/23/92
07/13/93
07/20/95

Phytoplankton Counts from Tabocas reservoir

Samples from March - 1996	Total Phytoplankton/mL	Cyanobacteria/mL (% of total phytoplankton)
Center	40,000	39,731 (99.3)
Margin	13,190	13,100 (99.3)
Raw water impounded to water treatment plant	21,420	20,882 (97.5)

Microcystin and Cylindrospermopsin content of carbon, ion exchange resin and sand filters from dialysis centers A and B in Caruaru, Brazil

Center/ Sample source	Microcystin (µg/g)	Cylindrospermopsin (µg/g)
Center A – carbon	1.0	19.70
Center A – anion resin	0.5	0.02
Center A – cation resin	2.1	0.05
Center A – sand	ND	ND
Center B – carbon	3.4	ND
Center B - sand	ND	ND

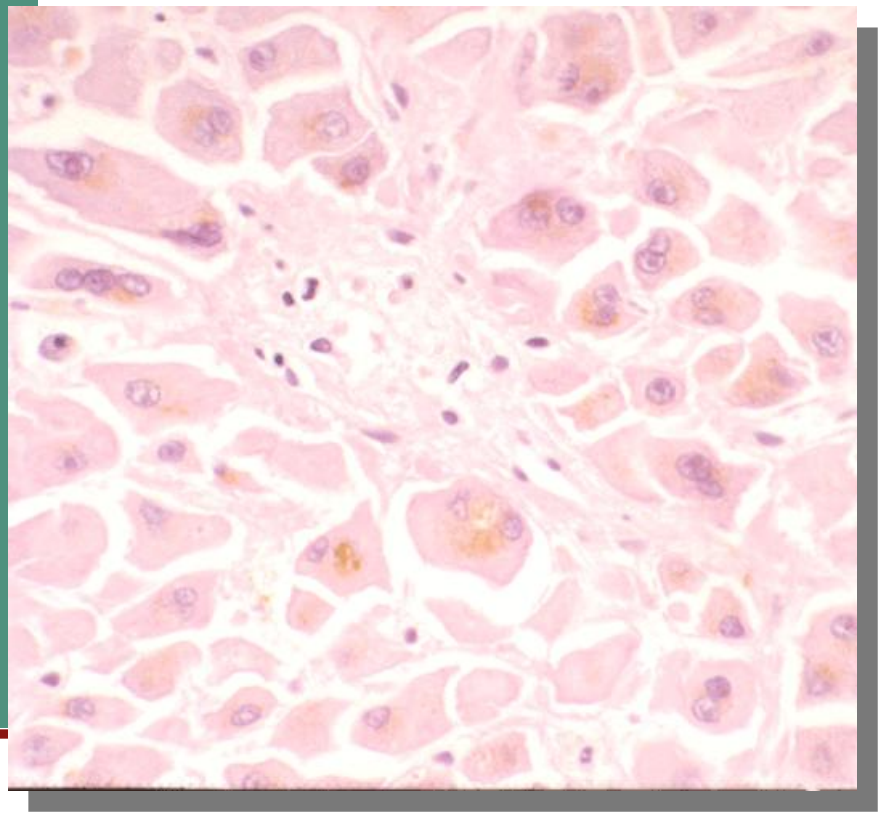
ND = not detected

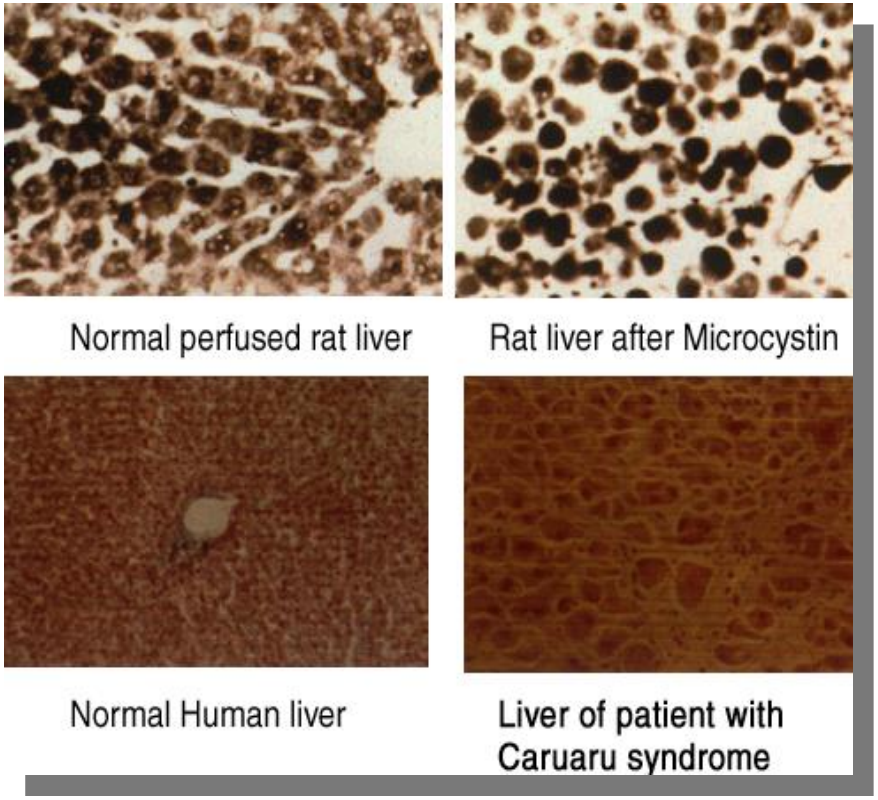
MICROCYSTIN VALUES OF LIVER AND SERUM FROM CARUARU PATIENTS

Month of Death (1996)	Number of victims/number of samples	Microcystin (ng/g)
February	4/7	257.7
March	11/14	255.4
April	8/12	179.6
May	3/4	272.5
July	1/1	471.8
August	4/6	216.8
September	3/3	219.9
October	2/2	50.2
December	1/1	160.4
Patients without Date of death	2/2	145.9
Average	38/52	223

Average of serum values	38/52	Microcystin 2.54 (ng/mL)

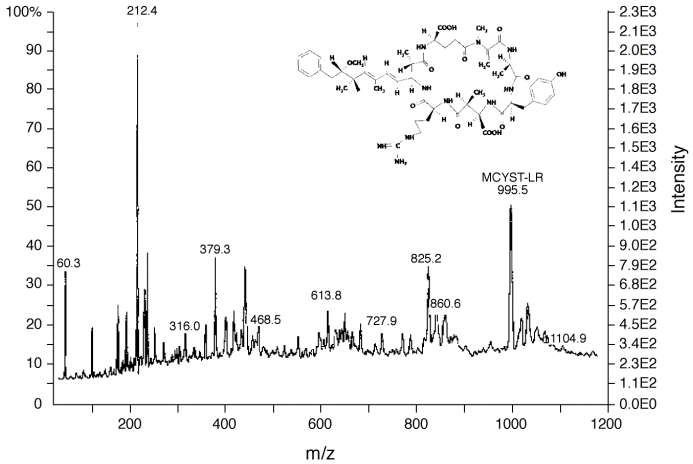
Histopathology

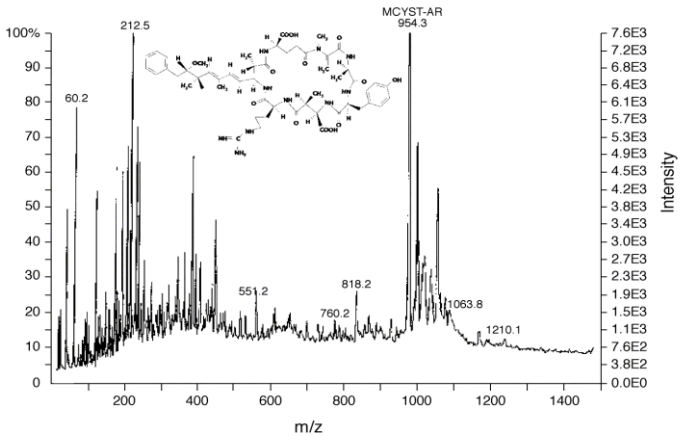


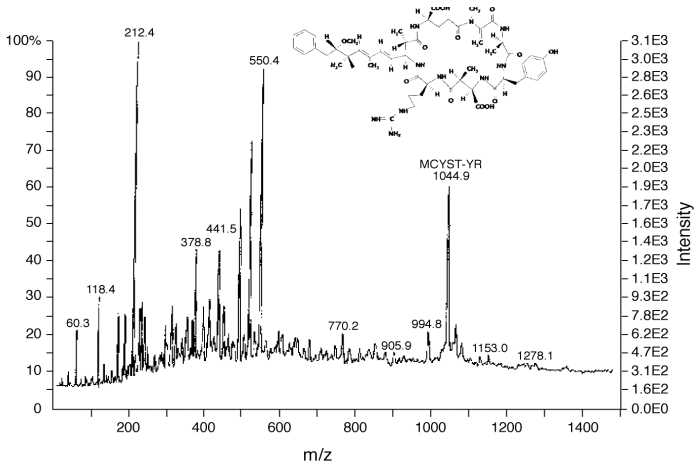


Disruption of liver plates, liver cell deformity, necrosis, apoptosis, cholestasis, cytoplasmic vacuolization, mixed leukocyte infiltration and multinucleated hepatocytes

MALDI/TOF-MS of liver extract from a Caruaru dialysis patient







Carmichael et al. 2001

Azevedo et al. 2002

LIVER FAILURE AND DEATH AFTER EXPOSURE TO MICROCYSTINS AT A HEMODIALYSIS CENTER IN BRAZIL

ELISE M. JOCHIMSEN, M.D., WAYNE W. CARMICHAEL, PH.D., JISI AN, M.SC., DENISE M. CARDO, M.D., PH.D., SUSAN T. COOKSON, M.D., CHRISTIANNE E.M. HOLMES, M.D., M. BERNADETE DE C. ANTUNES, M.D., DJALMA A. DE MELO FILHO, M.D., TEREZA M. LYRA, M.D., VICTORINO SPINELLI T. BARRETO, M.D., SANDRA M.F.O. AZEVEDO, PH.D., AND WILLIAM R. JARVIS, M.D.

Volume 338 Number 13 · 873

The New England Journal of Medicine

Human Fatalities from Cyanobacteria: Chemical and Biological Evidence for Cyanotoxins

Wayne W. Carmichael,[1] Sandra M.F.O. Azevedo,[2] Ji Si An,[1] Renato J. R. Molica,[3] Elise M. Jochimsen,[4] Sharon Lau,[5] Kenneth L. Rinehart,[5] Glen R. Shaw,[6] and Geoff K. Eaglesham[7]

Environ Health Perspect 109:663–668 (2001).

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Toxicology 181–182 (2002) 441–446

TOXICOLOGY

www.elsevier.com/locate/toxicol

Human intoxication by microcystins during renal dialysis treatment in Caruaru—Brazil

Sandra M.F.O. Azevedo [a,*], Wayne W. Carmichael [b], Elise M. Jochimsen [c], Kenneth L. Rinehart [d], Sharon Lau [d], Glen R. Shaw [e], Geoff K. Eaglesham [f]

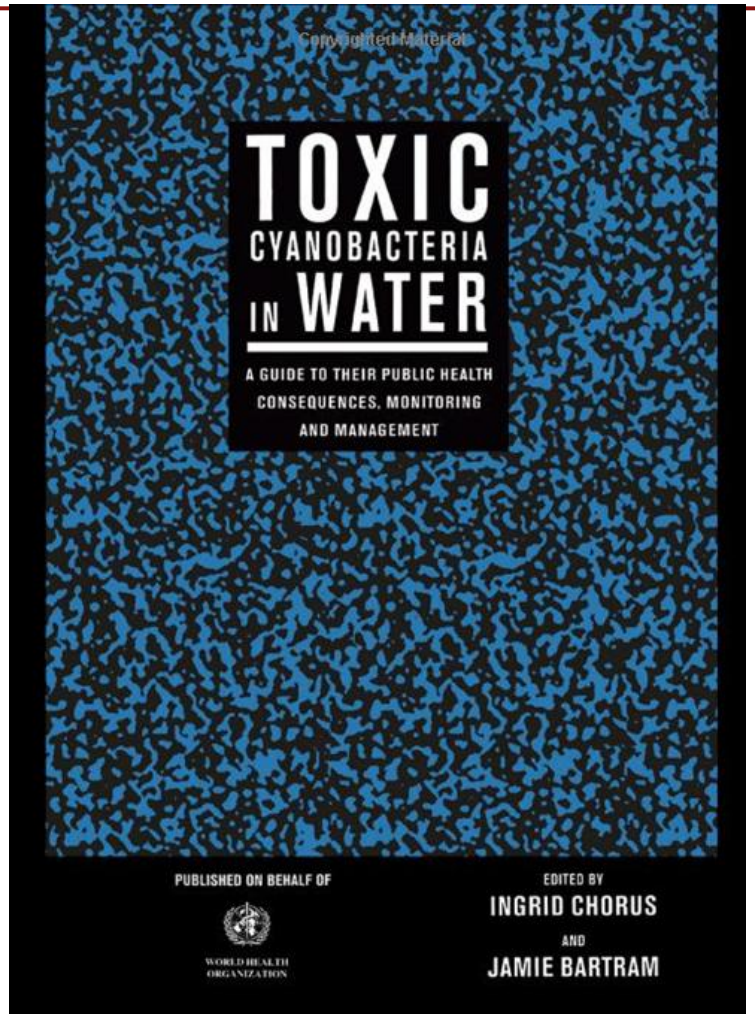
Algological Studies 103
(Cyanobacterial Research 2) | 9–29 | Stuttgart, November 2001

Background of the Caruaru tragedy; a case taxonomic study of toxic cyanobacteria

By Jiří Komárek[1, 4], Sandra M.F.O. Azevedo[2], Patricia Domingos[2], Jaroslava Komárková[1, 5] and Martin Tichý[3]

Description of cyanobacteria species found in Tabocas reservoir - phytoplankton samples from March to May 1996

- **Detection of several picoplanktonic species**
- **Description of a new specie – *Romeria caruaru***



MINISTÉRIO DA SAÚDE

PORTARIA N.° 1469, DE 29 DE DEZEMBRO DE 2000. (*)

Estabelece os procedimentos e responsabilidades relativos ao controle e vigilância da qualidade da água para consumo humano e seu padrão de potabilidade, e dá outras providências.

O Ministro de Estado da Saúde, no uso das atribuições que lhe confere o artigo 2º do Decreto nº 79.367, de 9 de março de 1977, resolve:

Art. 1º Aprovar a Norma de Qualidade da Água para Consumo Humano, na forma do Anexo desta Portaria, de uso obrigatório em todo território nacional.

Art. 2º Fica estabelecido o prazo máximo de 24 meses, contados a partir da publicação desta Portaria, para que as instituições ou órgãos aos quais esta Norma se aplica, promovam as adequações necessárias a seu cumprimento.

§ 1º No caso de tratamento por filtração de água para consumo humano suprida por manancial superficial e distribuída por meio de canalização e da obrigação do monitoramento de cianobactérias e cianotoxinas, este prazo é de até 36 meses.

§ 2º No período de transição deverão ser observadas as normas e o padrão estabelecidos na Portaria n.º 36/GM, de 19 de janeiro de 1990.

Art. 3º É de responsabilidade da União, dos estados, do Distrito Federal e dos municípios a adoção das medidas necessárias para o fiel cumprimento desta Portaria.

Art. 4º O Ministério da Saúde promoverá, por intermédio da Fundação Nacional de Saúde – FUNASA, a revisão da Norma de Qualidade da Água para Consumo Humano estabelecida nesta Portaria, no prazo de 5 anos ou a qualquer tempo, mediante solicitação devidamente justificada de órgãos governamentais ou não governamentais de reconhecida capacidade técnica nos setores objeto desta regulamentação.

Art. 5º Fica delegada competência ao Presidente da FUNASA para editar, quando necessário, normas regulamentadoras desta Portaria.

Art. 6º Esta Portaria entra em vigor na data de sua publicação.

JOSÉ SERRA

1

Since 2000: Brazilian regulations for drinking water quality include maximum values of cyanotoxins concentration acceptable in potable water

Microcystins – 1μg/L

Saxitoxins - 3μg/L

Cylindrospermopsin - 1μg/L

** Microcystin and saxitoxins concentration were included as a mandatory parameter.*

*** Cylindrospermopsin level was included as recommendation.*

Conclusions:

- ✓ **Examination of phytoplankton from the dialysis clinic's water source, analyses of the clinic's water treatment system, plus serum and liver tissue of clinic patients led to the identification of two groups of cyanobacterial toxins, the hepatotoxic cyclic peptide microcystins and the hepatotoxic alkaloid cylindrospermopsin. From liver concentrations and exposure volumes, it was estimated that 19.5 µg/L microcystin was in the water used for dialyses treatment. This is 19.5 times the level set as a guideline for safe drinking water supplies by the World Health Organization.**

- ✓ **Since many of the world's water supplies are subject to increasing levels of nutrients, it is highly probable that repeat episodes of cyanotoxin poisoning will occur unless measures control these eutrophication process are taken;**

- ✓ **These measures should consist of programs for watershed management to reduce nutrient inputs, cyanotoxin monitoring programs to alert authorities to the presence of cyanotoxins, and improvements in water treatment techniques to reduce or to remove cyanotoxins from finished water supplies.**

Acknowledgments:

- ✓ **Health authorities from Pernambuco State for assistance with epidemiology, data basing, specimen collection and shipment;**
- ✓ **Dr. F. S. Chu for helping to confirm ELISA analysis of serum;**
- ✓ **Dr. Victorino S. Barreto for histopathology assistance and offer slides;**
- ✓ **Dr. Renato J.R. Molica and JiSi An for assistance with chemical and ELISA analysis.**

Thanks for your attention!