Government of South Australia

SA Water

Australian Water Quality Centre

Cylindrospermopsin: Genotoxin and Potential Carcinogen

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Background - Occurrence

Palm Island, Australia - 1979

- Cyanobacterial bloom in water supply reservoir, treated with $CuSO_4$
- Cells lysed, toxin released, 140 people hospitalised
- Standard water treatment in place (coagulation, filtration, chlorination)
- *C. raciborskii* isolated from reservoir, cylindrospermopsin (CYN) eventually purified and characterised (1992)
- Effects in mice similar to those in Palm Island victims

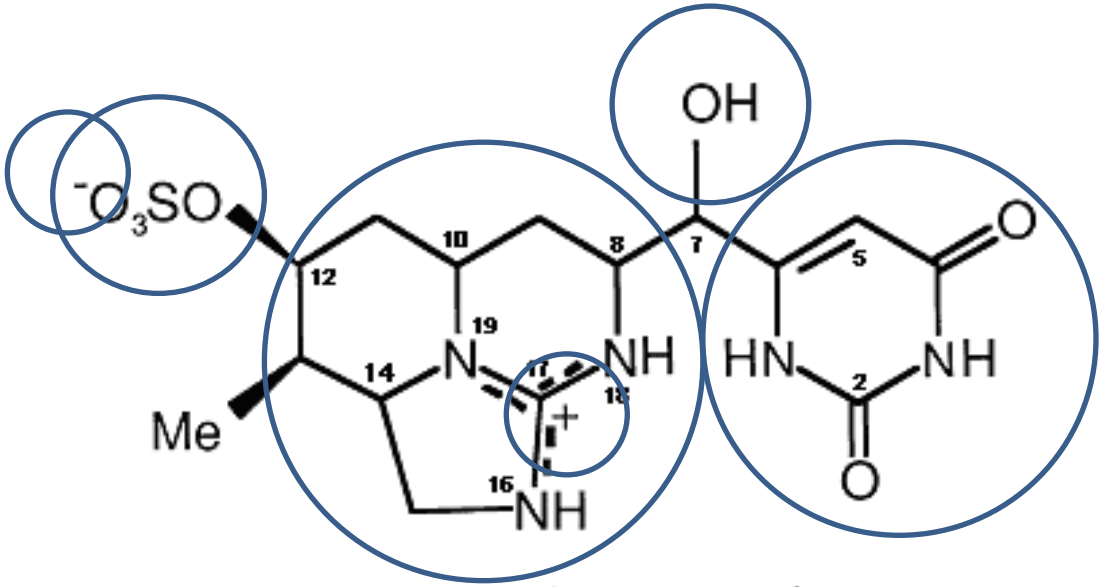
- Later found to be widespread in surface sources of drinking water

Background - Occurrence

US/World

- *C. raciborskii* &/or CYN identified in various States in US
- Both cells and CYN identified in Florida treated drinking water
- CYN as well as microcystins present in dialysis waters that killed 80 patients in Caruaru, Brazil
- *C. raciborskii* and other CYN producers considered invasive in Europe
- At least 6 other species now known producers of CYN

Background – Toxin Structure



Cylindrospermopsin

- Zwitterionic – highly water soluble
- Hydroxyl group epimeric or not present: 3 congeners
- Sulfate and guanidino reactive
- Uracil may mimic uranyl nucleotides or intercalate into DNA/RNA

CYN Toxicity – General Effects

Organs/Functions Affected

- Liver, kidneys, heart, lungs, thymus, spleen, vasculature/blood clotting, immunotoxicity, reproductive toxicity, genotoxicity
- Evidence for cumulative toxicity
- Evidence for carcinogenicity

Modes of Action

- Protein Synthesis Inhibition (parent compound)
- CYP450-mediated toxicity (toxic &/or reactive metabolites)
- Generation of Reactive Oxygen Species & reduced GSH synthesis (ROS defence)
- Cell death: Apoptosis at low concs, necrosis at higher concs

CYN Toxicity – General Effects

Gene expression studies (in vitro and in vivo, microarray and RT-PCR)

- Induction of genes involved in:
  - DNA repair and induction of apoptosis (p53-dependent)
  - Xenobiotic metabolism (CYP1A1, CYP1A2, CYP2A4)
  - Oxidative stress response (Nrf2-dependent)
  - Ribosomal proteins

- Down regulation of genes involved in:
  - Xenobiotic metabolism (most other families)
  - Blood coagulation

CYN Genotoxicity – damage to genes & chromosomes

Numerous studies show CYN-induced DNA/chromosomal damage (in vitro and in vivo)

- Induction of micronuclei
- Induction of DNA strand breaks
- DNA adduct formation
- Loss of whole chromosomes
- Chromosomal rearrangements
- Gene amplification

CYN Genotoxicity – damage to genes & chromosomes

Most of these effects likely involve metabolism of CYN by CYP450

- DNA strand breakage & micronucleus formation blocked by CYP450 inhibitors (SKF525A, omeprazole, ketoconazole)
- Specific CYP genes up regulated

Oxidative stress may be involved in causing some DNA damage but levels induced are not high enough to account for CYN's genotoxic potency

- Implies involvement of CYN metabolites

Some chromosomal effects may be secondary to protein synthesis inhibition by parent compound (chromatin & microtubule dysfunction)

Multiple genotoxicity modes of action may mean multiple mechanisms

CYN Genotoxicity – damage to genes & chromosomes

Most genotoxicity assays have shown effects in the range 0.1 – 10 µM, sensitivity likely dependant on the cell type's metabolic capability

However, SHE cell morphological transformation to a cancer-forming morphotype was induced by 0.24 pM CYN (ie 10^{-12} mole/L!)

- Note: this was the lowest concentration tested

CYN Carcinogenicity– induction of tumors in vivo

Only one study so far

- 2.3 or 8.3 mg CYN/kg mouse given orally up to 3 times 2 weeks apart. Most then given tumor promoter in food for 30 weeks
- 5 of 53 treated animals had evidence of carcinogenicity
- 0 of 27 control animals had evidence of carcinogenicity
- Fisher's Exact Test p = 0.16, RR = 6.2 (95% CI: 0.33 – 117)

- Hence results suggestive but not conclusive

- Need to treat 100 animals to confirm RR of 10 or 200 animals to confirm RR of 5

- Note: the standard carcinogenicity study protocol requires continuous exposure for 2 years – requires much more CYN

CYN Carcinogenicity– importance of proof

Neither WHO or US EPA have guidelines /MCLs for CYN in drinking water

Australian Drinking Water Guidelines include a "Health Alert Level" for CYN of 1 µg/L (2 nM) based on sub-chronic toxicity NOAEL (Humpage & Falconer 2003)

A NOAEL assumes a threshold below which an exposure to a toxin is safe

The risk assessment for genotoxic carcinogens usually assumes that no exposure is safe – calculates a concentration for an acceptable level of risk

CYN is *genotoxic* but need to determine if CYN is *carcinogenic* in vivo and at what concentration

MCL: Maximum Contaminant Level – legally enforceable regulatory limit
NOAEL: No Observed Adverse Effect Level

Public Health Significance

CYN (and other cyanotoxins) can, on occasions, breach normal drinking water treatment (low nM concs, lower concs generally not measurable)

CYN can co-occur with other cyanotoxins including microcystins

CYN caused transformation to carcinogenic morphology in SHE cells at sub-pM concentrations

Microcystins may block DNA-damage induced cell death (apoptosis) at pM concentrations

Hence, potential for synergism between these toxins at concentrations below current guideline values (even allowing for ~100-fold lower potency via oral route)

Current effort towards CYN carcinogenicity study

Cyanotoxins have been on the CCL since 1998

2001 US EPA expert group prioritised microcystins, cylindrospermopsin and anatoxin-a as highest priority cyanotoxins

Recent WHO meeting on Guidelines for Drinking Water Quality endorsed the need for a carcinogenicity study on cylindrospermopsin

Major inhibitor of progress has been availability of adequate toxin

Ingrid Chorus (Europe, WHO): seeking funding for CYN production

US EPA: in negotiations to conduct the carcinogenicity study

Extremely important that this effort is supported so that the public health risk from CYN can be quantified and appropriate drinking water standards established

Questions?

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