United States Environmental Protection Agency

Environmental forensic research for emerging contaminants in complex environmental matrices.

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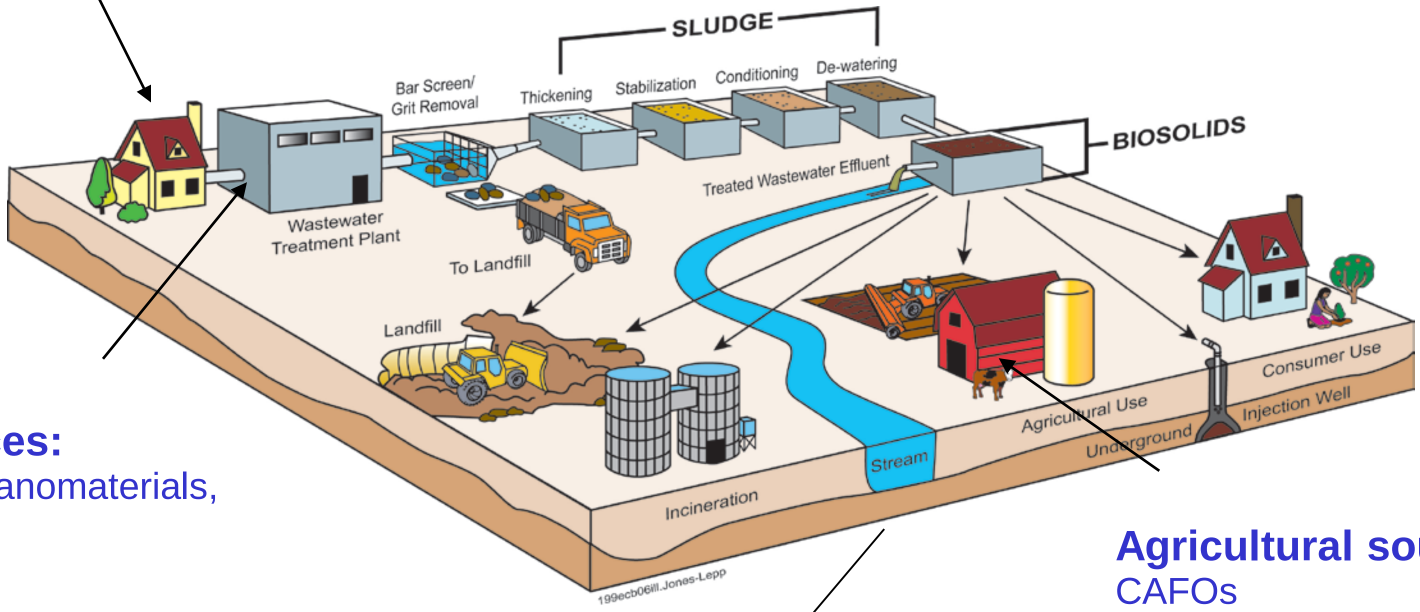
US EPA ORD National Exposure Research Laboratory – Las Vegas
Research Analytical Support
1970's – 2010's

NewJersey TomsRiver NewYork schools chickens SouthCarolina Pennsylvania FBI LoveCanal chemicalspills ExxonValdez oilspills organoarsenics dyes-spies ThreeMileIsland CIA SouthAfrica MetroPolice FDA organotins PCBs TimesBeach

Possible sources of emerging contaminants

Consumers: antibiotics, illicit drugs, nanomaterials in personal care products (e.g., sunscreens, sports wear), detergents (fluorescent brighteners), PVC pipe (organotins), surfactants (i.e., NPEOs, APEOs),



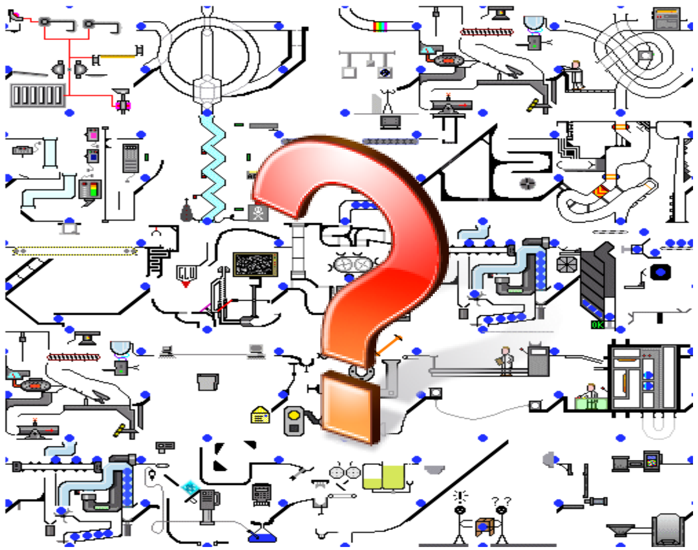
Industrial sources:
pharmaceuticals, nanomaterials, surfactants

Agricultural sources: farming, CAFOs

Naturally occurring: harmful algal bloom (HAB) toxins

Analysis and Identification of non-targeted emerging contaminants

Challenge – Identify unknown primary constituents in environmental samples



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Processes

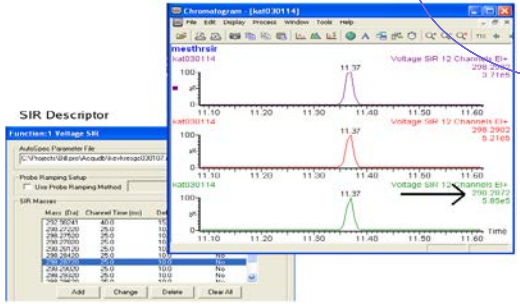
Water – source, wastewater
Sediments
Plant and fish tissues
Biosolids
Unknown chemical mixtures

Sampling

Sample processing

Analysis

Data interpretation



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Sample Processing

Solid

Solid/liquid manual extraction

Accelerated solvent extraction

pH adjustments or neutral

Extracts

Aqueous

pH < 3

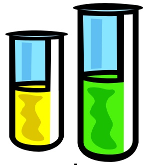
pH > 9

Solid phase extraction

Or neutrual

Solid phase extraction

Extracts



High resolution/accurate mass spectrometry screening

Polar: LC/MS

non-polar: GC/MS

Sample Analysis

MS/MS => fragmentation ions = identification

The steps to discovering non-targeted emerging contaminants

Initial Confirmation

- Use available software, mass spectral libraries and on-line databases (i.e., ChemSpider, NIST) to initially identify unknowns
- Mass spectral interpretation experts review the data

Final steps

- If possible procure standards for confirmation.
  - Multiple standards needed for isobaric ion confirmations
- Re-analyze to confirm initial identifications.

Case Studies

1. Gulf Oil spill – Deepwater Horizon Incident
2. Oklahoma Red River Fish Kills
3. Colorado River Basin Watershed
4. Lower Colorado River Basin Harmful Algal blooms

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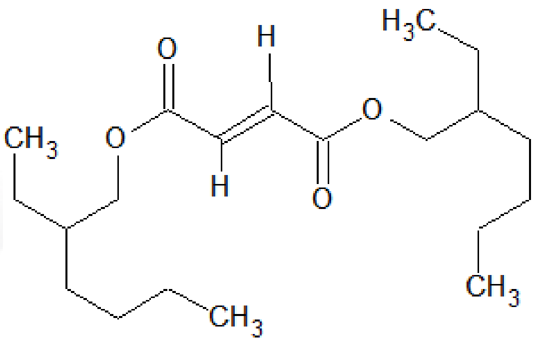
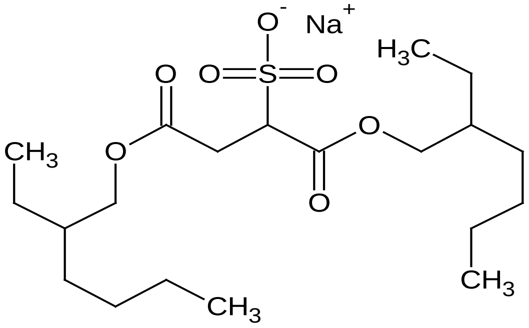
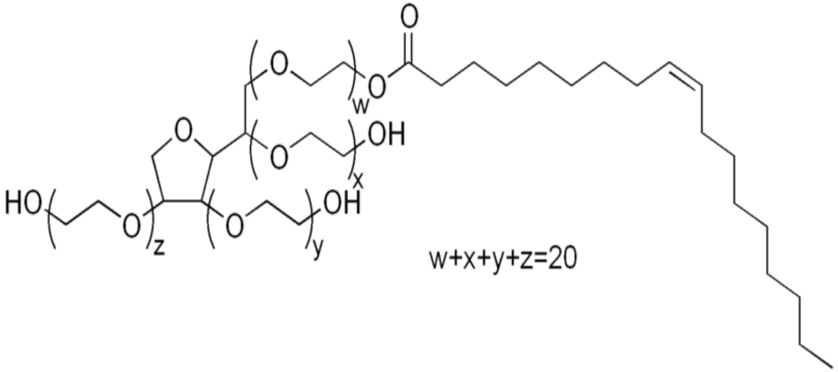
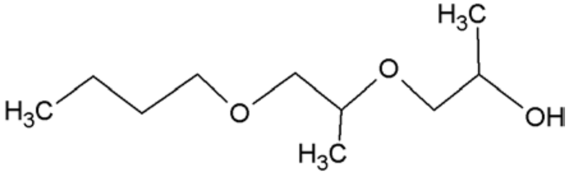
Case Study
Gulf Oil Spill – Deepwater Horizon Incident

Chemical Analysis of Dispersant(s)









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Case Study
Gulf Oil Spill – Deepwater Horizon Incident

Corexit 9500 was made up of a mixture of many different chemicals and chemical classes

Volatiles - Semi-Volatiles -Non-Volatiles

The major ingredients were: Solvents & Surfactants

with many impurities present

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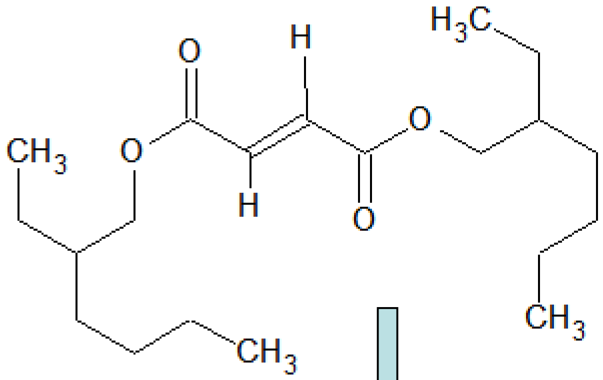
Case Study
Gulf Oil Spill – Deepwater Horizon Incident

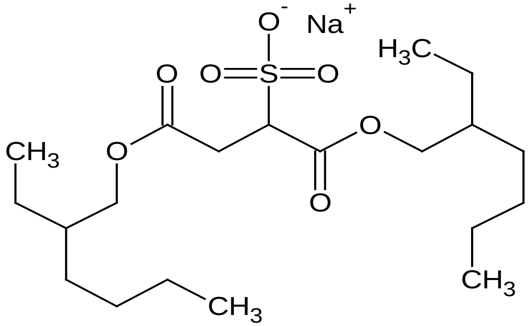
Chemical Analysis of:
1) Dispersant(s)
2) Dispersants in Seawater and Mousse
3) Toxicity well plates



Bis(2-ethylhexyl)fumarate

Discovered by GC/MS - semivolatile

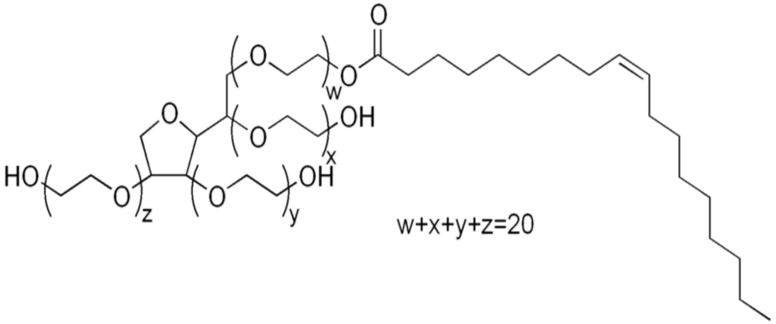
2x DOSS concentration



Dioctyl sodium sulfosuccinate

DOSS

Discovered by LC/MS
- non-volatile



Ethoxylated sorbitan trioleates

Discovered by LC/MS
- non-volatile

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Gulf Oil Spill – Deepwater Horizon Incident

Published list of Corexit 9500 ingredients

CAS #	Ingredient
57-55-6	1,2-Propanediol
577-11-7	Di(2-ethylhexyl) sodium sulfosuccinate (DOSS)
	Mon- and Tri- ethoxylated oleates
	Mon- and Tri- oleates
29911-28-2	2 Propanol, 1-(2-butoxy-1-methylethoxy)-
64742-47-8	Distillates (petroleum), hydrotreated light

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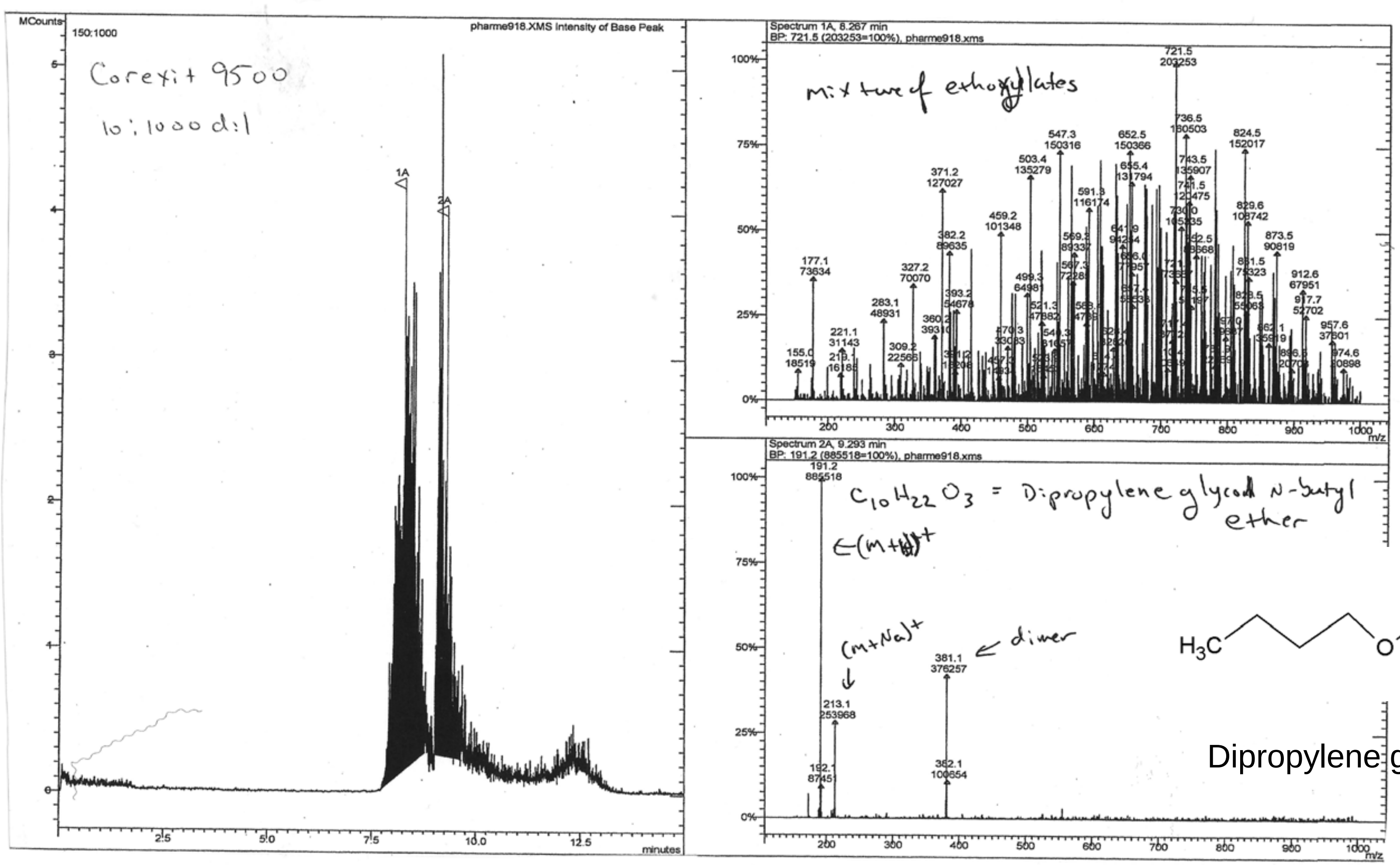
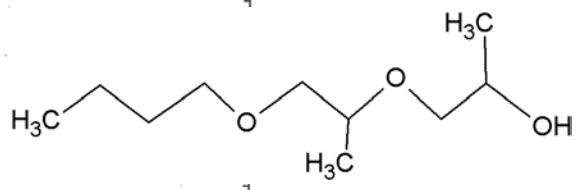
Identifying major non-volatile polar components in Corexit 9500 by LC-MS/MS





Dipropylene glycol n-butyl ether

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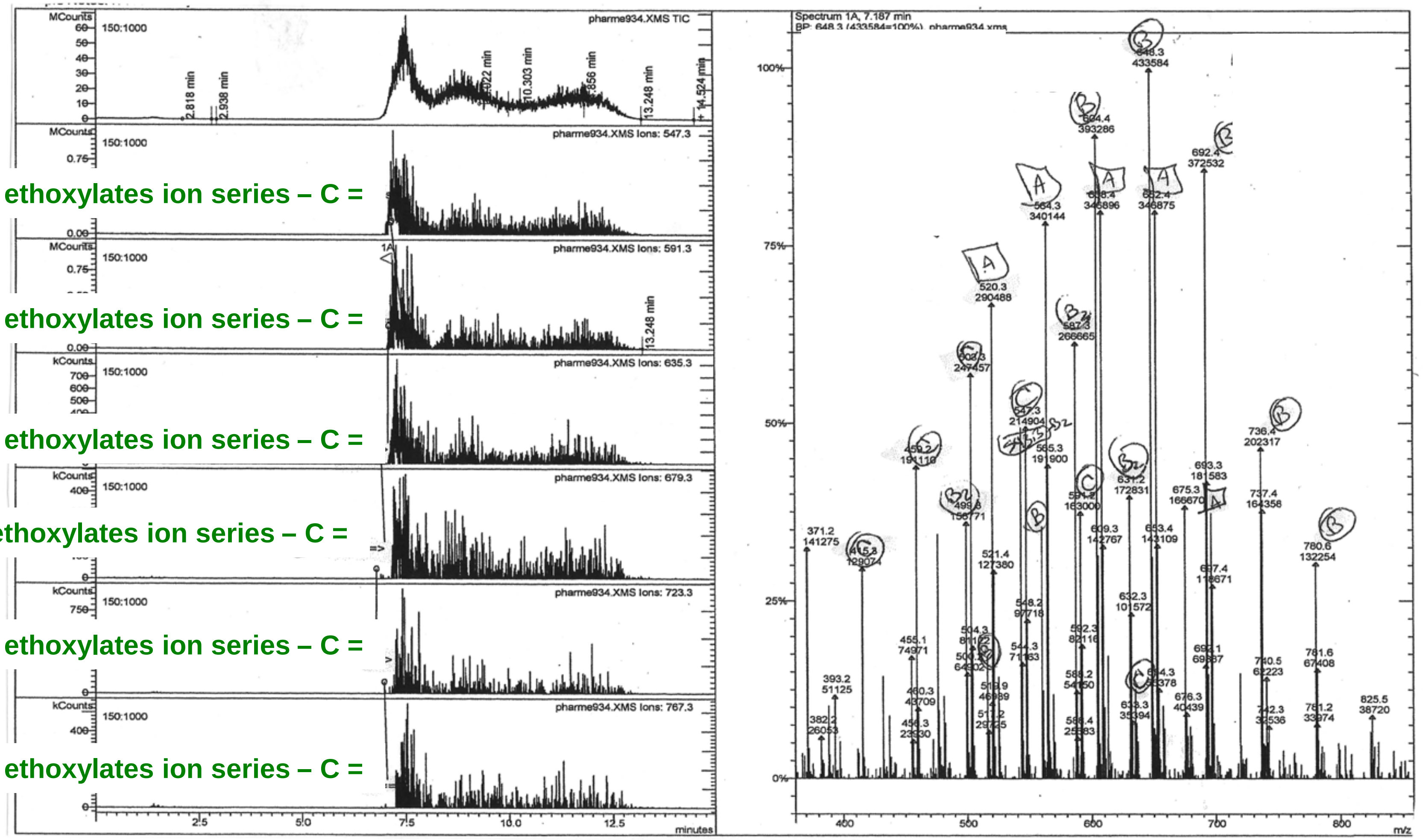
Gulf Oil Spill – Deepwater Horizon Incident

Contaminants in Corexit 9500: Nonylphenol ethoxylates and C_6EO_6



Nonylphenol ethoxylates ion series – C =

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Nonylphenol ethoxylates ion series – C =

A = $M+Na^+$
B = M+NH3
B2 = M+H
M= C_6EO_6
C = nonylphenol ethoxylates

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Gulf Oil Spill – Deepwater Horizon Incident

Other uses common Corexit constituents

- Polysorbate 80 – Food use: food emulsifier - particularly in ice cream. (courtesy of Wiki)
- Polysorbate 60 – Food use: food emulsifier – used in powdered drink preparations - hot cocoa mix (courtesy of Wiki)
- Dioctyl sodium sulfosuccinate (DOSS)
  - Common ingredient in consumer products, especially **laxatives** of the stool softener type, facilitates removal of excess earwax. (courtesy of Wiki)
  - Also useful for cleaning and peeling fruits and vegetables and cleaning food packaging. It is also used in various pharmaceutical products. (USFDA website)

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Gulf Oil Spill – Deepwater Horizon Incident

Other Corexit constituents

Dipropylene glycol n-butyl ether

- commonly used in surface coatings pesticides, industrial cleaners, resins, and chemical intermediates.
- Adverse effects: CNS solvent syndrome

Nonylphenol ethoxylates – impurity in Corexit

- Used in surfactants (i.e., laundry, dish detergents).
- Adverse effects: Breakdown in the environment to “nonylphenol”, which has the potential as endocrine disruptor and xenoestrogen.
- September 25, 2014 – EPA proposed a Significant New Use Rule to require Agency review before a manufacturer starts or resumes use of 15 nonylphenols (NPs) and nonylphenol ethoxylates (NPEs). This SNUR, when finalized, will provide EPA the opportunity to review and evaluate any intended new or resumed uses of these chemicals and, if necessary, take action to limit those uses. The public comment period for this proposal closed on January 15, 2015.

Bis(2-ethylhexyl) fumarate – unexpected contaminant – Starting material used to make the main ingredient DOSS

- Severe eye and skin irritant. Poison by intraperitoneal route. Combustible when exposed to heat/flame, reacts with oxidizers.

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Gulf Oil Spill – Deepwater Horizon Incident

Method Development and Application to Determine Dispersants in Seawater and Mousse

1. Focus was on DOSS, dipropylene glycol n-butyl ether, and nonylphenol ethoxylates from dispersant that were identified in the initial analytical phase.

2. Previously developed in-house extraction/analytical techniques for biosolids were modified for these compounds in these two matrices.

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Dispersant Toxicity Testing

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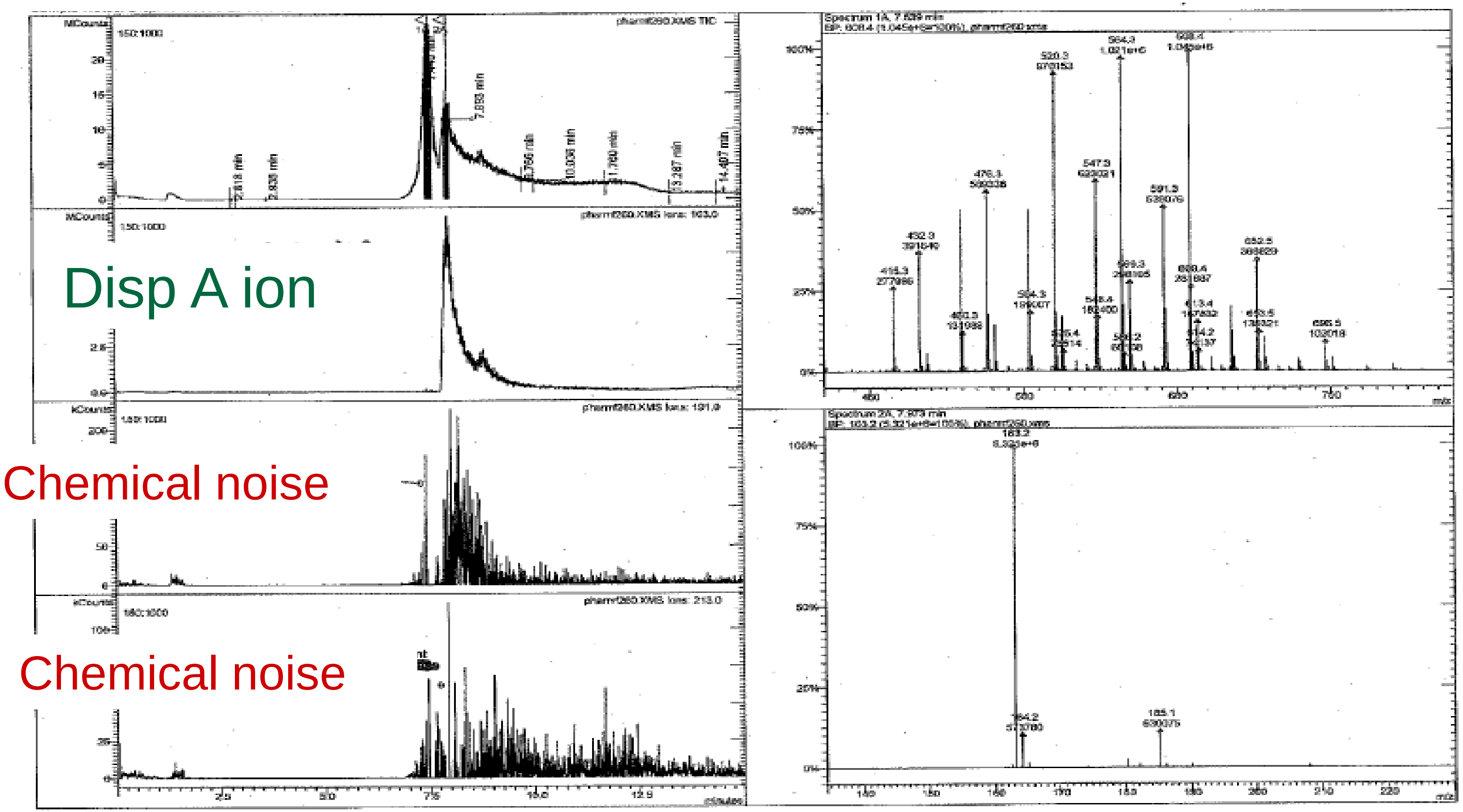
Gulf Oil Spill – Deepwater Horizon Incident

LC-MS Dispersant A

1:1000 dil



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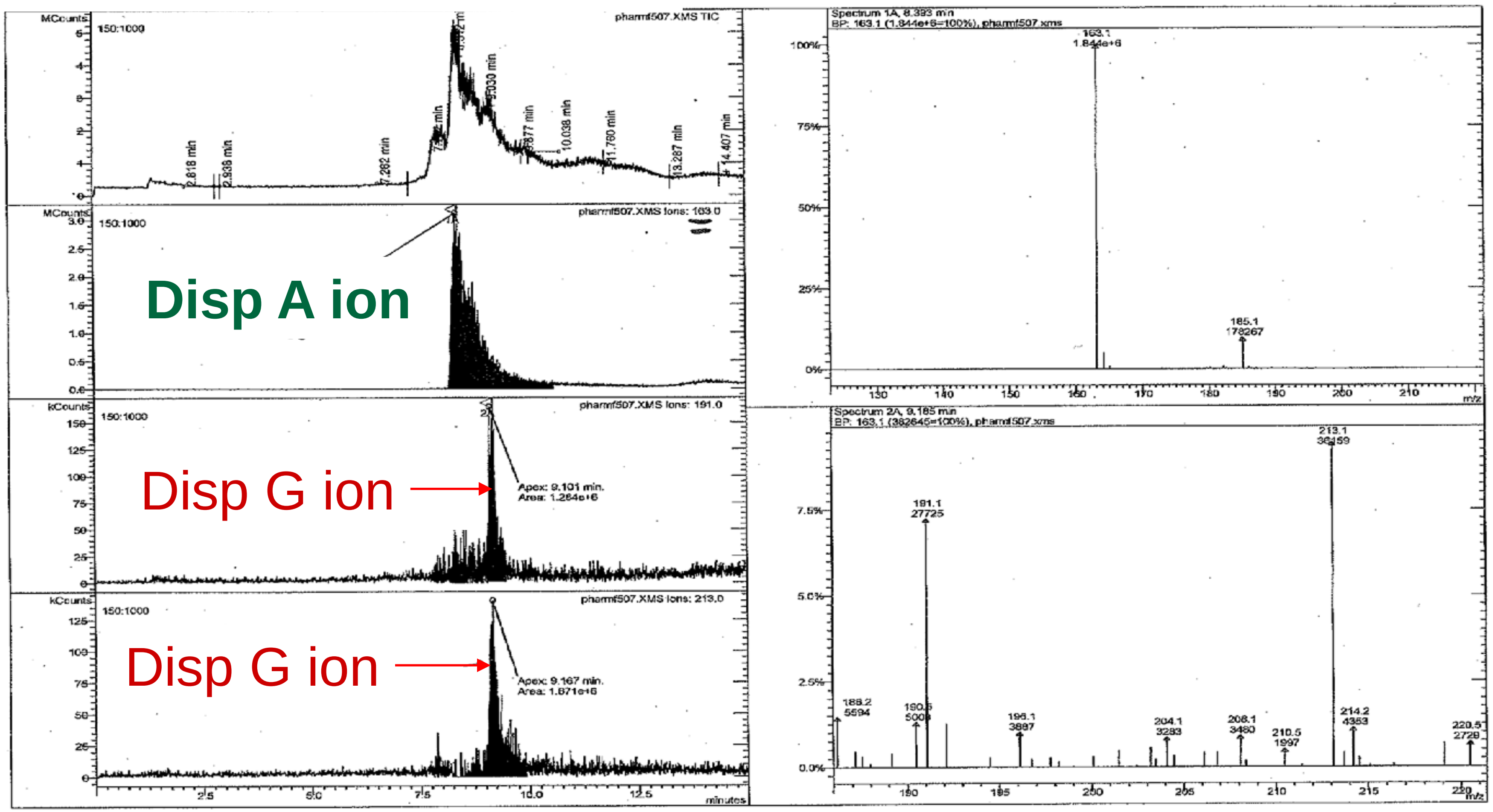
QA for Dispersant Toxicity Testing cont.

Print Date: 24 Jul 2010 14:07:32

LC-MS of Disp A well plate



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Gulf Oil Spill – Deepwater Horizon Incident

Summary

Chemical Analysis of:

1) Dispersant(s)

- Impurities were detected in the Dispersant
- Starting materials were 2x final product
- NPEOs were detected

2) Dispersants in Seawater and Mousse

- No dispersants were detected above LOD in samples

3) Toxicity well plates

- Contamination was found in toxicity testing well plates

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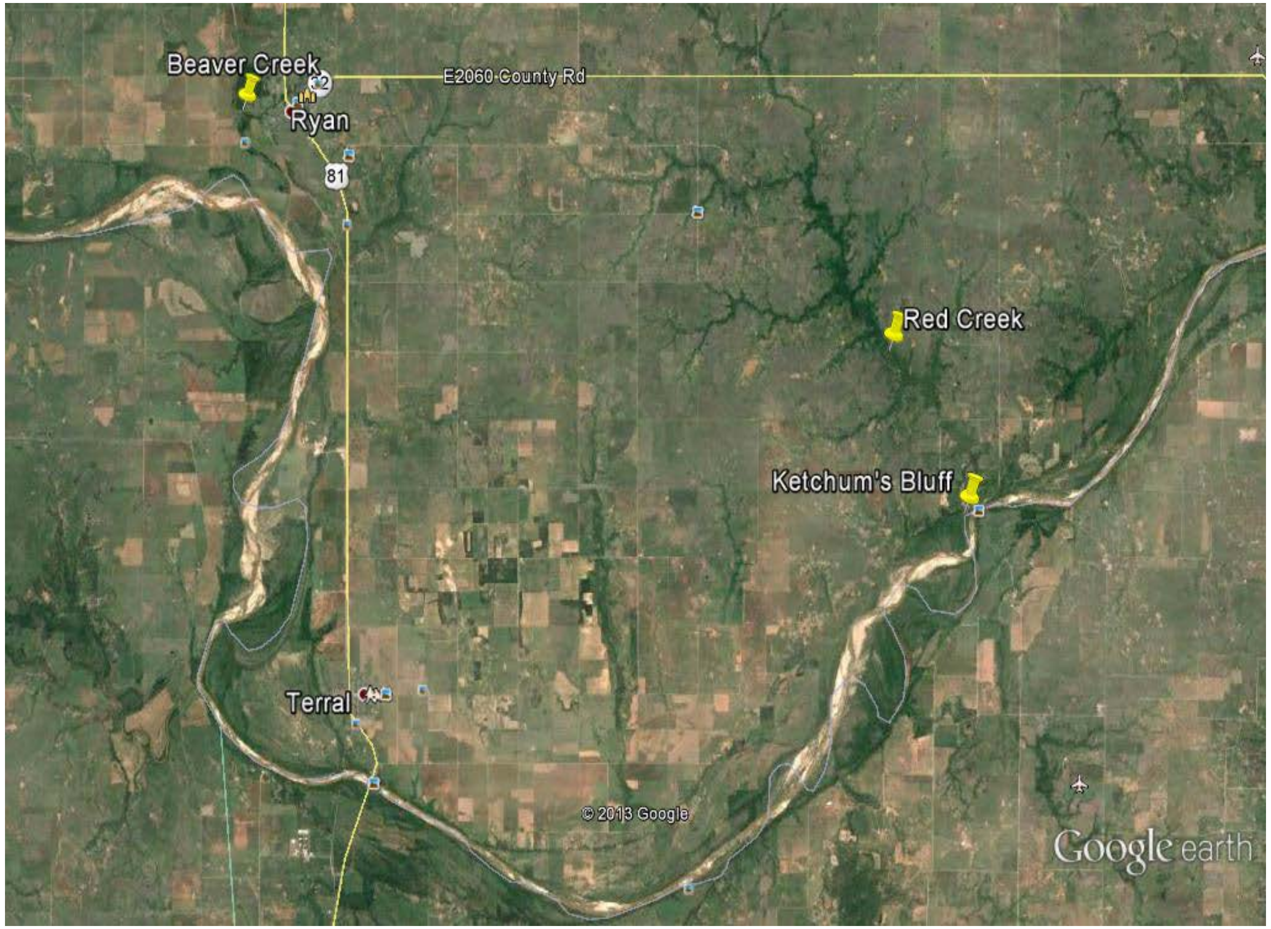
Case Study
Oklahoma Red River Fish Kill



Case Study
Oklahoma Red River Fish Kill

Background

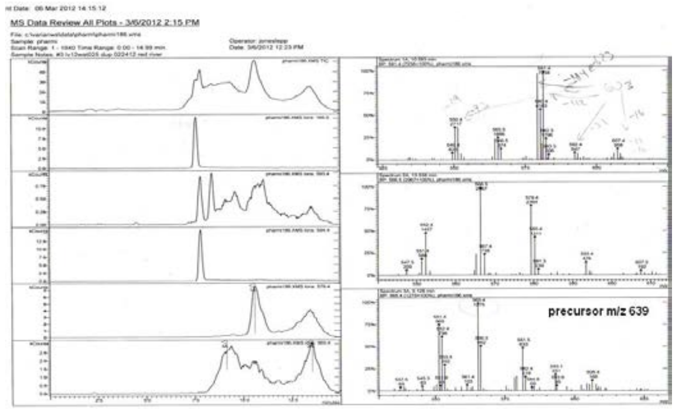
- Adjacent to Ketchum's Bluff on the Red River, OK was the site of three fish kills: July 2011, September 2011, and June 2012. In January 2013, the fourth fish kill, occurred a few km upstream on the Red River, along a minor creek – with a shared watershed.

- ORD (NERL-ESD/ECB) provided laboratory support for screening water samples for unknown toxins from fish kill event(s).

Case Study
Oklahoma Red River Fish Kill

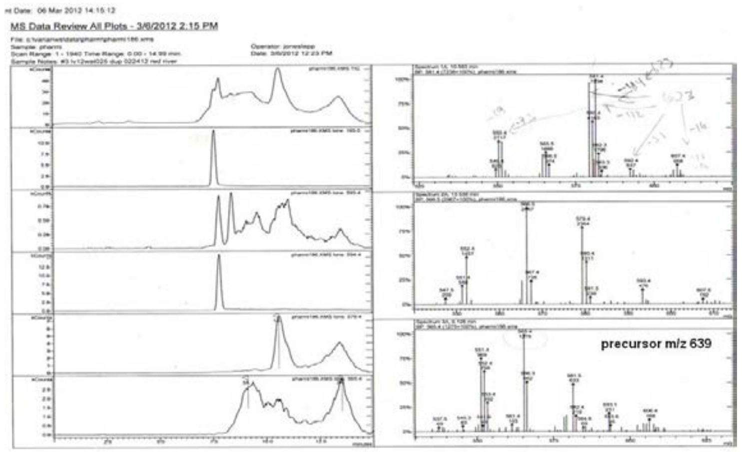
Identifying unknowns from waters taken during active fish kills

Fish kill IV Jan 2013

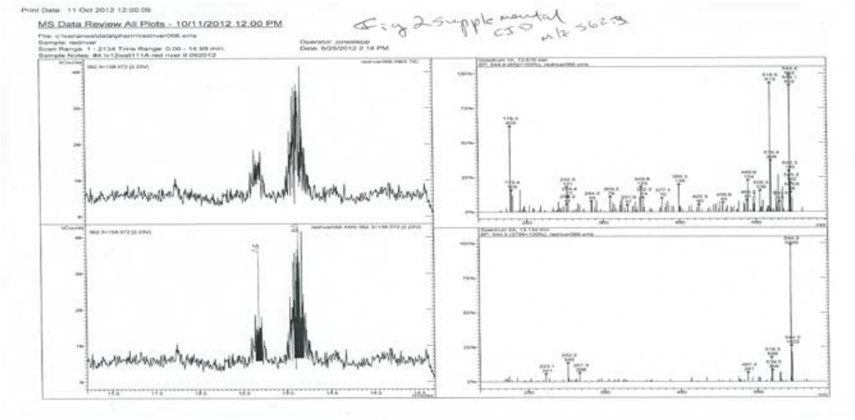


MS/MS spectra from three fish kills

Fish kill II Sept 2011

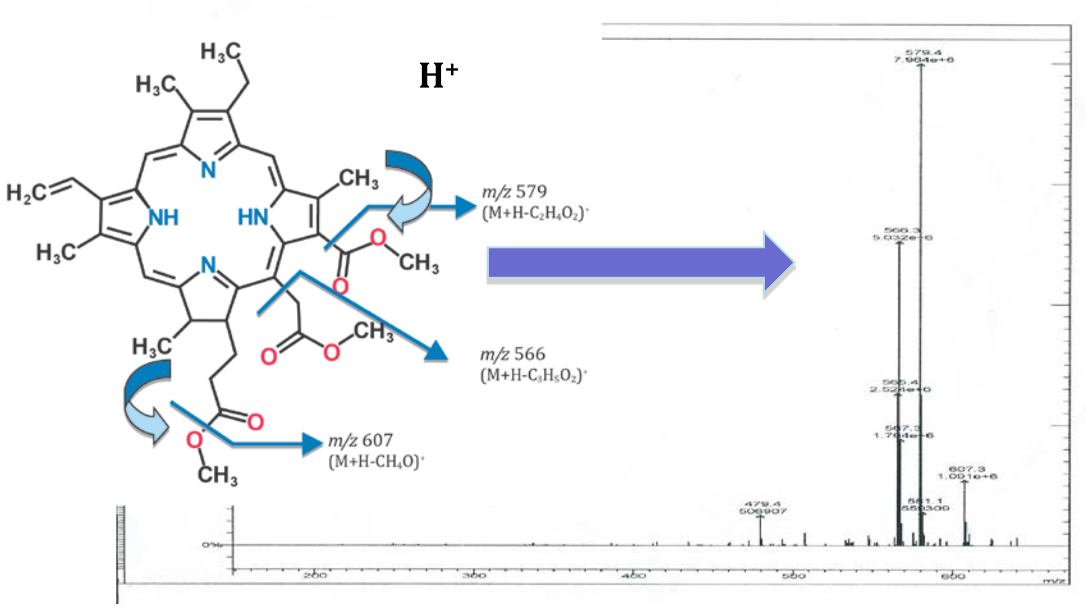


Fish kill III June 2012

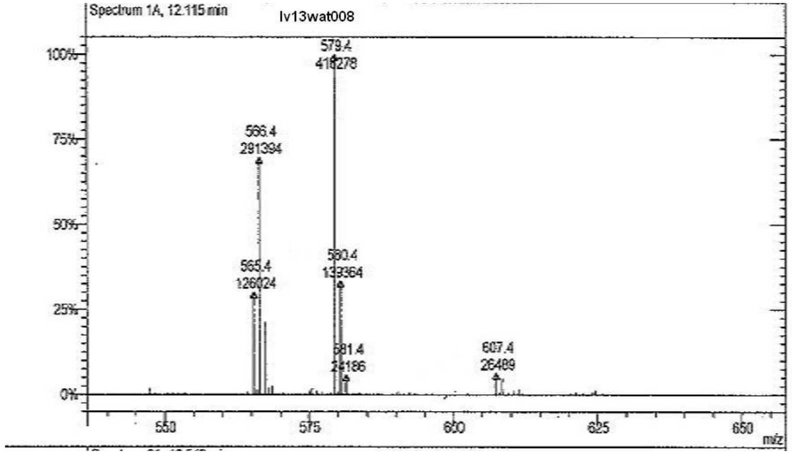


Case Study
Oklahoma Red River Fish Kill

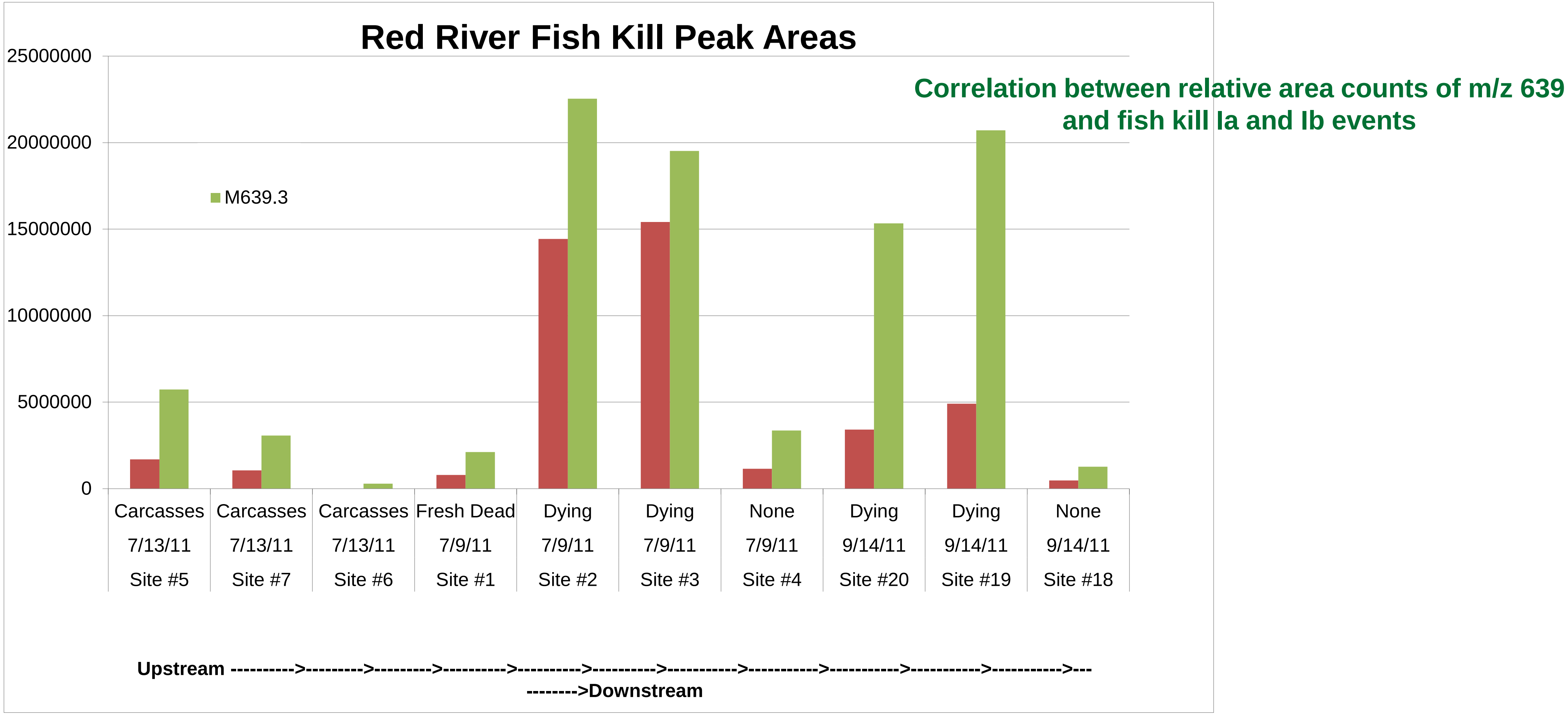
CID MS/MS LC-ITMS: Chlorin-e6-trimethyl ester standard, *m/z* 639.3 $(M+H)^+$



CID MS/MS LC-ITMS Unknown *m/z* 639.3 $(M+H)^+$ in fish kill water sample



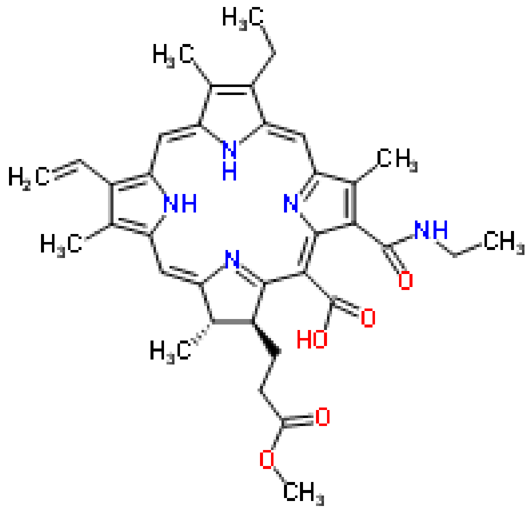
Case Study
Oklahoma Red River Fish Kill



Graph courtesy of Oklahoma Department of Environmental Quality

Case Study
Oklahoma Red River Fish Kill

Summary



•Compound detected at *m/z* 639.3 is chlorin e6 trimethyl ester

•Compound detected at *m/z* 624.3 is related but one of side groups replaced by an amide

•The unknown at *m/z* 826.7 $M^{+\cdot}$ [*m/z* 413.4 (M^{+2})] in fish kill IV is a diquaternary ammonium salt = $C_{46}H_{94}N_6O_6$ – class of chemicals are known to be toxic to aquatic organisms

•The other unknowns detected in fish kill III, are still unknown, but due to MS/MS product ions are probably another porphyrin series.

Tracing Sources of Emerging Contaminants in the Colorado River Basin

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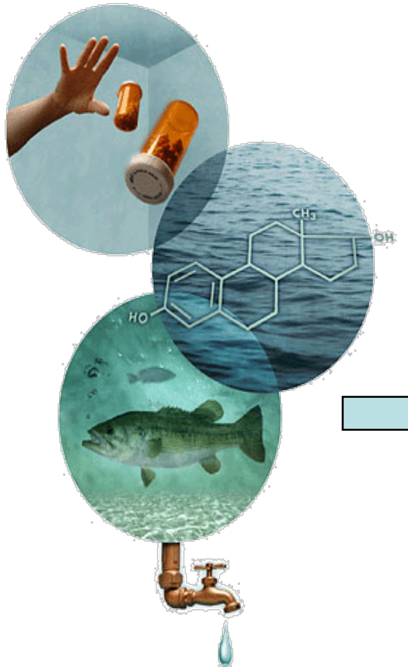
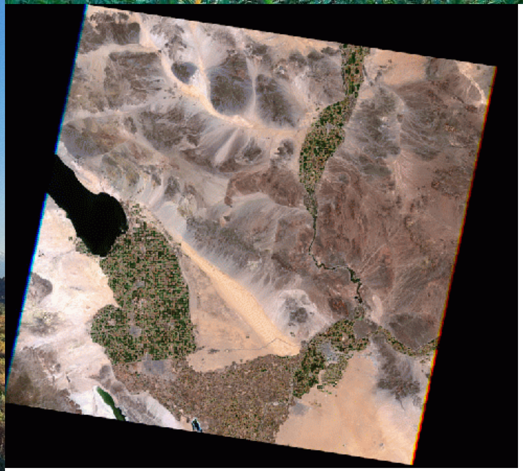


Glenwood Springs WWTP

Satellite view courtesy of GOOGLE maps



Satellite view courtesy of NASA landsat image

Yuma - Colorado River & New River – Salton Sea

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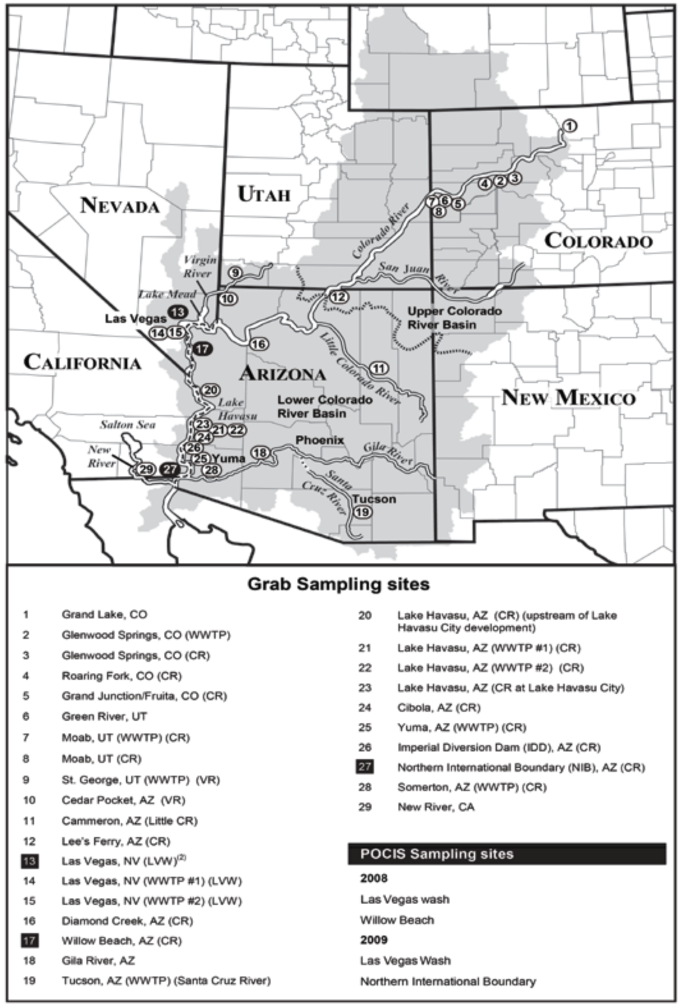
Tracing the Sources of Emerging Contaminants in the Colorado River Basin cont.

Research Objectives

- To characterize environmental sources of drugs (macrolide antibiotics, OTCs and illicit drugs) into the Lower Colorado River basin
  - Distribution
  - Ambient concentrations
  - Trends (spatial)
- To evaluate data for exposure analysis scenarios for risk assessors

Tracing Sources of Emerging Contaminants in the Colorado River Basin



	Grand Lake CO	Glenwood Spr CO WWTP	Glenwood Spr CO (CR)	St George UT WWTP Virgin River	Cedar Pocket AZ Virgin River 17 km DS	Las Vegas, NV two WWTPs	Las Vegas Wash, NV 15km DS	Lake Havasu two WWTPs	Tucson AZ WWTP	Yuma AZ WWTP	New River CA
Urobilin	ND	1400	ND			60		5			30
Azithromycin		900		150		2800		100	1300	770	
Clarithromycin						130		80	370		10
Roxithromycin									180		110
Clindaymycin				950		1150		550		740	
Methamphetamine		350				370	250	190	570	570	200
MDMA		100						70	1000		
pseudoephedrine		3300		430	290	3100		280			140
hydrocodone		900				330					

All values are ng/L (ppt)

Tracing Sources of Emerging Contaminants in the Colorado River Basin

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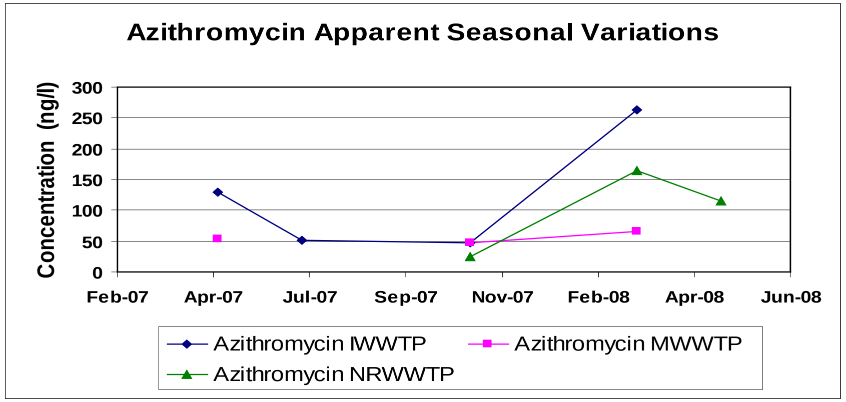
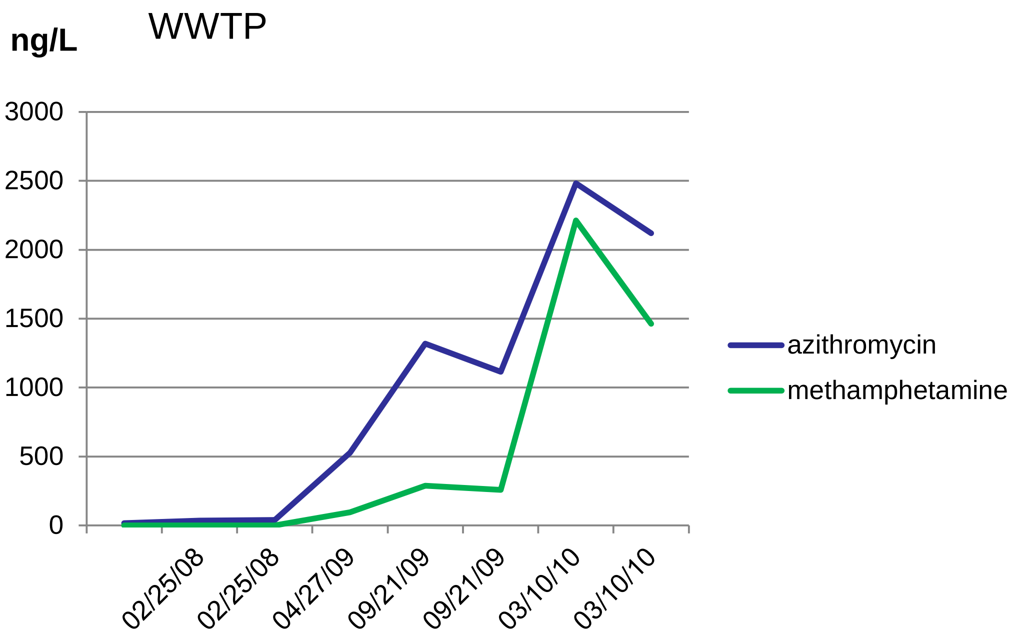
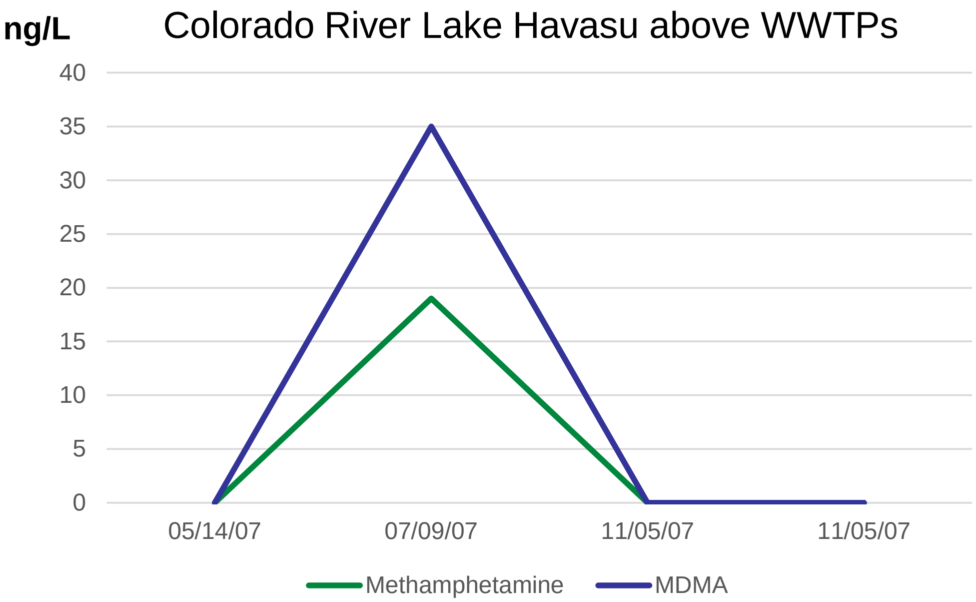


Seasonal variations





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Tracing Sources of Emerging Contaminants in the Colorado River Basin

Summary

- Some compounds, like azithromycin, can be thought of as pseudopersistent: always present in the wastestreams due to wide-spread anthropomorphic use.

- Compounds with high water solubilities, such as methamphetamine, MDMA and pseudoephedrine, can travel for several kilometers downstream from the WWTPs.

- Temporal variations in the release of different ECs at different times of the year can lead to an improved understanding of wastewater treatment technologies, such that engineering technologies could be tailored more specifically towards certain classes of compounds.

- Multi-use and recycling of wastewater effluent and the impact upon Southwestern water resources (e.g., Colorado River, Santa Cruz River, Gila River, etc.) increases the potential for cumulative increases of ECs into water supply sources.

- The characterization of ECs will become important for ecological and human health risk assessments and commodities valuation of water resources.

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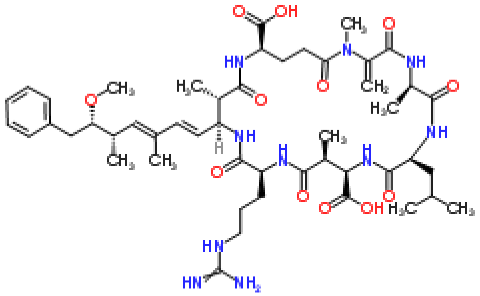
Lower Colorado River Basin Harmful Algal blooms



Lower Colorado River Basin Harmful Algal blooms

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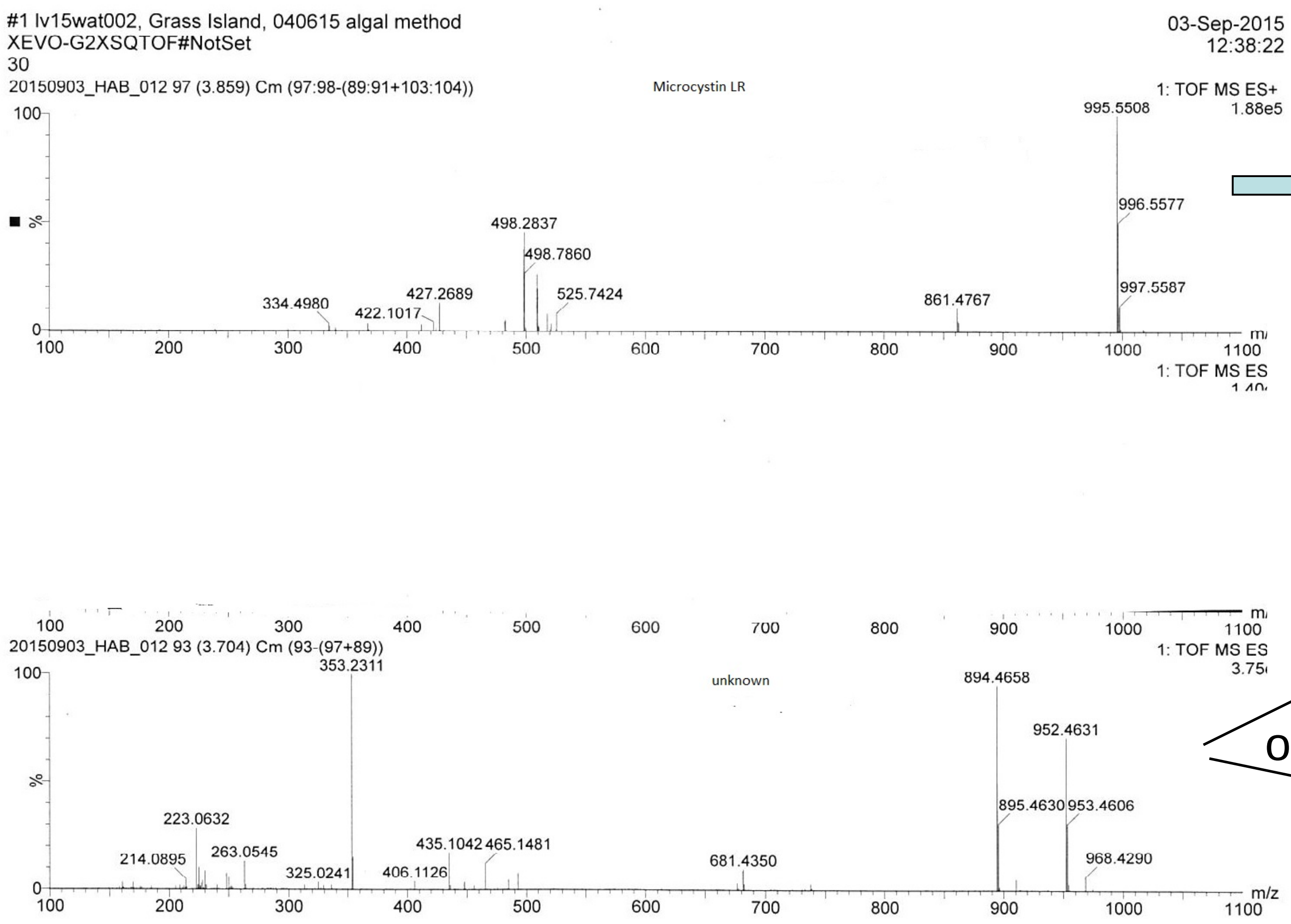
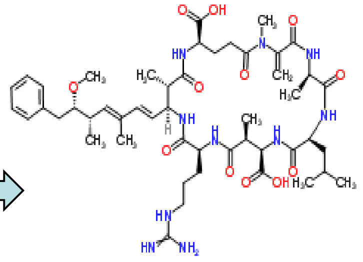
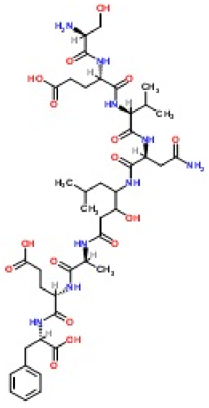
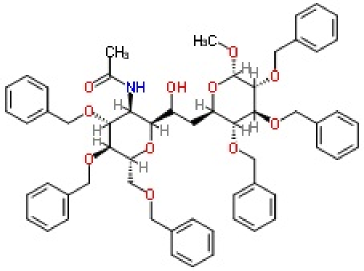


Microcystin-LR





or



Lower Colorado River Basin Harmful Algal blooms

Summary

- Increasing temperatures longer duration of algal blooms
- Other microcystin toxins potentially present, but as yet fully characterized
- Besides cyanobacteria, other algae have been identified by collaborators as present: Golden Algae, Euglenia. The toxins produced by these species have yet to be fully characterized
- Potential long-term human health effects:
  - Cyanobacteria blooms and non-alcoholic liver disease: evidence from a county level ecological study in the United States, Zhang et al, Env. Health 2015
  - Immunomodulatory potency of microcystin, an important water polluting cyanobacterial toxin, Adamovsky et al, ES&T 2015

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Vince Taguchi, retired, Ministry of the Environment, Canada

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David Alvarez, USGS, Columbia, MO
Charles Sanchez, Univ of Arizona, Yuma, AZ

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