

Aquatic Toxicity Tests and their Role in Environmental Protection in Ontario

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Introduction

- Toxicity testing 101
- Types of toxicity tests
- Toxicity testing vs. analytical testing
- Quality control (QC) in toxicity testing
- Regulations
- History of aquatic toxicology in Ontario
- Some ways we use toxicity testing in Ontario
- Have we made any improvements?
- What's next?

What is Aquatic Toxicology

- From a regulatory perspective it is the study of:
 - Environmental contaminants;
 - Heat, nutrients, inorganic chemicals, organic chemicals
 - MIXTURES!
 - And their effects;
 - Lethality, growth, reproduction, genotoxicity, bioaccumulation
 - Ecosystem changes (species abundance, diversity...)
 - On water-dwelling organisms
 - Bacteria, plants, animals

What are Aquatic Toxicity Tests?

- Laboratory or field exposures of aquatic organisms to a contaminant (either pure chemical or mixture)
- Use an aquatic organism to detect both the *presence* and *effects* of a toxicant
- Chemical analyses of effluents indicate presence, but not environmental/biological effects...

High Technology vs Low Tech



HIGH analytical complexity
Narrow Chemical focus

LOW analytical complexity
High biological complexity
Broad chemical focus



Toxicity Testing vs. Analytical Chemistry

- Toxicity Testing

- Measures effects on organisms, plants and animals
- Mixtures!
- Biological detectors
- Animals for Research Act
- Infrastructure

- Analytical Chemistry

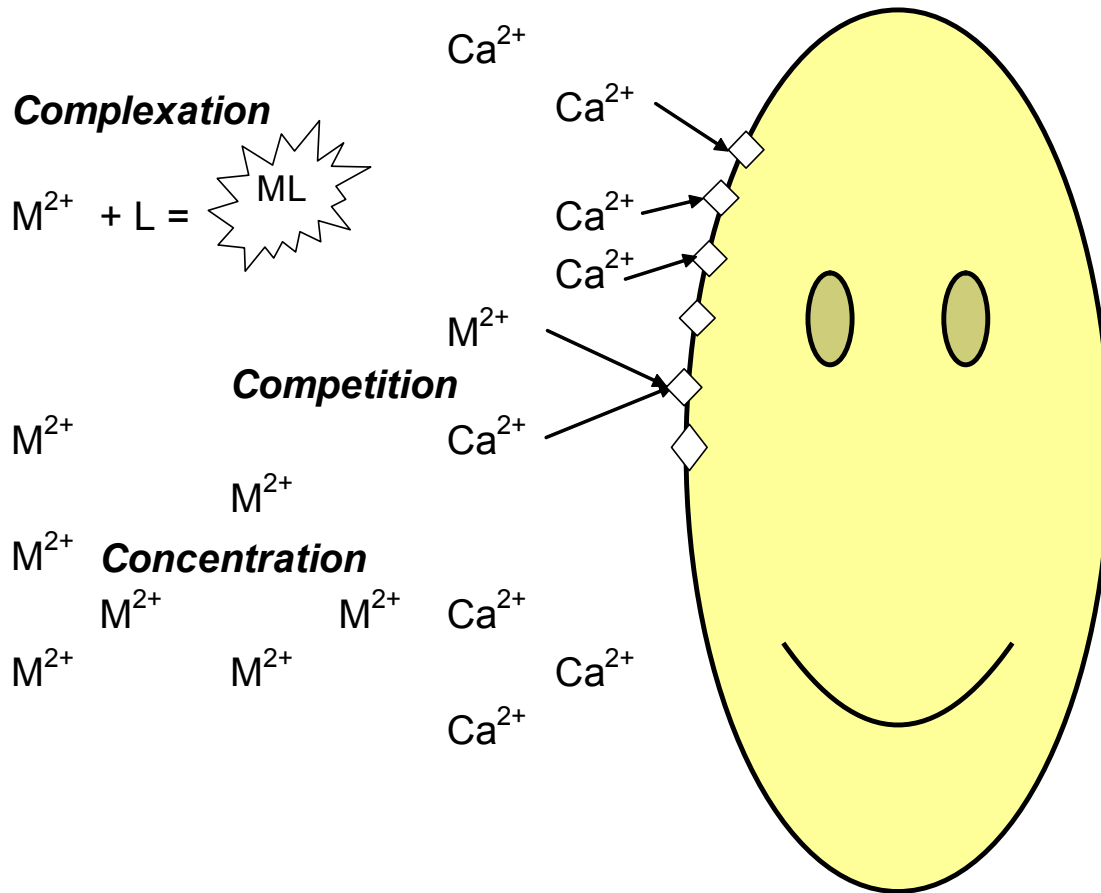
- Measures presence/absence and concentration
- Gives us an idea of what's causing effects (weight of evidence approach)
- Various – colorimetric, mass, etc.

Chemistry and biology work hand in hand to identify risk, and cause/source of the hazard.

How do They Work?

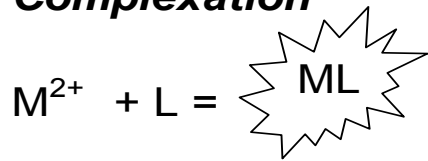
- Organisms respond to the three C's:
 - 1) Concentration (exposure)
 - How much toxicant is present
 - 2) Complexation
 - How much of the toxicant is bound to organic or inorganic ligands
 - 3) Competition
 - What substances are present to compete at the biological receptor
 - E.g., Metals...
-

Organism Responds to the Three C's of Toxicity



Organism Responds to the Three C's of Toxicity

Complexation



Competition

M^{2+}

M^{2+}

Ca^{2+}

M^{2+}

M^{2+}

M^{2+}

M^{2+}

Concentration

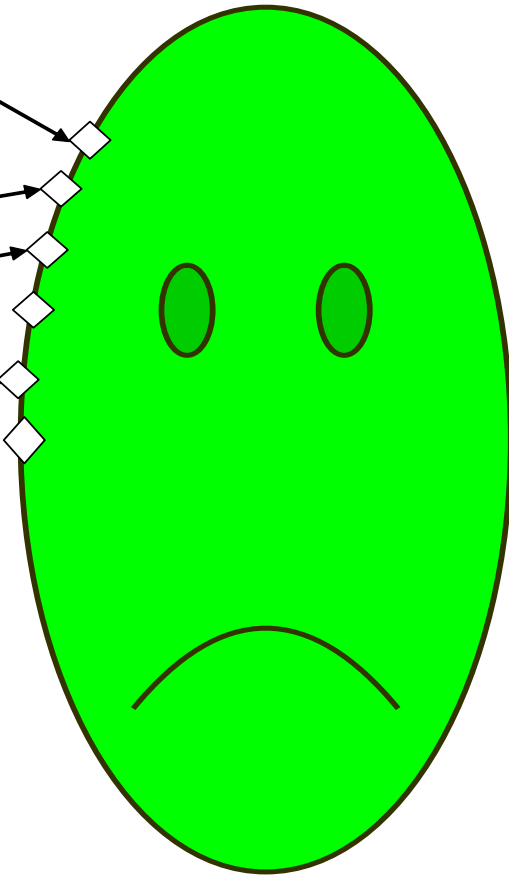
M^{2+}

M^{2+}

M^{2+}

M^{2+}

M^{2+}



Test Animals/Methods

- Select the correct tools for the job
- Look at the problem/question and decide which of a myriad of methods/test species would be appropriate



What Makes a Good Test Animal?

- Depends on the purpose, but generally:
 - Readily available in large numbers;
 - Lots known about biology/physiology;
 - Sensitive;
 - Representative;
 - Size;

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- For compliance/legal/monitoring purposes:
 - Must be present in Ontario (or ?)
 - Must represent community to be protected
 - “public appeal”
 - Methods must undergo strict quality control (QC)
 - Culture health
 - Reference testing
 - Audits
 - Proficiency testing

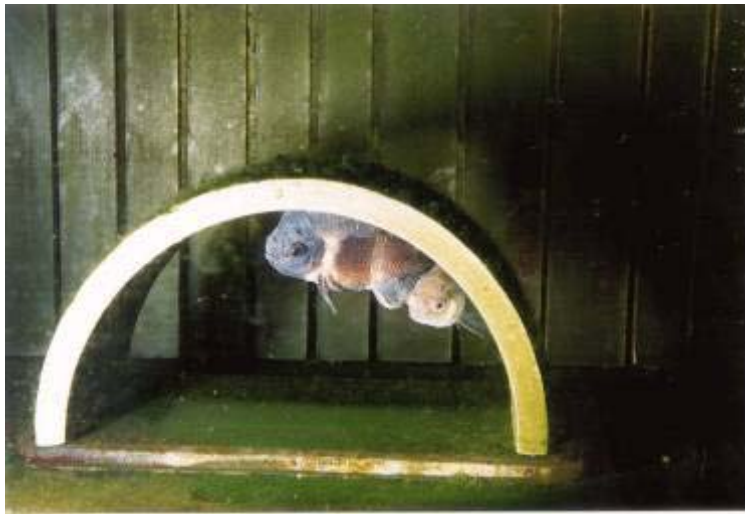
Types of Toxicity Tests

- Static Acute Lethality Tests
 - Single concentration and LC50 (lethal concentration that kills 50% of the test animals)
- Regulatory compliance tests



Types of Toxicity Tests

- Chronic/Sublethal Tests
 - Survival, growth, reproduction endpoints
 - 7 – 8 days to conduct
 - Monitoring only



Fathead minnow

Ceriodaphnia dubia



Sediment Testing



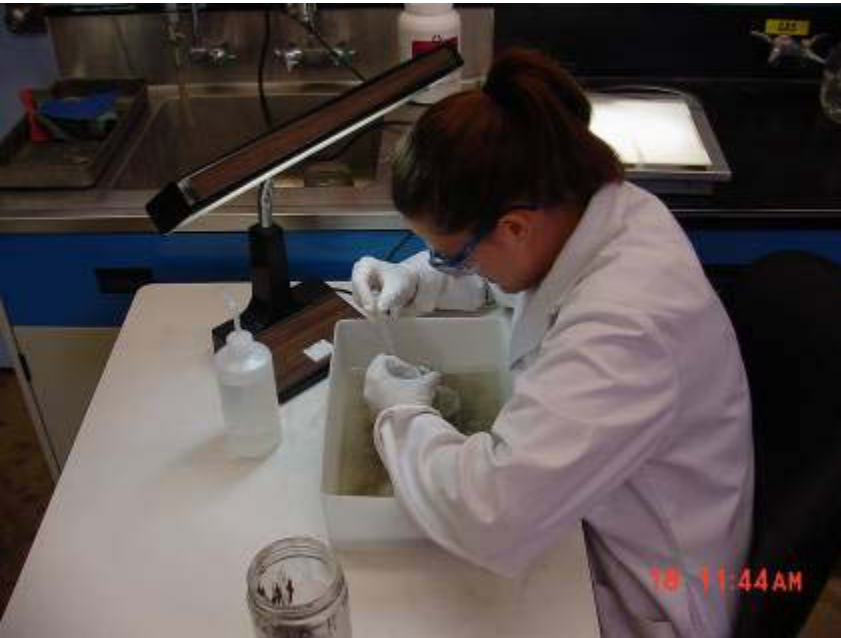
- Assists with assessing areas of historical contamination
 - Partitioning of contaminants (water/sediment/biota)



Sediment Testing

Chronic exposures

- 4 species, 3 exposure scenarios
- survival, growth, bioaccumulation



Interpreting Test Results

- Survival Effects
 - LC50 (concentration estimated to cause mortality to 50% of the test organisms)
 - Tells us how much toxicant is required to kill $\frac{1}{2}$ the organisms exposed
 - The higher the LC50, the lower the toxicity
 - Single Concentration Test
 - Determines the mortality of organisms exposed to an undiluted effluent
 - The higher the percent mortality, the more toxic the effluent

Quality Control (QC)

- Culture Health
 - % mortality in cultures
 - Number of offspring/brood
 - Time to first brood
- Routine water quality monitoring
- Standards Council of Canada (SCC) or Canadian Association for Environmental Analytical Laboratories (CAEAL) accreditation
 - Third party audits
 - Veterinary inspections
 - Under the Animals for Research Act
- Negative controls with every test
- Monthly QC checks

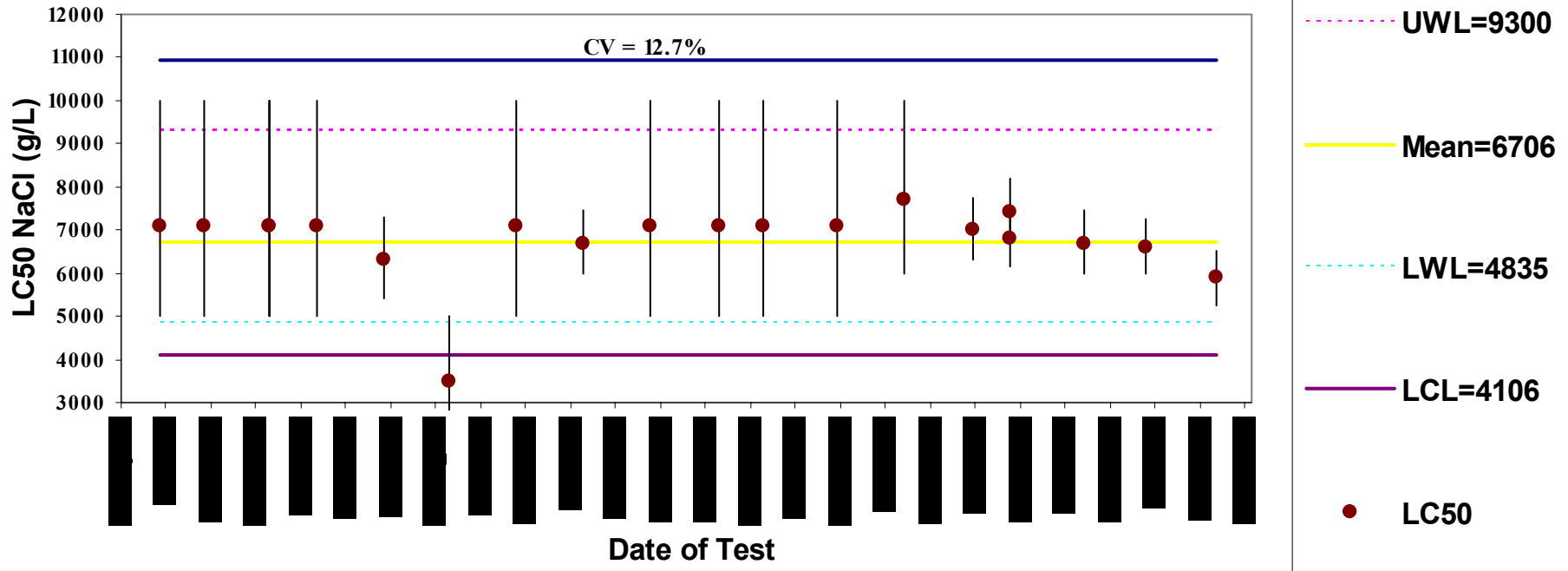


Quality Control

- Reference toxicant testing and control charting

UCL upper control limit
 UWL upper warning limit
 LCL lower warning limit
 LWL lower warning limit
 CV Coefficient of variance

Daphnia magna NaCl 48 hr LC50 Control Chart
 Mean and Limits Represent Antilog of Log LC50 Calculations



What do Chemists do For Us?

- QC
 - Measure culture water parameters
 - Measure food quality and contamination
 - Confirm reference toxicant concentrations
 - Supply internal proficiency evaluation (PE) samples
- Toxicity Assessment
 - Measure chemicals in toxic samples
 - Characterize unknown effluents/toxicants
- Sediment Assessment
 - Measure chemicals in sediments
 - Prepare and measure chemicals in biota (bioaccumulation)

Refocus Our Perspective

- Why are we doing what we are doing?
 - More sensitive analytical techniques, new analytes, metal speciation...
 - Measure/monitor/**reduce** environmental contaminants
 - *Balance industry with human and ecosystem health*

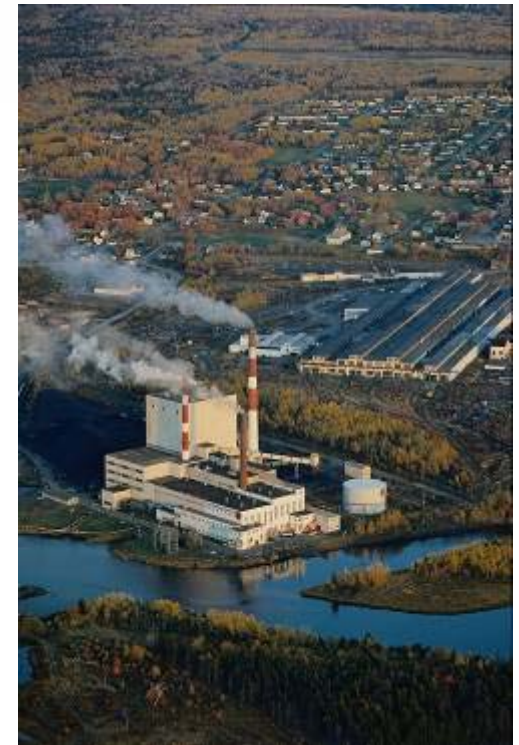


Regulations

- Our mandate comes from:
 - Ontario Environmental Protection Act (EPA)
 - Ontario Water Resources Act (OWRA)
 - Federal Fisheries Act (FA)
- *“no person shall discharge a contaminant or cause or permit the discharge of a contaminant into the natural environment that causes or is likely to cause an adverse effect”*
(EPA section 14.1)

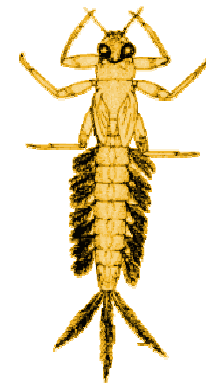
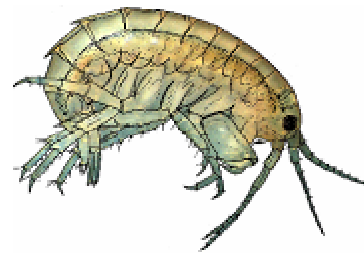
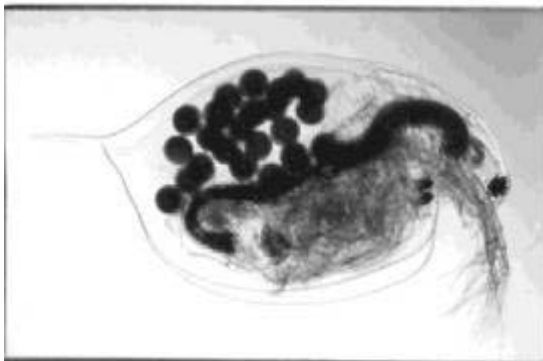
History

- 1960s and 70s
 - Acute lethality testing with fish
 - Primarily petroleum, pulp and paper, mining industries



History

- 1980s – 1990s
 - Municipal/Industrial Strategy for Abatement (MISA)
 - Monitoring regulation
 - Effluent Limits Regulations (under the EPA)
 - Chemical and toxicological characterization of effluents from >250 companies representing 10 industrial sectors
 - Development of national test methods



History – Present

- Effluent Limits Regulations
 - End of pipe controls
 - Sublethal data assessment
- Development of Provincial Water Quality Guidelines/Objectives (PWQO)
- Spill investigations
- Contaminated sites
 - Remedial Action Plan Sites (RAPS)
 - Contaminated sediments
- Toxicity Identification/Reduction Evaluations
- Method Development



Investigations

- Legal
 - Belle Island Landfill Leachate
 - Highly toxic (48 hour LC50 to *Daphnia* <0.0001%)
 - Toxic “soup” (1000s of chemicals identified)
- Assistance
 - Ministry of Transportation
 - Assess toxicity of various bridge cleaning solutions
 - Metro Toronto Works
 - Help choose an appropriate polymer and dose to assist settling
- Wastewater Treatment Plants (WWTP)
- Food Processing Plant Effluents
 - Part of a large team looking at effluent characteristics, including toxicity

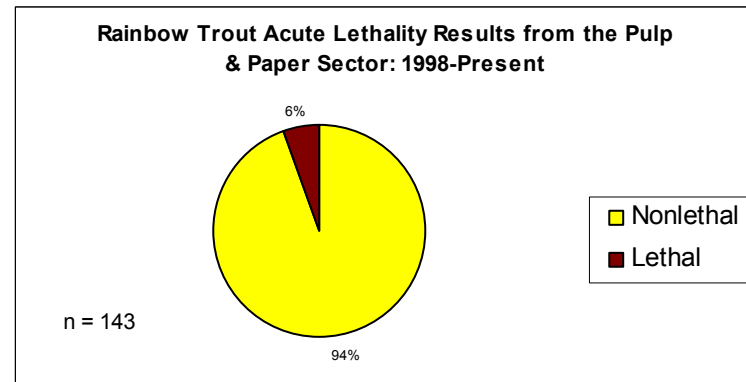
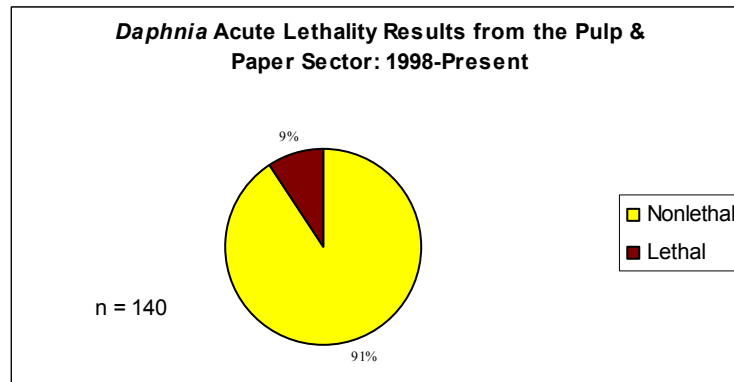
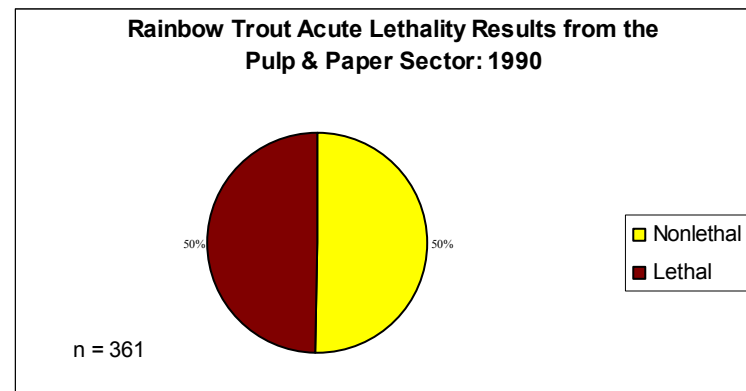
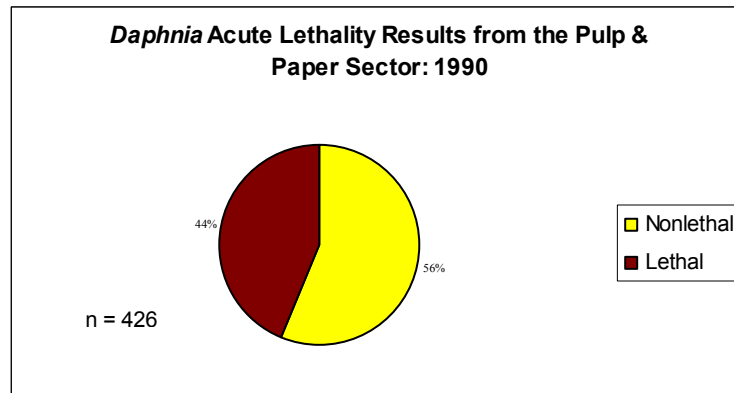


Investigations

- RAPS
- Ecological Risk Assessments (ERA)
 - Aquaculture (cage culture facilities)
 - High copper, zinc, nutrient, Biological Oxygen Demand (BOD)
 - Aluminum smelter decommissioning
 - Aluminum contamination
 - Pulp and paper plant decommissioning
 - Mercury
 - Manufacturing plant decommissioning
 - Polychlorinated biphenyls (PCBs) in river sediments and biota
 - Randle's Reef
 - Polycyclic aromatic hydrocarbons (PAHs), metals, BOD and ammonia

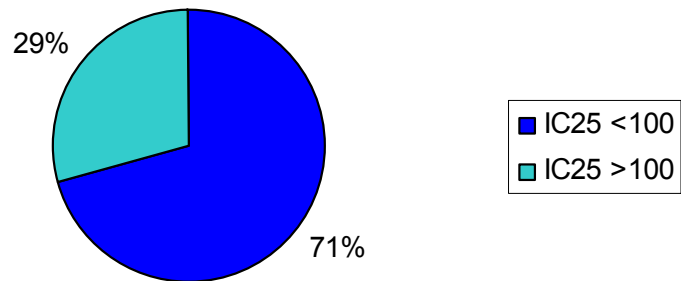


Has There Been Improvement?



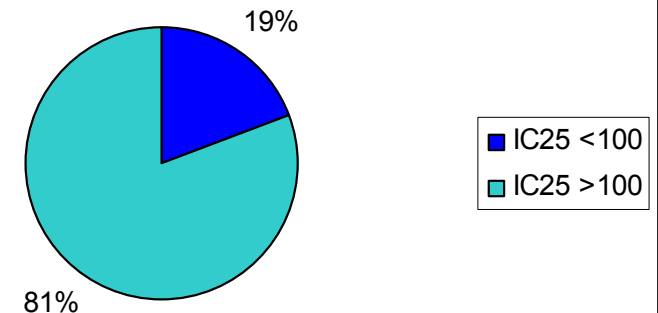
Sublethal Monitoring 1998 – 2002

***C. dubia* Sublethal Toxicity Results
from the Metal Mining Sector**



n = 78

***P. promelas* Sublethal Toxicity Results
from the Metal Mining Sector**



n = 78

IC25 inhibitory concentration

What's Next?

- Support investigation and enforcement activities.

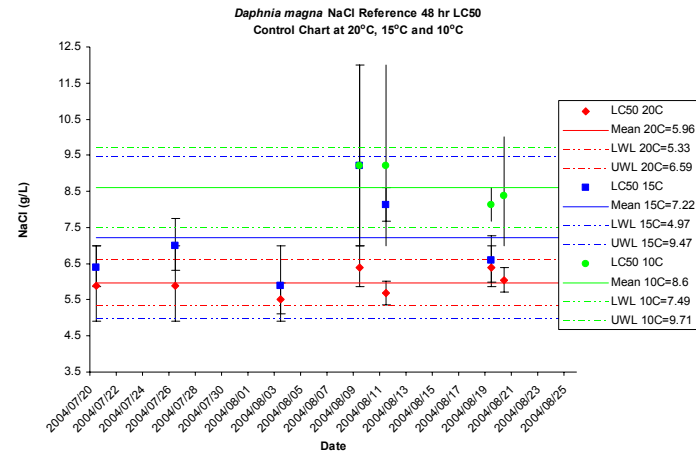


Effects of temperature on toxicity of ammonia to *Daphnia magna* cultured at different temperatures.

Test Temperature (°C)	13 LC50 (CL) (mg/L total ammonia)	13 LC50 (CL) (mg/L NH3)	20 LC50 (CL.) (mg/L total ammonia)	20 LC50 (Confidence Limit (CL)) (mg/L NH3)
10	199 (178, 222)	0.23 (0.21, 0.26)	162 (145, 181)	0.19 (0.17, 0.21)
15	251 (225, 280)	0.43 (0.39, 0.48)	137 (93-202)	0.24 (0.16, 0.35)
20			150 (100, 210)	0.38 (0.25, 0.53)

What's Next?

- Support PWQO and Canadian Council of Ministers of the Environment (CCME) guideline development
 - Fill in data gaps (e.g., ammonia toxicity in cold water and low pH)
 - Concern with mining and WWTP in northern communities



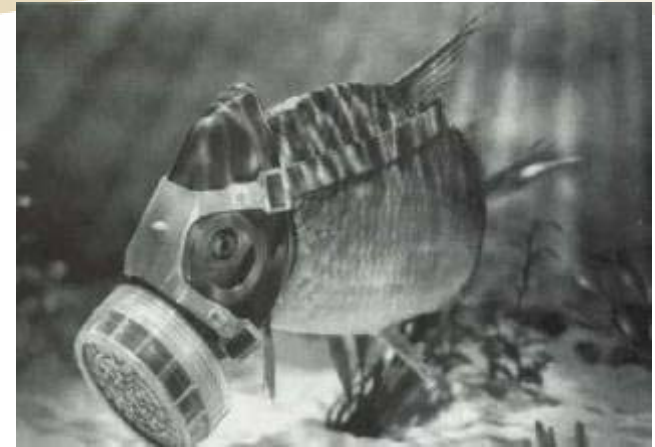
What's Next?

- Method Development
 - In Canada, method development is often done on a national level through guidance from Intergovernmental Ecotoxicology Group (IGETG)
- Presently includes “tweaking” and harmonizing sediment toxicity test methods, and pH stabilization in trout tests



What's Next?

- Monitor persistent and bioaccumulative substances
 - No well developed method available
 - Rely on field studies (\$\$\$)



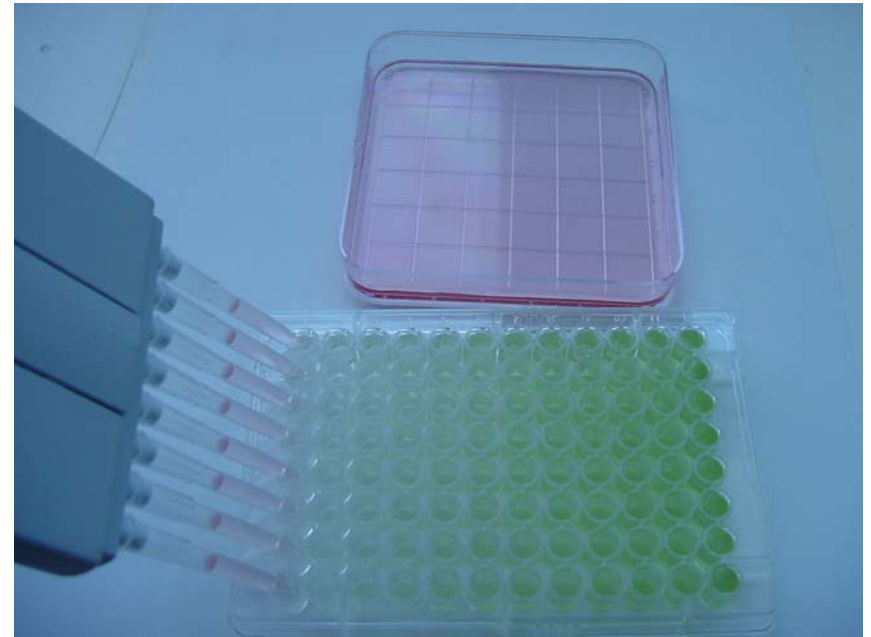
What's Next?

- Reduce number of animals used in testing
 - Animals for Research Act
 - E.g., cell culture assays using fish liver or gill cell lines



What's Next?

- Ecosystem Protection
 - The types and numbers of tests used to monitor and protect the environment based on:
 - Available methods
 - Available resources
 - Perceived importance
 - Sample size to collect
 - Need a plant test!



Plant Tests

- Micro-scale
 - Selenastrum microplate technique
- Macro-scale
 - Lemna



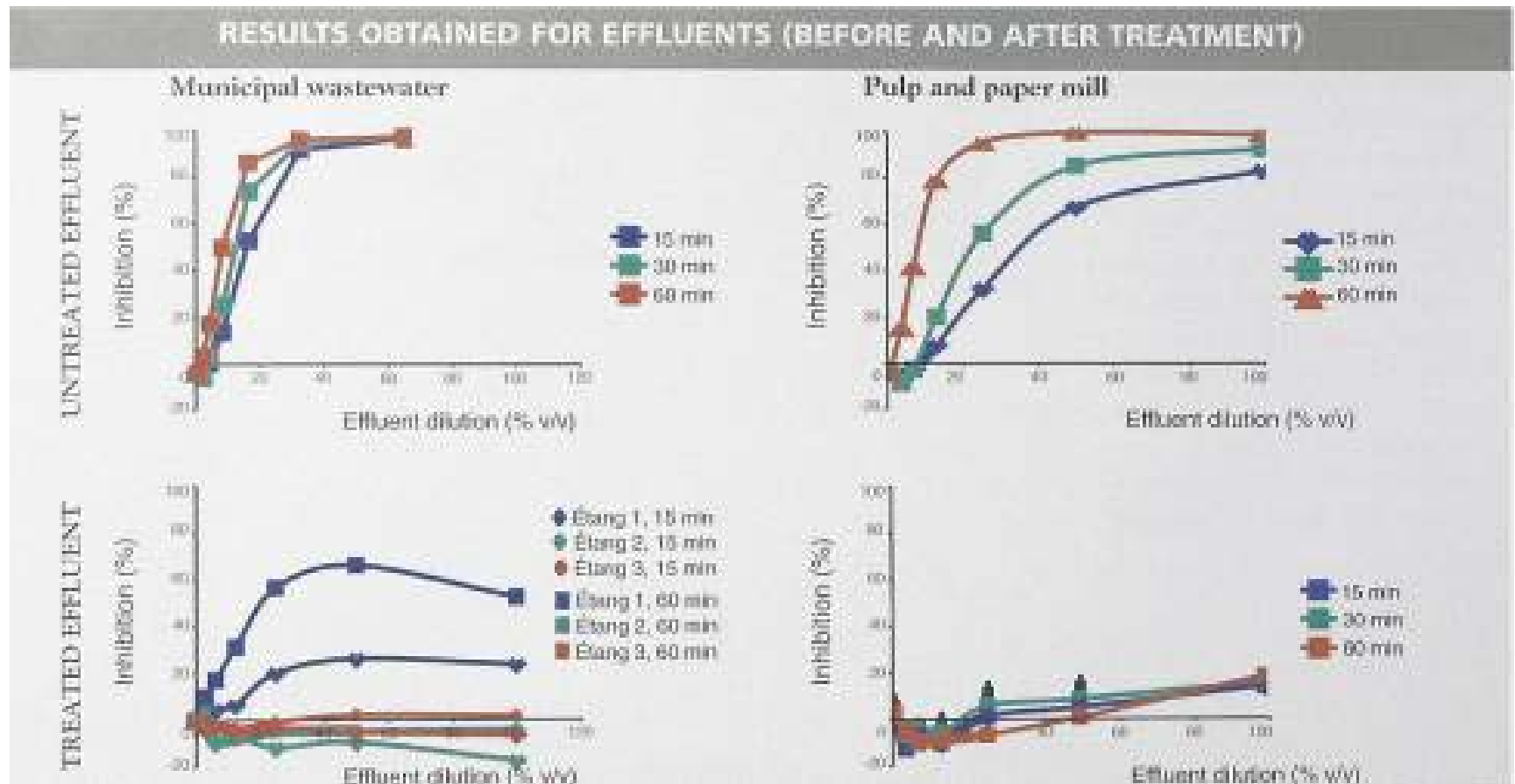
What's Next?

- Screening tools
 - Reduce workload in areas of expensive and difficult analyses required by regulation.
 - Luminotox
 - Using algae
 - Microtox
 - Using bacteria
 - Rotox
 - Using rotifers



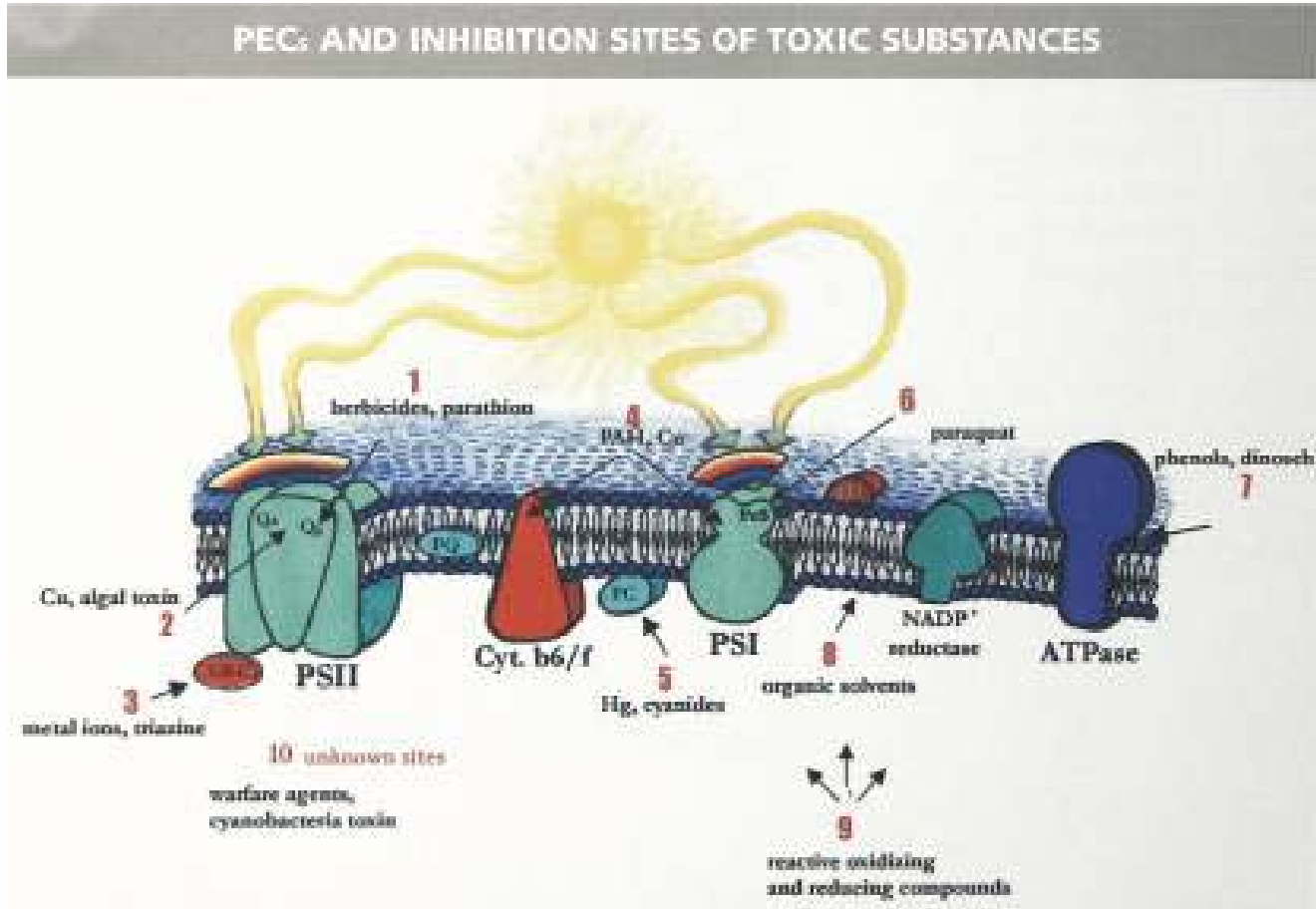
Luminotox

- Potential as a screening tool, or rapid test



Luminotox

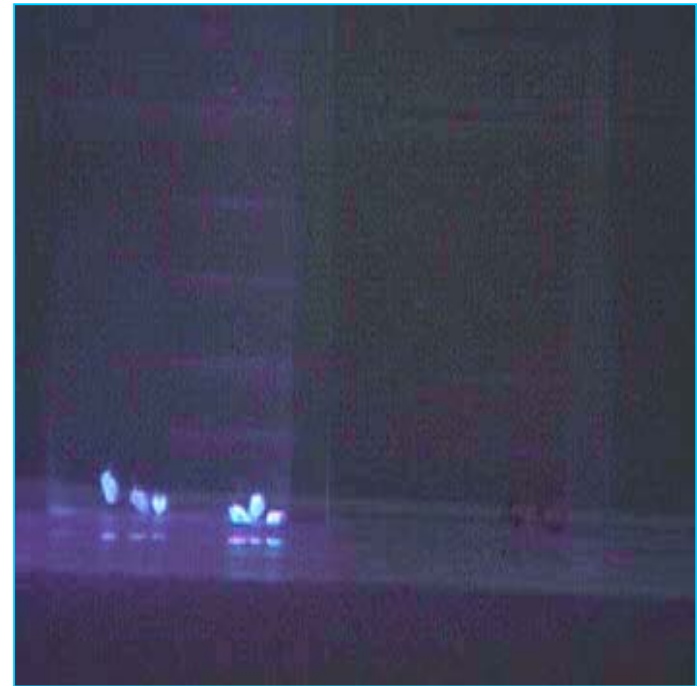
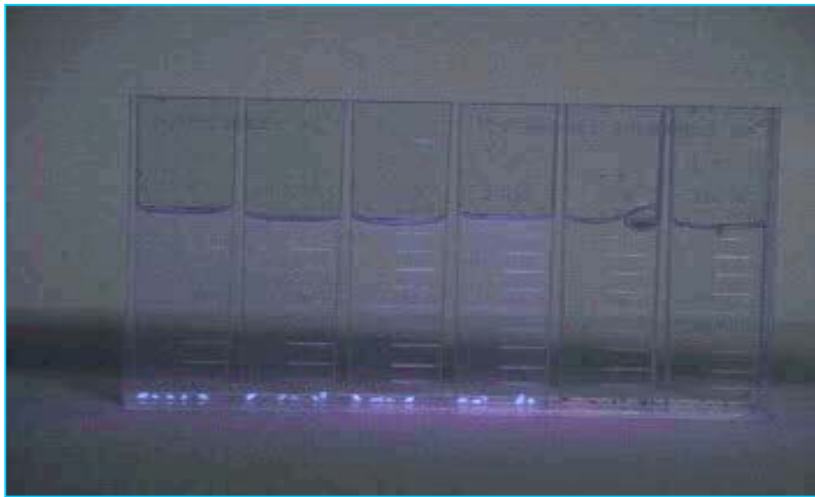
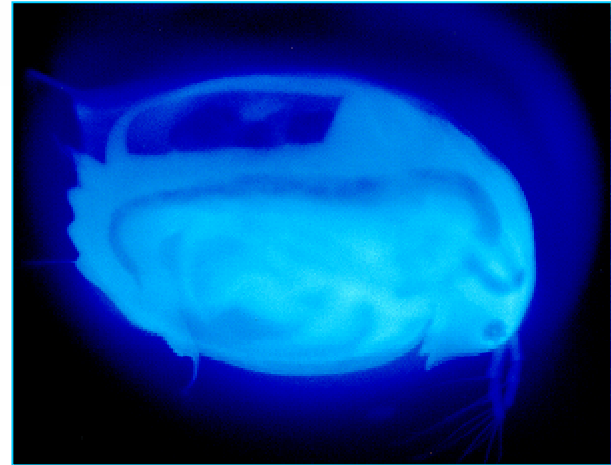
- Looks at chlorophyll disruption



PEC Photosynthetic enzyme complex

Rapid Tests

- Industry request
 - Wish to know if a discharge is toxic without waiting 2 – 4 days for results.



Modelling

- Quantitative Structure Activity Relationships (QSARS)
 - Extrapolates expected toxicities of compounds from data from one compound in the groups and their chemical structural relationships.
- Biotic Ligand Model (BLM)
 - Predicts copper toxicity (to trout) based on water quality relationships to toxicity
- Toxic Equivalents/Toxic Units (TU)
 - Bases toxicity of a compound on it's relative toxicity to another similar compound

Conclusions

- Toxicity tests have the advantage of looking at the environmental/biological effects of mixtures
 - Choose the right “instrument” for the job
 - There have been definite improvements in effluent quality over the past 10 years
 - Still a long way to go (e.g., chronic toxicity concerns, bioaccumulation, personal care products in sewage discharges, new toxins, etc.)
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Acknowledgements

- The staff of the Aquatic Toxicology Unit for their dedication
- D. Rogers (Kinectrics) for the slides on the IQ method, and L. Novak (Stantec) for the slides on the Microtox, and algal methods.

Thank You!

- Any Questions?



High Tech Fishing



Low Tech Fishing