ALS Laboratory Group

ANALYTICAL CHEMISTRY & TESTING SERVICES

Environmental Division

Rapid Bioassay for Oil-Contaminated Soil

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Right solutions….

….Right partner

We Need Your Soil!

- Oil-Contaminated Soil
- Soil with some site-remediation history
- Failing CCME Tier I
- Weathered F2-F4

ALS-HydroQual Joint Project

ALS Environmental

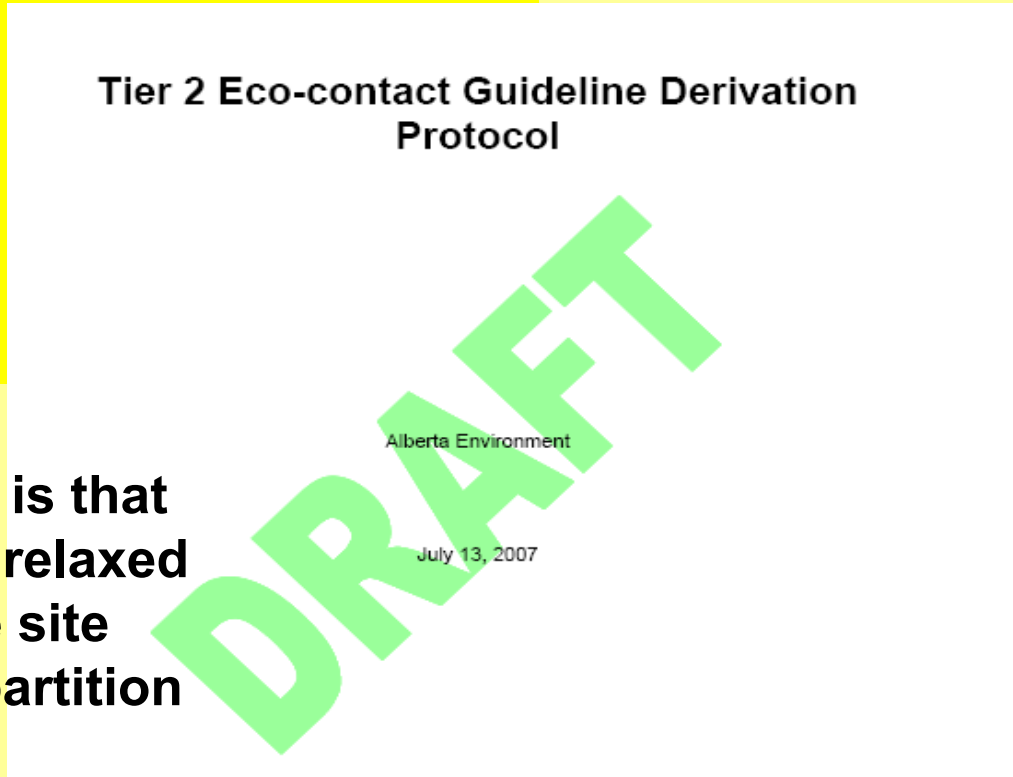
HydroQual Laboratories Ltd
Applied Biology for Environmental Management

Sponsored by AUPRF (PTAC)

PTAC

Tier 2 Site Specific Remedial Objective

- Eco-Contact Guideline Derivation Protocol
- Weight of Evidence (WoE) approach
- At least 10 bioassays required
- 2 invertebrate & 2 plant species
- Cost at least $ 10 k per site



The WoE claim being made is that Tier I thresholds should be relaxed due to ageing of PHC at the site (weathering, degradation, partition to OM, sorption on clay)

Earthworm bioassays

- Costly and time-consuming
- Earthworm survival, 1 month
- Earthworm reproduction, 2 months
- Cost $3 k per site
- We'll send you free results!!!!

HydroQual
Laboratories Ltd
Applied Biology for
Environmental Management

The Microtox™ bioassay is rapid and relatively inexpensive

Regular TAT = 3 days, cost = $150

The actual Microtox test takes 15 minutes

The rest of the TAT is sample login, prep, QA/QC, data entry and reporting

- **The luminescent test organism is susceptible to hydrocarbons, toxic metals, biocides, sulphides etc.**

- **These properties and the speed of the test have led to its use in drilling waste testing (since 1993).**

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Light intensity readings at 4 dilutions

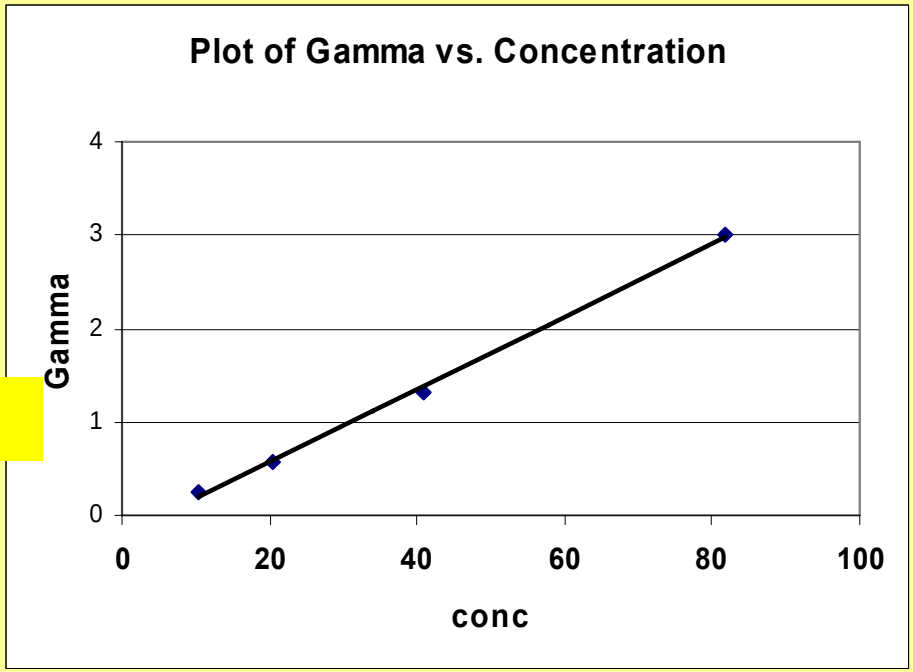
	0	10.2	20.5	40.9	81.8 %
I_o	95	92	90	93	96
I_t	95	73	57	40	24

Gamma = light lost ÷ light remaining

Gamma at 81.8 % = $(I_o - I_t)/I_t$
= (96-24)/24 = 3.0

Calculate EC50 value (= toxicity of sample) by plotting Gamma against concentration

At the EC50, Gamma = 1 (light intensity halved)



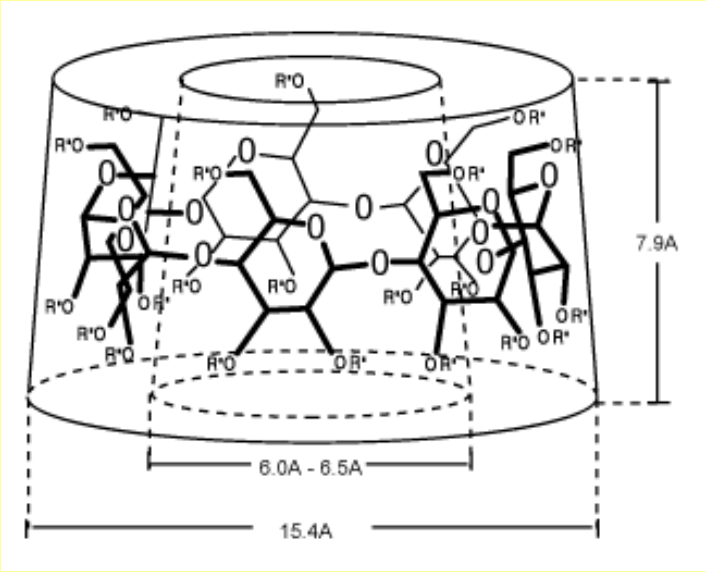
EC50 ≈ 30 %

And now for something completely different . . .

“There’s a hole in this cake!”

Cyclodextrin

"There's a <u>hole</u> in this molecule!"



- **β-CD cone built from 7 glucose rings**
- **Hydrophilic exterior makes the complex soluble**
- **"Waxy" interior pocket can "sorb" a PHC molecule**
- **Soil PHC extraction method developed by ALS (2004)**
- **CD sorbs <u>available</u> PHC (< 20% of aged <u>total</u> PHC)**
- **Earthworm bioassay & CD-PHC data correlated**
- **Available PHC data can be used in Tier 2 assessment**

Cyclodextrin info, Miles Tindal, Axiom Environmental

“Total” petroleum hydrocarbons in cyclodextrin extract of soil (F2-F4) quantified by solvent extraction (hexane) & GC-FID

Our proposed alternative is to run a Microtox bioassay on the cyclodextrin extract. Advantages:

- **Simpler than quantifying available PHC by GC/FID**
- **Soil extraction (1 h) & bioassay (1 h) done in one run**
- **Results correlate with 2-month earthworm bioassay ?**
- **Microtox result will thus warn if Tier 2 testing at the site in question (cost > $10 k) would probably fail**
- **Initially a screening test . . .**

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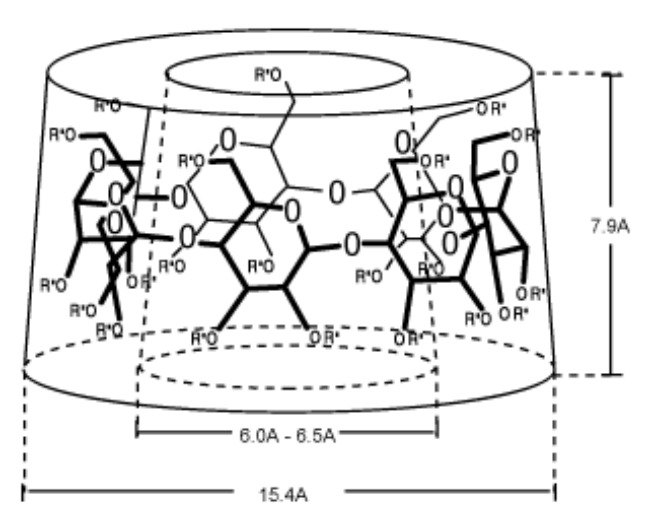
Straight β-cyclodextrin extracts of oily soil



- Non-toxic towards Microtox test
- unless % oil in soil > 2 %
- (like 1:1 water extracts)

The follwing slides are a sequence of Microtox plots for CD-extracts of soils spiked with F2-F4 PHC . . .

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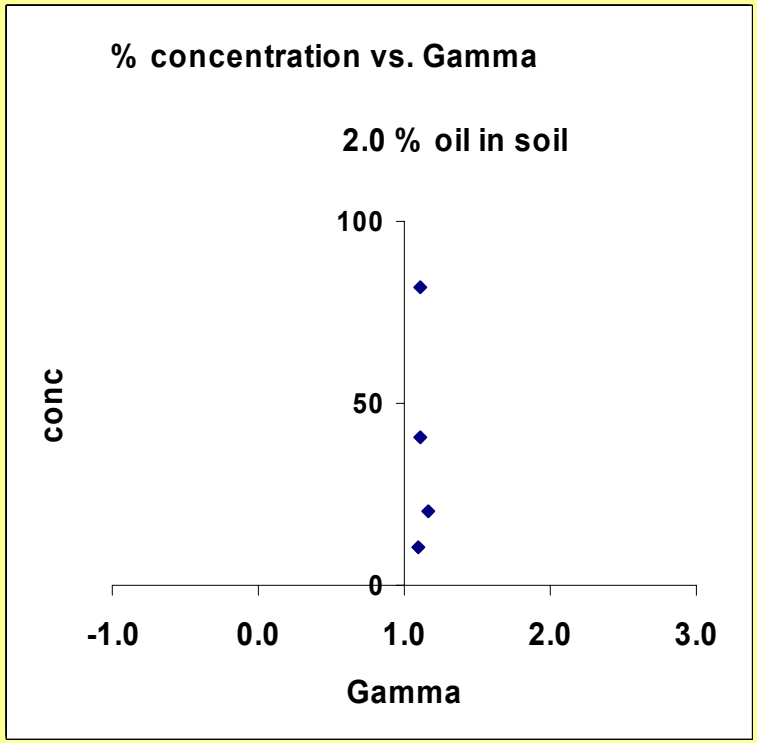
Straight β-cyclodextrin soil extracts



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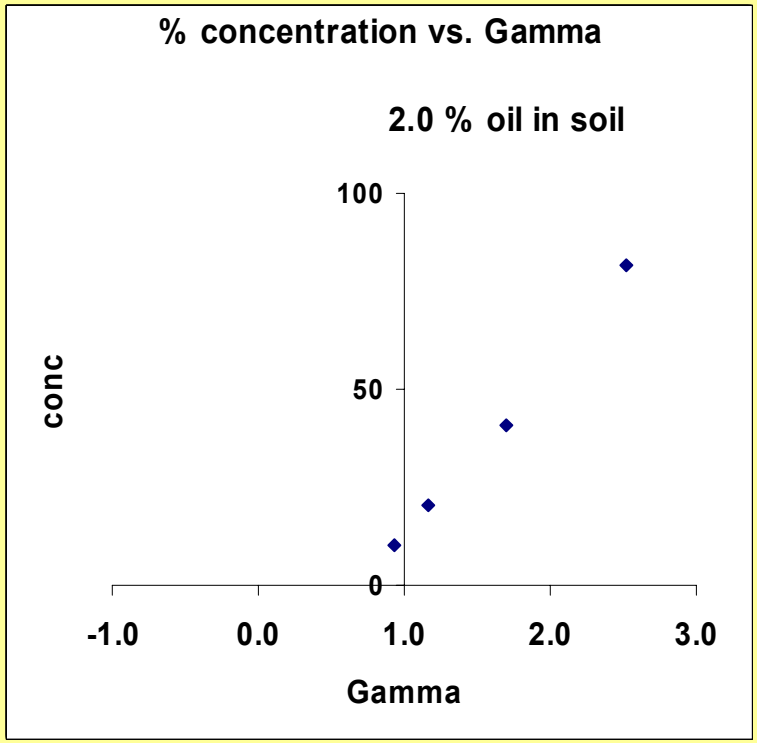
“Activated” β-cyclodextrin soil extracts



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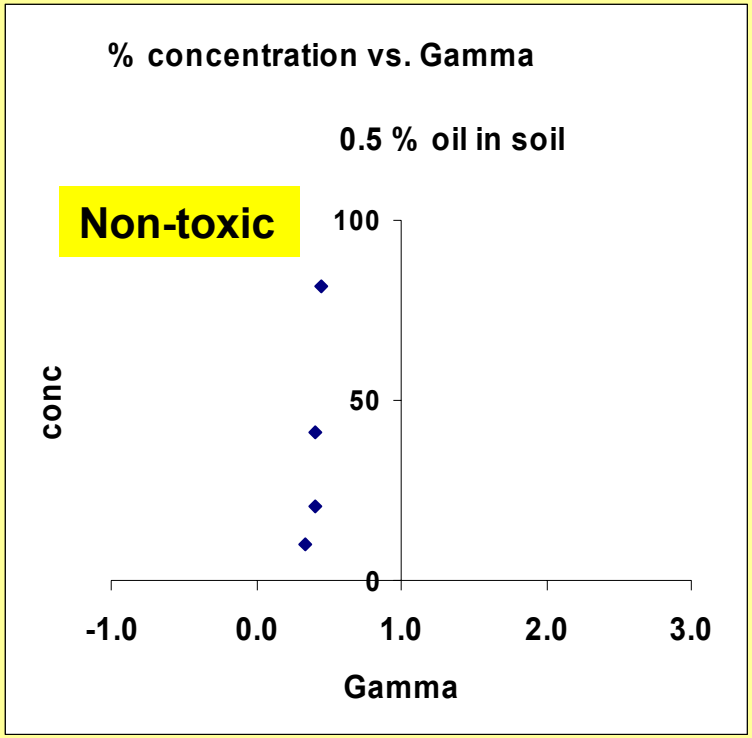
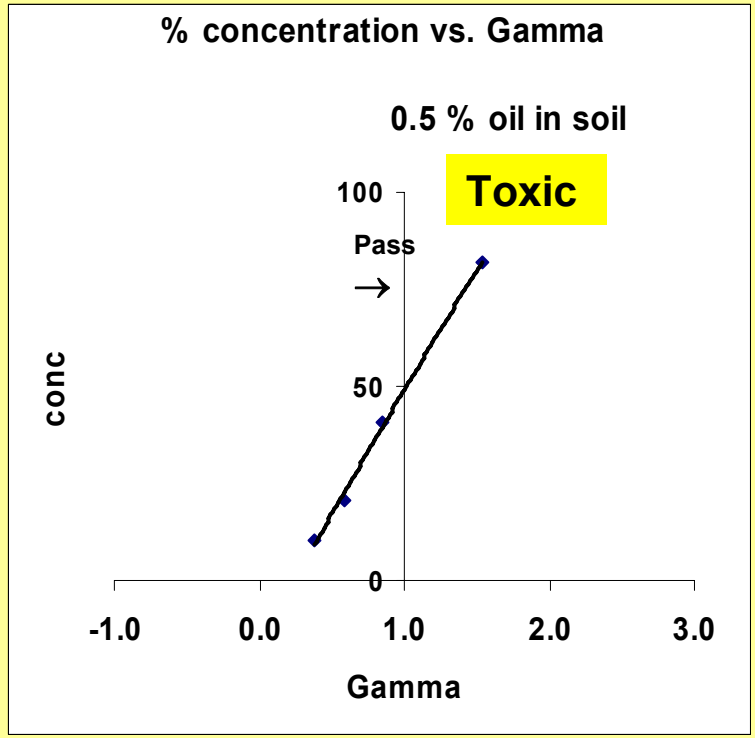
Straight β-cyclodextrin extract



Activated β-cyclodextrin extract



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The "Activation" Step

- Weakens or breaks up the CD-PHC inclusion complex
- The "released PHC" is now toxic to the Microtox organism
- But how can acidification & plain re-neutralization work?
- Try to make the "activated" CD-PHC complex more toxic
- Vary the conditions of the acid treatment (hydrolysis?) step
- Enzyme-catalyzed hydrolysis
- May be better to use γ-cyclodextrin & amylase enzyme
- Enzyme buffer solutions toxic to the Microtox organism

You Soil Donors have nothing to lose!

Here's what you will get (at no cost)
in exchange for sending us your "Tier 2" soils:

- **GC-FID results for F2-F4 in your soil**
- **Earthworm reproduction bioassay results**
- **Information on how the Cyclodextrin Extraction / Microtox test correlates with earthworm reproduction**
- **Project information – overall conclusions**
- **Acknowledgement**

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