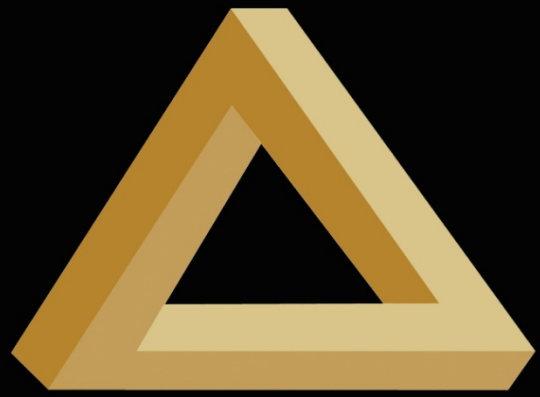

TRIUM

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EVALUATION OF THE MICROTOX® TOXICITY TESTING SYSTEM: DOES IT BELONG IN THE ENVIRONMENTAL INDUSTRY?

DR. ERIK J. MARTIN, PH.D., DABT

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BIOGRAPHY

Dr. Erik J. Martin, Ph.D., DABT

Technical Director, Toxicology & Health Sciences

- Ph.D. in Toxicology from Queen's University, Kingston, ON.
- Diplomat of the American Board of Toxicology (DABT).
- Expertise inc. environmental and mechanistic toxicology, and HHERA.
- Have conducted health / risk assessments across Canada and the US as well as internationally.
- Have experience with many different chemicals including PAHs, dioxin/furans, VOCs, metals, and PHCs.

BACKGROUND

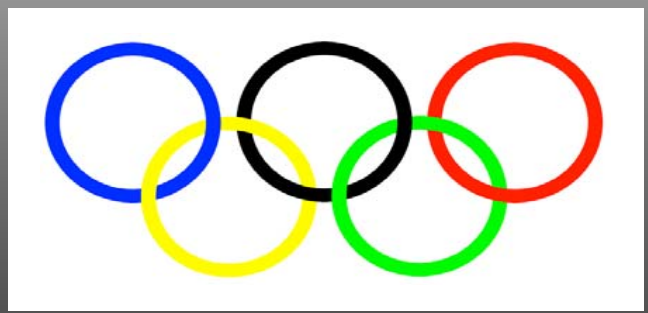
- ERCB *Directive 50* identifies Microtox as the standard for evaluating the toxicity of drilling products and wastes.
- Evaluated Microtox toxicity testing system as part of a larger study to assess drilling muds at closed sumps in a Western Asia location.

Three Phases

- *Phase 1* - Laboratory precision
  - Baseline EC_{50} values for SRMs
- *Phase 2* - Effects of time and temperature
- *Phase 3* - Microtox on 60 sump samples

MICROTOX® ORIGIN

- Microtox originally used to monitor drinking water supplies where accidental or deliberate contamination is a concern.
  - 1984 Los Angeles Olympics
  - 2000 Democratic National Convention
  - Pentagon, Washington, DC – following 9/11

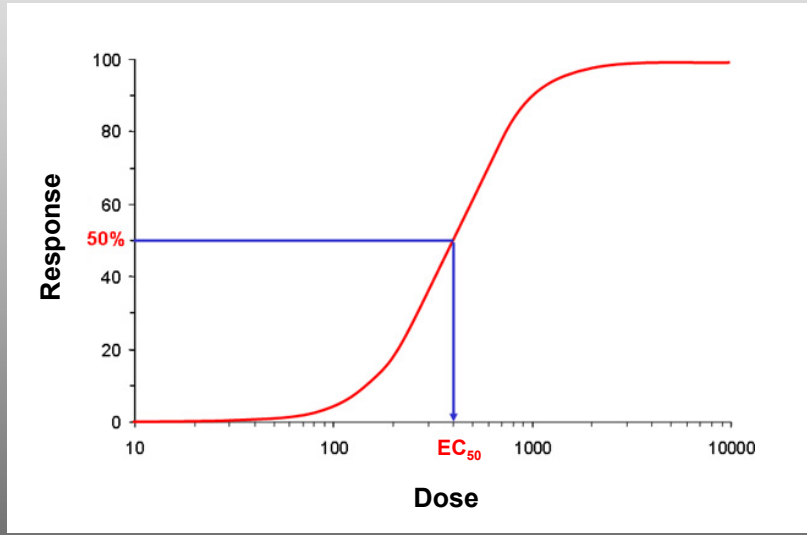


MICROTOX®

- Microtox® toxicity testing technology is a biosensor-based measurement system for toxicity.
- Microtox test systems are based on the use of luminescent bacteria (*Vibrio fischeri)* which produce light as a by-product of normal metabolism.
- Any inhibition of normal metabolism - exposure to toxic substance - results in a decreased rate of luminescence.
- ⬆ level of toxicity = ⬆ inhibition of light production.

TERMINOLOGY

- **EC_{50}** - Effective Concentration (sample conc.) that causes a 50% reduction in light emission.



Aqueous extraction of solid samples

↓

Create serial dilution

↓

Determine EC_{50} (15°C, 15 min)

↓ → PASS → $EC_{50} \geq 75\%$

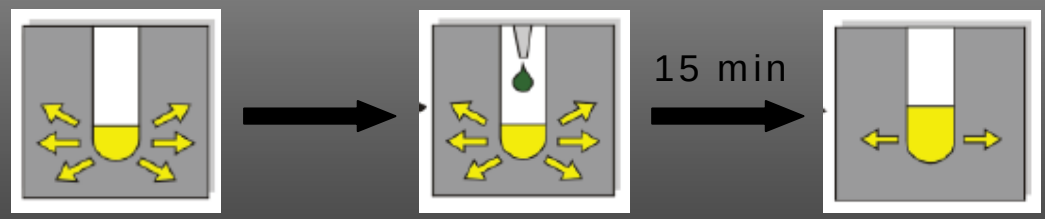
Charcoal treatment

↓ → PASS → $EC_{50} \geq 75\%$

Sample aeration

↓ → PASS → $EC_{50} \geq 75\%$

Sample filtration



PHASE 1

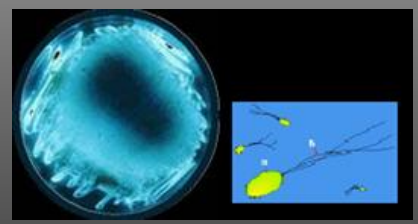
PHASE 1

- Seven standard reference material (SRM) samples sent to the laboratory for Microtox testing.

  Purpose:

  1. Test laboratory precision
  2. Establish baseline EC_{50} values – laboratory accuracy

- Baseline EC_{50} values necessary for evaluation of SRM samples included with samples from Western Asia.



PHASE 1 RESULTS

SRM ID	Original P/F	EC50	Charcoal P/F	EC50	Filtration P/F	EC50
DM-1-003*	Fail	0.76	Fail	0.99	Pass	>81.9
DM-1-043*	Fail	1.22	Fail	3.59	Pass	>81.9
DM-1-068*	Fail	1.83	Pass	>81.9	na	na
DM-1-107	Fail	0.40	Fail	2.13	Pass	>81.9
DM-1-147*	Fail	0.48	Fail	6.39	Pass	>81.9
DM-1-187*	Fail	1.65	Fail	11.6	Pass	>81.9
DM-1-225	Fail	1.13	Fail	5.07	Pass	>81.9

*** Samples run in different laboratory**

PHASE 1 RESULTS

- One anomalous sample (i.e., DM-1-068). Here, toxicity appears to be related to hydrocarbons.
- **Explanation:**

  "…*failed to run a proper viability test on the bacteria*…"
- For all other samples, toxic constituents appear to be adsorbed onto the filtering media.
  - Associated with suspended solids?
  - Offers little information re: characterization of the toxic component(s).

PHASE 1 RESULTS – PRECISION & ACCURACY

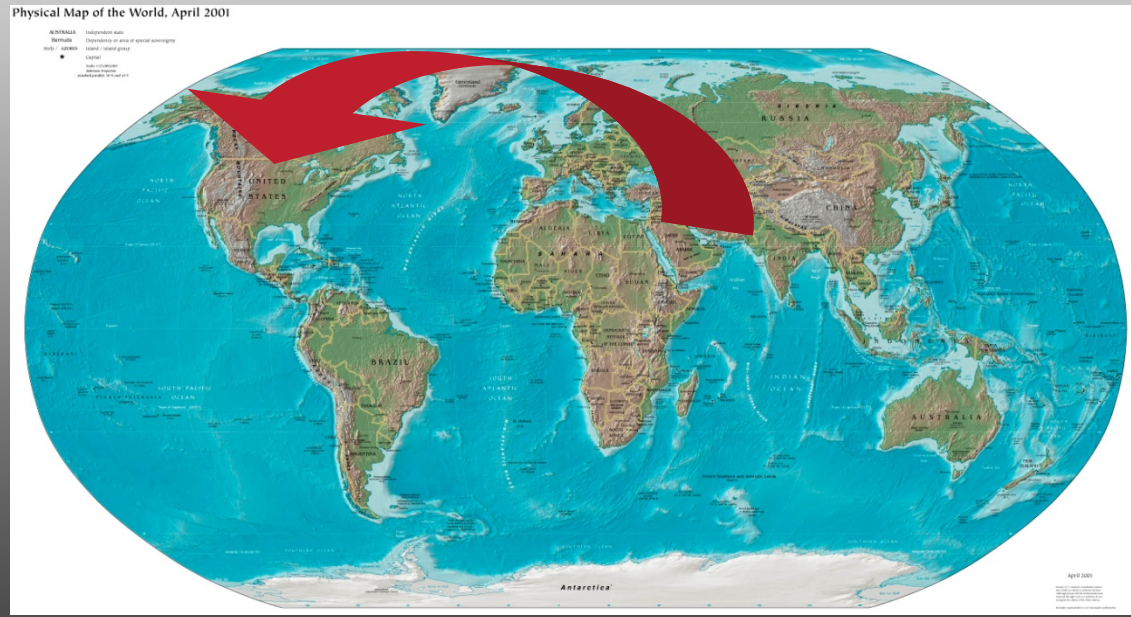
Baseline EC_{50}

- Original Microtox Assay
  - Range: 0.4 – 1.65 %
  - Mean: 0.94 %
  - 95% UCL: 1.44 %
  - SD: 0.48
- Charcoal Treatment
  - Range: 0.99 – 11.6 %
  - Mean: 4.96 %
  - 95% UCL: 8.94 %
  - SD: 3.79

PHASE 2

PHASE 2

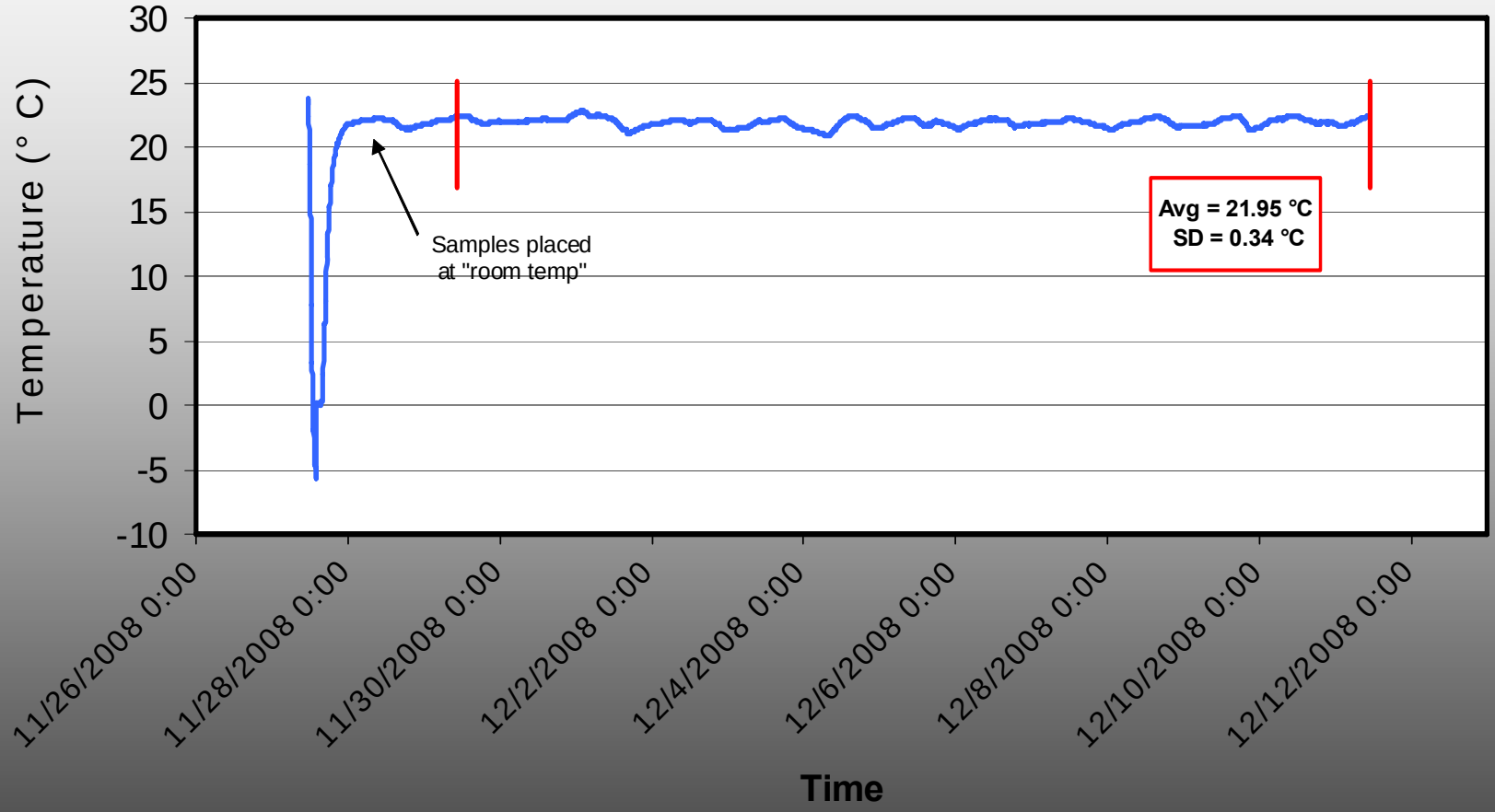
- Study to determine the effects of time and temperature on results generated using the Microtox test system.
- Particularly important for our field investigation as samples were shipped from Western Asia to Canada.



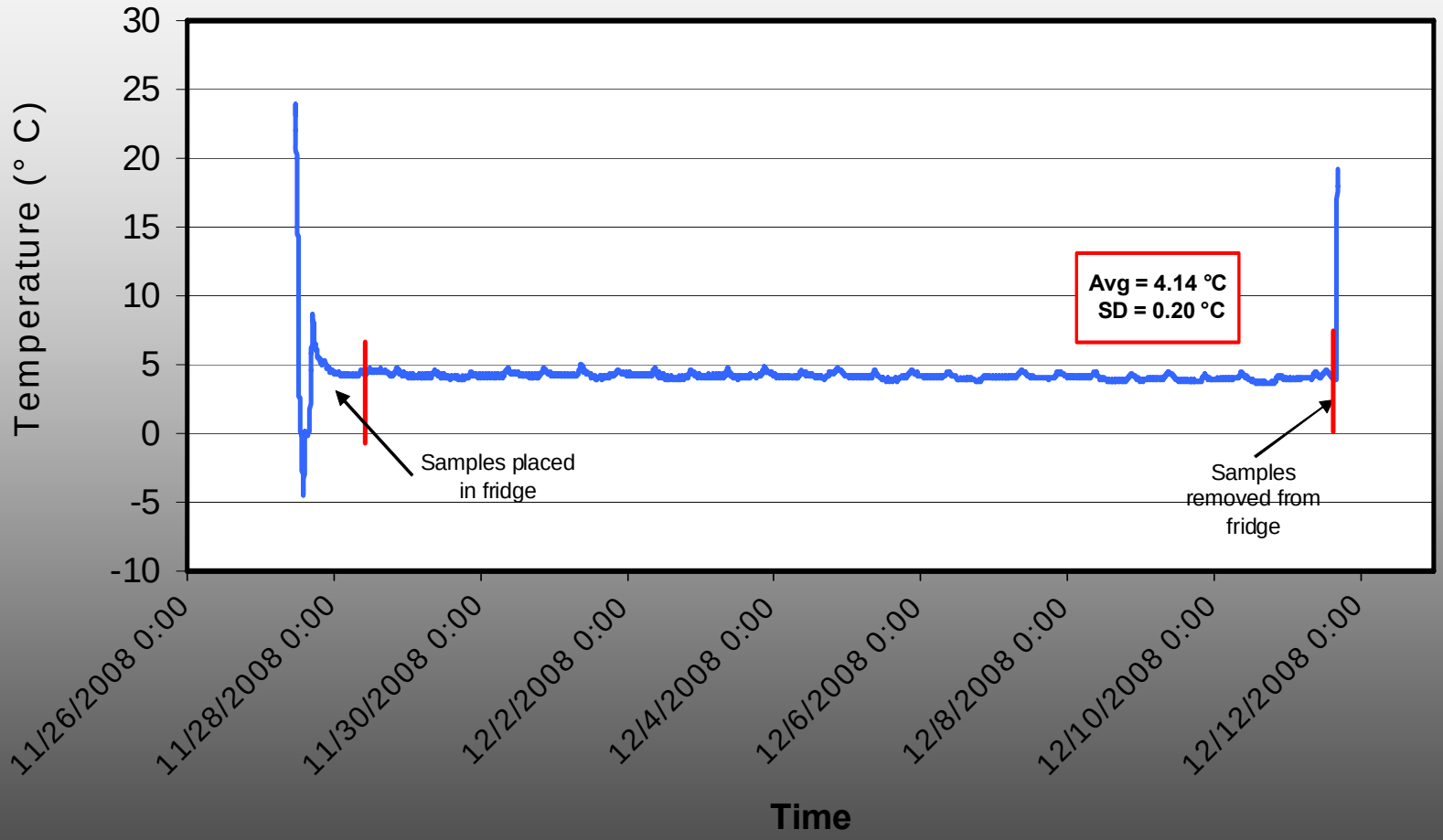
PHASE 2 - METHODOLOGY

- Fifteen SRM soil samples sent to the lab for Microtox toxicity testing:
  - five samples stored at 22°C (room temperature; RT)
  - five stored at 4°C (fridge)
  - five stored at -20°C (freezer)
- A thermochron was stored with each group of samples to log the temperature over the course of the study.
- On days 1, 3, 5, 7 and 9, one soil sample stored at RT, 4°C and -20°C underwent Microtox testing.

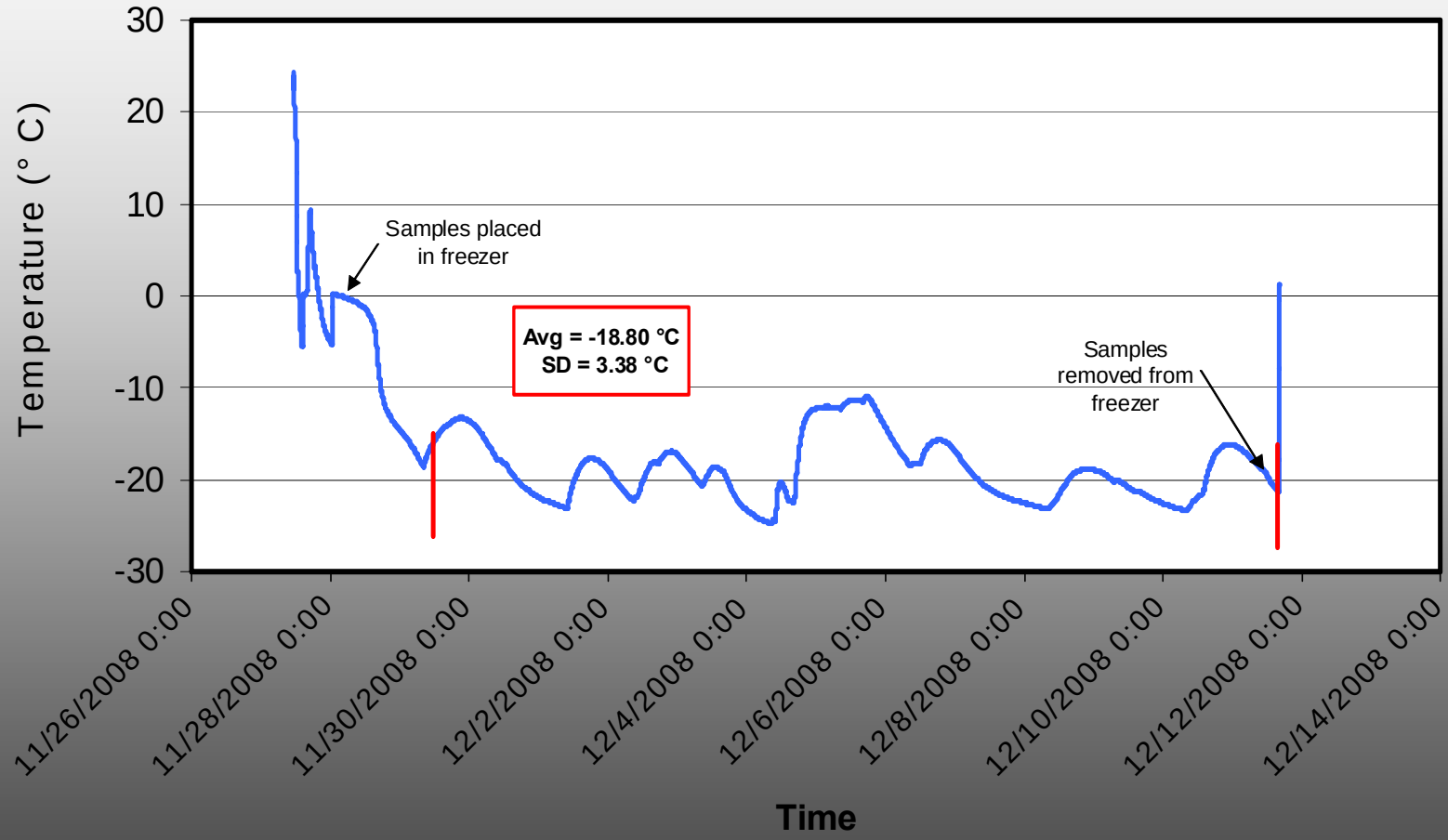
PHASE 2 RESULTS
THERMOCHRON DATA FOR RT



PHASE 2 RESULTS
THERMOCHRON DATA FOR 4°C



PHASE 2 RESULTS
THERMOCHRON DATA FOR -20°C



PHASE 2 RESULTS
ROOM TEMPERATURE

	Day 1	Day 3	Day 5	Day 7	Day 9
SRM ID	**DM-1-009**	**DM-1-015**	**DM-1-012**	**DM-1-021**	**DM-1-018**
Original Microtox	Fail	Fail	Fail	Fail	Fail
Charcoal Microtox	Fail	Fail	Fail	Fail	Fail
Aeration Microtox	Fail	Fail	Fail	Pass	Pass
Filtration Microtox	Pass	Pass	Pass	na	na

PHASE 2 RESULTS
ROOM TEMPERATURE

- Results for days 1, 3 and 5 are the same as baseline.
- Days 7 and 9, sample extracts pass following aeration.
- ⬇ in toxicity following aeration with O_2 generally indicates that toxic components can be oxidized (and rendered less toxic), volatilized, or degassed.

Rationalization of Results

- Toxic components are more readily oxidized over time?
- Aeration on days 1, 3 & 5 insufficient to rid of all VOCs, but aeration + volatilization is sufficient by days 7 & 9?

PHASE 2 RESULTS
FRIDGE, 4°C

	Day 1	Day 3	Day 5	Day 7	Day 9
SRM ID	**DM-1-010**	**DM-1-013**	**DM-1-019**	**DM-1-016**	**DM-1-022**
Original Microtox	Fail	Fail	Fail	Fail	Fail
Charcoal Microtox	Fail	Fail	Fail	Fail	Pass
Aeration Microtox	Fail	Fail	Fail	Fail	na
Filtration Microtox	Pass	Fail*	Fail**	Pass	na

*** Duplicate passed; ** EC_{50} = 58.5 (Pass at >81.9)**

PHASE 2 RESULTS
FRIDGE, 4°C

- Results for days 1 and 7 are the same as baseline.
- For day 3, the duplicate sample passed the Microtox test following filtration.
- For day 5, **no** sample extracts passed the Microtox test.
- For day 9, the sample extract passed the Microtox test after charcoal treatment.

Rationalization of Results

- Combination degradation + charcoal sufficient to cause sample to pass?

PHASE 2 RESULTS
FREEZER, -20°C

	Day 1	Day 3	Day 5	Day 7	Day 9
SRM ID	**DM-1-011**	**DM-1-017**	**DM-1-023**	**DM-1-014**	**DM-1-020**
Original Microtox	Fail	Fail	Fail	Fail	Fail
Charcoal Microtox	Fail	Fail	Fail	Fail	Fail
Aeration Microtox	Fail	Fail	Fail	Fail	Pass
Filtration Microtox	Pass	Pass	Fail*	Pass	na

*** EC_{50} = 66.1 (Pass at >81.9)**

PHASE 2 RESULTS
FREEZER, -20°C

- Results for days 1, 3 and 7 are the same as baseline.
- For day 5, **no** sample extracts passed the Microtox test. However, after filtration the EC_{50} = 66.1 (Pass at >81.9).
- For day 9, the sample extract passed the Microtox test after aeration.

Rationalization of Results

- Toxic components are more readily oxidized over time?
- Aeration on days 1, 3 & 5 insufficient to rid of all VOCs, but, aeration + volatilization is sufficient by day 9?

PHASE 2 CONCLUSIONS

General Trends

- *Effect of time* – by day 7 to 9 following the start of the study, Microtox results appeared compromised.

  ∴ Field samples should be analyzed within 7 to 9 days of sample collection.

- *Effect of temperature* – at RT samples appeared compromised at day 7 while for -20°C samples appeared compromised at day 9.

  ∴ Field samples should be kept as cold as possible.

PHASE 3

PHASE 3

- Sixty sump samples were collected from Western Asia and subjected to the Microtox toxicity test.

PHASE 3 RESULTS

- All sump samples collected either passed the original Microtox test or passed after charcoal treatment.

  ∴ either non-toxic or toxicity associated with hydrocarbons.

- General trend where samples collected from sumps receiving hydrocarbon-based muds contained the highest levels of PHCs and failed the Microtox test.

***Interesting find*:**

- Charcoal treatment caused samples (max TPH = 87,200 mg/kg) to pass Microtox, but not SRMs (average TPH = 90,659 mg/kg) ∴ PHCs not driving SRM toxicity?

RESULTS FOR SRMS SUBMITTED WITH SAMPLES FROM SITE

SRMs submitted with samples from Western Asia had similar Microtox results as baseline SRMs.

SRM ID	DM-1-025	DM-1-168	DM-1-211
Original Microtox	Fail	Fail	Fail
Charcoal Microtox	Fail	Fail	Fail
Aeration Microtox	Fail	Fail	Fail
Filtration Microtox	Pass	Fail*	Pass

* EC_{50} = 35.2 (Pass at >81.9)

ACCURACY & PRECISION

Samples from Site

- Original Microtox Assay
  - Range: 0.28 – 3.39 %
  - Mean: 1.93 %
  - 95% UCL: 5.81 %
  - SD: 1.56
- Charcoal Treatment
  - Range: 0.35 – 4.7 %
  - Mean: 1.97 %
  - 95% UCL: 7.88 %
  - SD: 2.38

Samples from Baseline Study

- Original Microtox Assay
  - Range: 0.4 – 1.65 %
  - Mean: 0.94 %
  - 95% UCL: 1.44 %
  - SD: 0.48
- Charcoal Treatment
  - Range: 0.99 – 11.6 %
  - Mean: 4.96 %
  - 95% UCL: 8.94 %
  - SD: 3.79

SUMMARY

- Based on Phase 2 SRM study, samples should undergo Microtox testing within 7 days of collection, and should be kept cold en-route to the laboratory.
- Microtox can be useful as a screening tool, however:
  - Need to follow protocols closely
  - Laboratory data should be closely scrutinized
  - SRMs can be used to ensure laboratory accuracy
- The Microtox assay provides very little information concerning the identity of toxic components.

QUESTIONS?

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TOXICITY ASSAYS

- **Trout**
  - *Pro*: higher organism, potentially 'real' receptors
  - *Con*: lengthy testing period, extensive storage/living area, $$
- **Earthworm Assay**
- **Daphnia (water fleas)**
  - *Pro*: short test (7-days), validated protocols, sensitive
  - *Con*: labour intensive, poor understanding of health/survival requirements, fragile organisms
- **Fathead minnows**
  - *Pro*: 7-day test method validated by US EPA
  - *Con*: fragility may lead to shock/death when exposed to 'non-toxic' samples.
- **Terrestrial plants**

PETROLEUM HYDROCARBON CONTENT OF SRMS

	F1	F2	F3	F4	TPH
DM-1-004	100	34,300	56,000	2,940	93,340
DM-1-044	110	31,500	51,200	2,450	85,260
DM-1-069	100	33,100	54,000	2,520	89,720
DM-1-108	110	33,100	54,000	3,680	90,890
DM-1-148	110	34,900	57,000	2,790	94,800
DM-1-188	90	35,300	57,700	2,650	95,740
DM-1-226	100	31,400	51,200	2,160	84,860
Average	102.9	33,371	54,443	2,741	90,659

Units are mg/kg