



# Identification of Particulate-Associated PAH Toxicity to *Ceriodaphnia dubia*

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## Background

- Surface water discharge from former industrial site into fish-bearing stream
- Historical uses
  - Multiple industrial users
  - Also used for landfill
- Transportation corridor
  - Railroad and highway
- Contaminants of concern
  - Metals; Organics (PAHs); High TSS also an issue

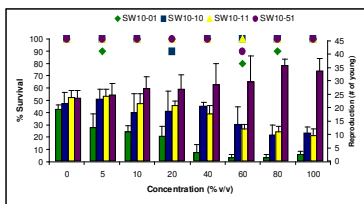
## Methods

- Samples tested for toxicity with freshwater invertebrate (*Ceriodaphnia dubia*) and green algae (*Pseudokirchneriella subcapitata*)
- Environment Canada Guidelines were followed
- Four samples were tested with both species

Test organism	<i>Ceriodaphnia dubia</i>
Test organism source	30-hour culture
Test type	Daily Static-Renewal
Test organism age	<24 hr old neonates produced within 12 hr
Test duration	7 ± 1 day
Test Chamber and solution volume	20 mL test tube; 15 mL
Test concentrations (% sample)	0, 5, 10, 20, 40, 60, 80, 100%
Number of replicates; Number of organisms/chamber	10; 1
Control/dilution water	20% Perrier water (hardness 80-100 mg/L CaCO <sub>3</sub> )
Test temperature	25 ± 1°C
Feeding	Daily, with 0.1 mL <i>Pseudokirchneriella subcapitata</i> and 0.05 mL of YCT
Light intensity	100 to 600 lux
Photoperiod	16 hours light/ 8 hours dark
Test protocol	Environment Canada (2007a), EPS 1/RM21
Test acceptability criteria for controls	≥80% survival; ≥15 young per surviving control; ≥60% of controls producing three or more broods

## Initial Results

- No adverse effects observed in all samples tested with green algae
- Three of the four samples exhibited adverse effects with *C. dubia*; primarily associated with the reproductive endpoint



## What was cause of toxicity?

- Conducted Toxicity Identification Evaluation
- USEPA guidelines used in focused approach
- Phase 1 General characterization of cause
  - EDTA: chelates various metals
  - Solid phase extraction (SPE) with C18 column: non-polar organics
  - Filtration: 0.45 um to remove particulates
  - pH10 + filtration: precipitate metals
  - pH 10 + filtration + C18: residual organics
  - Centrifugation



Reproduction of *C. dubia* in solutions of SW10-01 subjected to various TIE treatments: Phase 1

Treatment	Reproduction (# of young) Mean ± Standard Deviation
Control	10.8 ± 9.1
Untreated	0.0 ± 0.0
EDTA (25 mg/L)	1.2 ± 1.1
Filtration	18.2 ± 10.3
pH 10 + filtration	27.8 ± 2.4
pH 10 + filtration + C18	21.2 ± 6.1
C18	26.2 ± 2.6

- EDTA not effective
- pH 10 + filtration + C18 no more effective than filtration alone
- Suggests toxicity associated with particulate phase
- Wanted to confirm results using lower EDTA concentrations, as well as centrifugation;
  - Confirm major class of contaminants (metals) was not associated with toxicity, and rule out potential testing artifacts.

## Phase 1 Verification

Reproduction of *C. dubia* in solutions of SW10-01 subjected to various TIE treatments: Phase 1 Verification

Treatment	Reproduction (# of young) Mean ± Standard Deviation
Control	16.2 ± 5.9
Untreated	1.8 ± 1.3
EDTA (5 mg/L)	2.0 ± 1.4
EDTA (10 mg/L)	1.2 ± 1.8
Filtration	13.4 ± 5.9
Centrifugation (10 min)	21.6 ± 2.1
Centrifugation (30 min)	16.6 ± 4.0

## Phase 2

- Phase 1 Confirmed:
  - EDTA not effective: *metals not indicated*
  - Both filtration and centrifugation were effective: *particulate contribution to toxicity confirmed*
- Question: Is toxicity only a function of particulates, or are there particle-bound contaminants present?
- Centrifuge sample (1L)
- Extraction of particulates
  - pH 4 (metals)
  - Methanol (organics)
- Centrifuge, and
- Test supernatants for toxicity (nominal 2X)
- Verify effectiveness of centrifugation on toxicity of remaining two samples



Reproduction of *C. dubia* in extracts of particulates associated with SW10-01, and supernatants from samples SW10-10 and SW10-11.

Treatment	Reproduction (# of young) Mean ± Standard Deviation
Control	19.6 ± 2.2
Methanol control (2%)	13.6 ± 2.7
SW10-01 untreated	2.4 ± 4.3
SW10-01 acid extract	18.8 ± 4.4
SW10-01 methanol extract	0.6 ± 1.3
SW10-10 untreated	11.8 ± 2.3
SW10-10 centrifuged	17.0 ± 5.9
SW10-11 untreated	9.8 ± 1.5
SW10-11 centrifuged	18.4 ± 3.6

- Centrifugation removed toxicity in two remaining samples; particulate-associated toxicity confirmed for all toxic samples
- Weak-acid extract of particulate not toxic; suggests particulate-bound metals not contributing to toxicity
- Methanol extract of particulate similarly toxic to untreated sample; suggests bound organics responsible for toxicity
- Focus on organics vs particulates—why?
  - Toxicity decreased over time, but particulate concentration remained constant
  - Methanol extract of particulates exhibited toxicity
- Which organics?
  - Analytical results suggested PAHs were elevated (approximately 20 ug/L) in samples
  - Methanol extract contained approximately 1.5 ug/L, suggesting most PAH relatively tightly bound

## Verification

- Multiple organics could be contributing to toxicity
- Test methanol extract with and without UV irradiation – PAHs increase in toxicity with UV
- Separate organics into discrete MeOH:water fractions and test with UV irradiation

96-hr survival (%) of *C. dubia* exposed to a methanol extract of particulates from SW10-01 under UV and shaded conditions

Concentration (%)	Shaded	UV exposed
Control	100	100
Methanol control	100	100
0.25X	100	0
0.5X	100	0
1X	20	0

96-hr survival (%) of *C. dubia* exposed to a gradient of methanol extracts of particulates from SW10-01 under UV and shaded conditions

Concentration MeOH(%)	Shaded	UV exposed
Control	100	100
Methanol control	100	100
80	100	100
85	100	100
90	100	100
95	100	80
100	100	0
100	100	0
100	100	100

- Toxicity enhanced by UV irradiation, suggesting PAHs
- Toxicity recovered primarily in consecutive 100% methanol fractions, and to a lesser extent in the 95% fraction, suggesting relatively non-polar compound(s)
- PAHs in MeOH:water fractions compared to identify potential contributors to toxicity

## Conclusions

- LMW PAHs were not associated with pattern of mortality, peaked in the 90 and 95% fractions (data not shown)
- Toxicity largely related to distribution of HMW PAHs in fractions
- Candidate compounds included benzo(a)pyrene and benzofluoranthene
- PAHs in sample were dominated by HMW PAHs, suggested PAHs sorbed to particulates were most likely responsible for original toxicity
- If needed, studies with suspect compounds could be used to confirm effects concentrations.