

DISCLAIMER: PTAC does not warrant or make any representations or claims as to the validity, accuracy, currency, timeliness, completeness or otherwise of the information contained in this report , nor shall it be liable or responsible for any claim or damage, direct, indirect, special, consequential or otherwise arising out of the interpretation, use or reliance upon, authorized or unauthorized, of such information.

The material and information in this report are being made available only under the conditions set out herein. PTAC reserves rights to the intellectual property presented in this report, which includes, but is not limited to, our copyrights, trademarks and corporate logos. No material from this report may be copied, reproduced, republished, uploaded, posted, transmitted or distributed in any way, unless otherwise indicated on this report, except for your own personal or internal company use.

A rapid bioassay for predicting toxicity of PHC-contaminated soil, Phase 3

Final Report

AUPRF Project #13-AU-SGRC-06

April 30, 2014

Authors:

John Ashworth and Lisa Watt
ALS Laboratory Group
9936 67 Avenue
Edmonton, AB T6E 0P5

Leila Oosterbroek and Bryon Shore
HydroQual Laboratories Ltd.
4-6125 12 Street SE
Calgary, AB T2H 2K1

Gladys Stephenson and Robin Angell
Stantec
70 Southgate Drive, Suite 1
Guelph, ON N1G 4P5

Table of Contents

Summary..... 4

Introduction..... 5

Materials and Methods 6

 Cyclodextrin..... 6

 Amylase. 6

 Soils. 6

 Soil extraction..... 8

 Enzyme-catalysed hydrolysis and Microtox® testing. 8

 Verification of Microtox® sensitivity to γ -CD extracted PHC concentrations. 8

 Earthworm bioassays. 8

Assessment Method 9

Results and Discussion 10

 Verification of Microtox® sensitivity to γ -CD extracted PHC concentrations. 10

 Donated soils..... 11

 Stantec-donated soils..... 13

 Bioassay results and PHC values. 15

Conclusions..... 16

Literature References 17

List of Tables and Figures

Table 1. Physical and chemical characterization of the nine donated soils	7
Table 2. Physical and chemical characterization of the Stantec-donated soils	7
Table 3. Rapid bioassay and chronic earthworm test results for the nine donated soils	12
Table 4. Agreement between the rapid bioassay and chronic earthworm endpoints for the nine donated soils	13
Table 5. Rapid bioassay and chronic earthworm test results for the six Stantec-donated soils....	14
Table 6. Agreement between the rapid bioassay and chronic earthworm endpoints for the six Stantec-donated soils	15
Table 7. Comparison of rapid bioassay and chronic earthworm endpoint results with solvent-extractable PHC content.....	16
Figure 1. Potential bioassay agreement scenarios for the earthworm survival endpoint.....	10
Figure 2. Verification of Microtox® sensitivity to increasing PHC spike concentrations extracted using γ -CD.....	11

List of Appendices

Appendix 1. Chronic earthworm toxicity results for nine donated soils

Appendix 2. AUPRF Project # 913751 (Phase 1) final report

Appendix 3. AUPRF Project # 09-9159-50 (Phase 2) final report

Summary

A rapid (2-h), Microtox®-based bioassay intended for soils contaminated with petroleum hydrocarbons (PHC) above Tier 1 remediation criteria, but potentially sufficiently weathered to be candidates for Tier 2 site-specific assessment, was developed and refined during the first two phases of this project. This assay was not intended to replace any of Environment Canada's battery of Tier 2 tests, but rather to identify soils with toxic levels of bioavailable PHC prior to initiating testing. It was hoped that screening these soils prior to testing would avoid the unnecessary time and expense associated with conducting the full battery of ecotoxicity tests necessary to achieve regulatory closure.

Phase 3 of this project was an expansion of efforts conducted during the first two phases to correlate results of the rapid bioassay with results obtained from the earthworm survival and reproduction bioassay required as part of the full Tier 2 testing battery. While tentative agreement between the rapid bioassay and the earthworm survival endpoint was observed in Phase 2, no correlation was observed for the other two test endpoints (juvenile production and weight). Phase 2 trials using peat-amended soils showed that soil texture/porosity may exacerbate or otherwise interact with PHC levels to influence observed toxicity.

The rapid bioassay and earthworm survival and reproduction test, as well as physicochemical characterization, were conducted on nine donated soils. Six of eight additional soils donated by Stantec, for which earthworm and/or soil chemistry data were already available, were also subjected to rapid bioassay testing. Two of the eight Stantec soils were unable to undergo the rapid bioassay due to their high moisture content. HydroQual Laboratories Ltd. (HydroQual) and ALS Laboratory Group (ALS) performed duplicate rapid bioassay testing on a subset of samples to ensure reliability of results. Similarly, HydroQual and Stantec performed duplicate testing on one of the donated soils to ensure reliability of chronic earthworm test results.

Results for both the rapid bioassay and earthworm 35-day survival endpoints agreed for all but one of the soils tested. In the case where the tests disagreed, the rapid bioassay identified the soil as toxic while the earthworm test did not (i.e., a false positive result). Agreement for 63-day juvenile reproduction was achieved less than half the time (5 of thirteen tests), with false negative results (i.e., the rapid bioassay did not identify the soil as toxic when in fact it was) forming the majority of disagreements. Results for 63-day juvenile weight were generally positive, with agreement in the majority of cases (10 of thirteen tests). Disagreements were comprised of two false negatives and one false positive result.

While the rapid bioassay results as a whole were generally predictive of those obtained during the earthworm testing, the lack of correlation with juvenile reproduction data and the presence of both false positive and false negative results preclude the rapid bioassay as a blanket indicator of soil toxicity as outlined in the Tier 2 criteria. Given an understanding of these limitations, however, it may be possible to use the rapid bioassay in conjunction with other

information to help determine whether contaminated soils are likely to pass the earthworm survival and reproduction component of the Tier 2 test battery.

Introduction

Decisions regarding remediation or disposal of petroleum hydrocarbon (PHC)-contaminated soils are based on solvent-extractable PHC concentrations, which may exceed established guidelines (CCME 2008) despite residual PHC having become biologically unavailable (and thus non-toxic to biota) with time through weathering (Reid et al. 2000; Axiom 2005). The goal of this project was to develop a fast and relatively inexpensive screening-level bioassay to predict soil toxicity caused by bioavailable PHC. Provided that a correlation between the screening test and one or more terrestrial bioassays exists, this information would aid in deciding whether soils exceeding CCME-mandated PHC thresholds are likely to satisfy the eco-contact requirements of the full Tier 2 battery of terrestrial toxicity tests, thus justifying the expense of this further testing.

In brief, the rapid bioassay involves shaking a representative soil sample with aqueous hydroxypropyl- γ -cyclodextrin (CD) to recover bioavailable PHC; the centrifuged extract is then treated with amylase before being subjected to a standard Microtox[®] test, which employs the luminescent marine bacteria *Vibrio fischeri*. Previous research has shown that hydroxypropyl- β -CD solution primarily extracts bioavailable PHC from contaminated soils (Reid et al. 2000, Axiom 2005), however previous phases of this project found little relation between Microtox[®] test results and hydroxypropyl- β -CD solution PHC extract concentrations, presumably due to occlusion by the hydroxypropyl- β -CD structure. Hydroxypropyl- γ -CD has previously been reported to be susceptible to enzyme-catalyzed hydrolysis when exposed to amylase (Jodál et al. 1984), and hydroxypropyl- γ -CD solution, followed by an amylase digestion step to hydrolyse the CD structure following PHC extraction, was shown to be a suitable alternative to hydroxypropyl- β -CD solution in Phase 2 of this project. Final reports from both previous phases of this project are included as appendices to this report.

Phase 3 of this project was an expansion of efforts conducted during the first two phases to correlate results of the rapid bioassay with results obtained for the Environment Canada earthworm survival and reproduction bioassay (2004) required as part of the full Tier 2 testing battery. The chronic earthworm bioassay is the longest (56 or 63 days) and most expensive test required by the Tier 2 testing battery, and it is therefore essential that screening test results are predictive of earthworm survival and reproduction to have value for industry. Tentative agreement between the rapid bioassay and the earthworm survival endpoint was observed in Phase 2, however no correlation was observed for the other two test endpoints (juvenile production and weight). As only one soil tested during Phase 2 had significant solvent-extractable PHC, however, it is possible that these results may not be indicative of the predictive capacity of the rapid bioassay. As such, a third phase, using additional donated soils, was

initiated to determine the degree to which the rapid bioassay may predict chronic earthworm bioassay results.

Materials and Methods

Cyclodextrin. Gamma cyclodextrin (Hydroxypropyl γ -cyclodextrin; CAS 128446-34-4) was purchased from Sigma Life Sciences. Weighed amounts of γ -CD were magnetically stirred into ultra pure water and then made up to the volume required for an 8% solution (8 g per 100 mL).

Amylase. Liquid formulation Fungamyl[®] 800L, which contains α -amylase from the fungus *Aspergillus oryzae*, was purchased from Sigma Life Sciences. This preparation was previously used by Jodál et al. (1984) hydrolyze γ -CD. The Fungamyl was stored at 4 ± 2 °C.

Analysis of a sample indicated that Fungamyl contains 120 g NaCl per litre. Before adding Fungamyl to aqueous CD, it was diluted (2 parts by volume) with ultra pure water (1 part), in order to both lower its viscosity (thus making it easier to pipet reproducibly), and to reduce the salinity of Fungamyl to a level suitable for conducting routine Microtox[®] testing.

Soils. Nine real-world soils were donated to the project for Microtox[®] and chronic earthworm testing. These soils were analyzed for various inorganic and organic parameters, and were found to range widely in PHC content (Table 1). Four soils had elevated levels of Ba, while another two were low in organic matter (OM), characteristics that may be of concern for earthworm reproduction; all soils were subjected to earthworm testing. Microtox[®] testing was also performed on six additional soils donated by Stantec for which earthworm and/or soil analytical data had already been collected. Parameters for these soils are included in Table 2.

Table 1. Physical and chemical characterization of the nine donated soils.

Sample ID	Description	Texture	OM (%)	pH	EC (ds/m)	SAR	Elements (mg/kg)	F2 (mg/kg)	F3 (mg/kg)	F4 (mg/kg)
13-1258-1	Greenfill	Sandy Loam	1.8	7.5	5.74	6.61	***	70.4	121	14.5
13-1258-2	sample 1 11-31-69-3W6	Loam	2.5	7.5	3.32	1.75	***	13.2	32.3	<5.0
13-1259-1	sample 1	Clay Loam	1.3	8.0	0.598	1.11	***	39.8	78.8	5.7
13-1259-2	sample 2	Sandy Loam	1.7	7.7	1.38	0.26	***	9.8	269	109
13-1361	sample 2	Loam	6.2	7.0	6.41	4.02	Ba - 1070	36.9	47.5	10.4
13-1362-1	Gregg River 4-6-49-24W5	Sandy Loam	4.1	5.6	1.98	0.23	Ba - 833	18.2	694	30.5
13-1362-2	McLeod River 4-13-50-23W5	Loam	8.4	6.2	1.98	0.18	Ba - 1160	8	119	85
13-1362-3	OBED 8-31-52-22W5	Loam/Sandy clay loam	2.6	7.3	1.11	0.53	Ba - 2780	29	744	35
13-1367	2-31-88-8W5	Sandy Clay Loam	2.7	7.0	3.13	0.96	***	31.4	389	130

***Below Tier 1 criteria (Alberta Environment 2010)

Table 2. Physical and chemical characterization of the Stantec-donated soils.

Sample ID	Description	Texture	OM (%)	pH	EC (ds/m)	F2 (mg/kg)	F3 (mg/kg)	F4 (mg/kg)
13-1162-1	ON Ref. (Control)	Sand	0.2	5.48	13.1	5	5	5
13-1162-2	ON Plot 1 (Contaminated)	Sand	0.9	6.11	27.4	3620	2500	748
13-1162-5	MB TP11-1 (Control)	Clay loam	4.4	7.72	255	5	110	18
13-1162-6	MB TP2-1 (Contaminated)	Silty clay loam	7.5	8.24	479	31	2500	500
13-1162-7	MB TP5-1 (Contaminated)	Silt loam	10.3	7.87	856	120	5400	1000
13-1162-8	MB TP9-1 (Contaminated)	Silt loam	11	7.96	681	130	8100	1600

*F2 and F3 fractions approximated from EPH 10-19 and EPH 19-32, respectively.

Soil extraction. Moist soil subsamples (equivalent to 5.0 g dry soil) were treated with 40 mL of 8 % CD solution. This combination of soil:solution ratio and CD concentration (Axiom 2005) provided a large stoichiometric excess of CD over total PHC in the soils tested. Extraction was done in plastic centrifuge tubes shaken end-to-end at 120 excursions per minute for 30 minutes, the time that gave the best recovery of added PHC in previous work (Axiom 2005). The resulting suspension was centrifuged for 15 min. at 3,000 x g and the clear supernatant (CD extract) transferred to a glass vial.

Enzyme-catalysed hydrolysis and Microtox® testing. CD soil extracts thus obtained (4 mL) were treated with 0.4 mL of 2:1 Fungamyl-water mixture at room temperature (21 ± 2 °C) and a Microtox® test was performed within 1 h, using the 4-serial dilutions ISA procedure (10.2, 20.5, 40.9 and 81.8 %), with aqueous phenol as the reference standard (WCMUC 1994). No pH-adjustment or colour-correction was required. The endpoint of interest for the rapid bioassay was the EC₅₀ (15); the effective concentration at which the organisms displayed a 50% decrease in response (i.e., light output) at 15 minutes. Microtox® testing was duplicated at HydroQual and ALS for a number of samples to ensure reproducibility of results.

Verification of Microtox® sensitivity to γ -CD extracted PHC concentrations. Duplicate Microtox® testing was performed at HydroQual and ALS on artificial PHC spike samples extracted using the γ -CD extraction and α -amylase hydrolysis method outlined in the previous sections. Spikes of 50, 100, and 200 mg/L PHC were created by serially diluting a weathered diesel sample with 8% γ -CD solution. The spikes were extracted, hydrolyzed, and tested using Microtox® to verify test sensitivity to increasing PHC concentrations. Additional 50 mg/L PHC samples were extracted using γ -CD but were not hydrolyzed using α -amylase to verify the necessity of the digestion step. Finally, control samples (deionized water) were treated with γ -CD, hydrolyzed with α -amylase, and tested using Microtox® to determine any toxicity caused by the extraction process itself.

Earthworm bioassays. Chronic earthworm bioassays were conducted at HydroQual using modified Environment Canada (2004) methods. Modifications were applied to the numbers of concentrations tested for each soil, and not to other test parameters. After homogenization, the raw soils were successively diluted with artificial soil so as to obtain 90.9, 45.5, 22.7 and 11.4 % concentrations of each original soil. Lab controls (100 % artificial soil) were also prepared. Following soils preparation, testing was initiated according to the Environment Canada method (2004). Adult survival was assessed after 35 days, and juvenile production and growth were assessed after 63 days. For each soil, adult survival, juvenile production (number of juveniles), and juvenile growth (dry mass per juvenile) were assessed for each concentration relative to controls (i.e., controls set to 100%), and LC₅₀, LC₂₅, IC₅₀ and IC₂₅ values were calculated for each parameter as appropriate. LC₅₀ and LC₂₅ values are the lethal concentrations at which 50% or 25% of the organisms, respectively, no longer survive. IC₅₀ and IC₂₅ values are the inhibitory concentrations at which 50% or 25% of the organisms, respectively, are inhibited/affected for a defined endpoint (i.e. juvenile production and growth). A duplicate chronic bioassay for one of the samples (13-1361) was conducted at Stantec to ensure reproducibility of results.

Assessment Method

Agreement between the rapid bioassay and chronic earthworm bioassay results was assessed on the basis of the Tier 2 eco-contact criteria. The Tier 2 assessment criteria pertinent to the chronic earthworm bioassay for natural areas, agricultural, and residential/parkland land uses are as follows (Alberta Environment 2007):

1. When statistically significant differences are identified between site and reference soils, the differences must be $\leq 25\%$ for at least 75% of the endpoints [among all bioassays]; and
2. Test organism mortality must be no greater in site soil than reference soil; and
3. Invertebrate reproduction in site soil treatments must not be less than 50% of that in reference soil; and
4. No more than one endpoint per test species may exceed a 25% difference between site and reference soils.

Criteria for commercial and industrial land uses are similar, with the criteria specifying $\leq 25\%$ differences between endpoints relaxed to $\leq 50\%$:

1. No more than 25% of the endpoints shall have a statistically significant difference between site and reference soils of $> 50\%$; and
2. Test organism mortality must be no greater in site soil than reference soil; and
3. Invertebrate reproduction in site soil treatments must not be less than 50% of that in reference soil; and
4. No more than one endpoint per test species may exceed a 50% difference between site and reference soils.

For the earthworm survival endpoint, the tests were determined to be in agreement if i) both the EC_{50} (15) for the rapid bioassay and the LC_{25} for the chronic earthworm test were greater than the highest concentration tested ($> 82\%$ and $> 91\%$ for the rapid bioassay and earthworm test, respectively) (non-toxic); or ii) both the EC_{50} (15) for the rapid bioassay and the LC_{25} for the chronic earthworm test were less than the highest concentration tested (toxic). In cases where the tests disagreed, the result was deemed a false positive if the rapid bioassay determined the soil as toxic when the earthworm test did not, and a false negative when the rapid bioassay found the soil to be non-toxic when the earthworm test yielded a toxic result (Figure 1).

		Earthworm Bioassay LC ₂₅	
		<91%	>91%
Rapid Bioassay EC ₅₀ (15)	<82%	Agree - Toxic	False Positive
	>82%	False Negative	Agree - Non-toxic

Figure 1. Potential bioassay agreement scenarios for the earthworm survival endpoint.

Agreement for the juvenile reproduction endpoint was determined similarly to earthworm survival, except the reproduction IC₅₀ was used in place of the LC₂₅ endpoint. As the threshold for a toxic result for juvenile weight varies depending on the desired land use, agreement between the rapid bioassay and the chronic earthworm test was assessed against both thresholds (IC₂₅ and IC₅₀).

Determination of agreement between the rapid bioassay and results for the Stantec-donated soils was performed slightly differently. Earthworm data for these soils were obtained from screens such that each soil was tested at full strength (i.e., no dilutions) and data were not compared against a reference to determine LC/IC values. However, the suite of soils contained samples from two provinces (Ontario and Manitoba), with one soil from each province corresponding to a control site (Table 2). As such, these soils were assessed by comparing mean survival, reproduction, and juvenile weight values for the contaminated soils within each province against those obtained for the corresponding controls to determine whether a 25% and/or 50% effect was observed (as applicable). Additionally, agreement between the two bioassays for the earthworm survival endpoint was also possible within each control soil as these endpoints were scored on a closed (percentage) scale.

Results and Discussion

Verification of Microtox® sensitivity to γ-CD extracted PHC concentrations. Results of the verification testing are shown in Figure 2. Microtox® testing of control samples treated using the γ-CD extraction and α-amylase hydrolysis steps did not exhibit any toxicity. Similarly, 50 mg/L PHC spikes extracted using γ-CD but not hydrolyzed using α-amylase did not exhibit any toxicity, either. This result confirms the necessity of the hydrolysis step to free bound PHC from the γ-CD structure. Finally, the 50, 100, and 200 mg/L PHC spikes exhibited increasing toxicity to Microtox® as expected. Results obtained by HydroQual and ALS were similar for all samples tested.

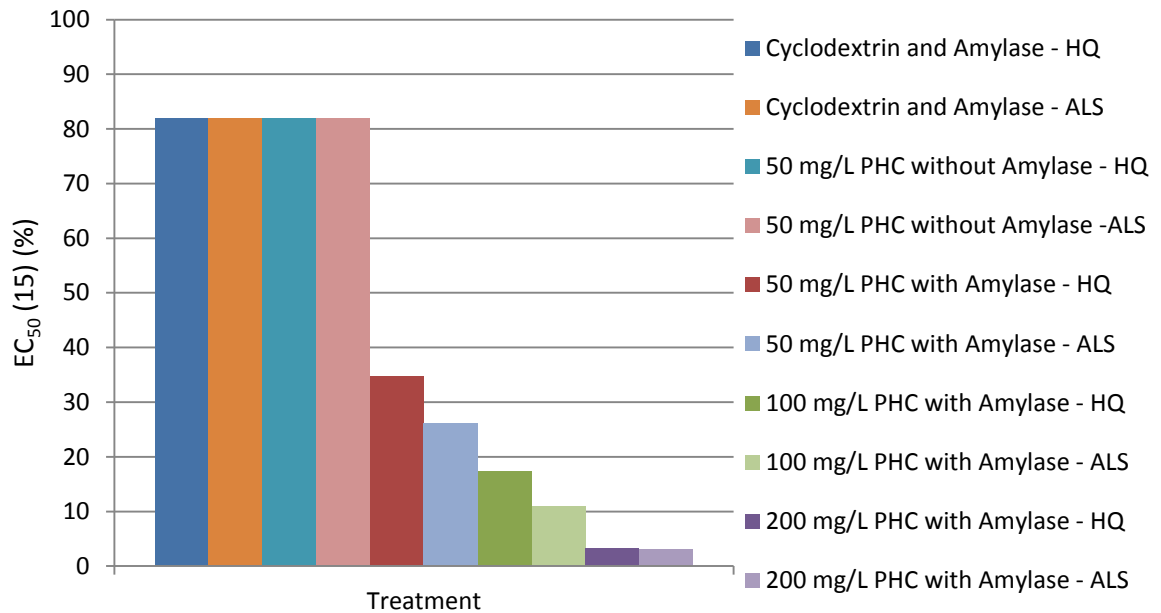


Figure 2. Verification of Microtox® sensitivity to increasing PHC spike concentrations extracted using γ -CD

Donated soils. Of the nine donated soils from contaminated field sites, none had solvent-extractable PHC in excess of Tier 1 criteria (3,600 mg/kg F2-F4; Alberta Environment 2010) (Table 1). Three, however, had F3 fractions in excess of Tier 1 criteria (300 mg/kg).

Results of the rapid bioassay and chronic earthworm test for the nine donated soils are shown in Table 3. Notably, results of the duplicate rapid bioassays conducted at HydroQual and ALS agreed for all nine soils tested. Results of duplicate testing of soil 13-1361 at HydroQual and Stantec also agreed for all chronic earthworm test endpoints. Results for all individual chronic earthworm tests are included in Appendix 1.

Table 3. Rapid bioassay and chronic earthworm test results for the nine donated soils.

Sample ID	Description	Rapid Bioassay EC ₅₀ (15min) (%)		Earthworm Survival	Juvenile Production	Juvenile Weight	
		HQ data	ALS data	LC ₂₅ (%)	IC ₅₀ (%)	IC ₅₀ (%)	IC ₂₅ (%)
13-1258-1	Greenfill	<10	<10	14	<11	22	<11
13-1258-2	sample 1 11-31-69-13W6	>82	>82	>91	42	>91	>91
13-1259-1	sample 1	>82	>82	>91	53	>91	>91
13-1259-2	sample 2	>82	>82	>91	76	>91	>91
13-1361	sample 2	15	<10	>91*	>91*	>91*	>91*
13-1362-1	Gregg River 4-6-49-24W5	>82	>82	>91	34	19	<11
13-1362-2	McLeod River 4-13-50-23W5	>82	>82	>91	>91	>91	>91
13-1362-3	OBED 8-31-52-22W5	>82	>82	>91	40	40	20
13-1637	2-31-88-8W5	>82	>82	>91	>91	>91	>91

*Similar results (i.e., no observed effects relative to lab controls) were obtained for tests conducted at both HydroQual and Stantec.

Agreement between the rapid bioassay and the chronic earthworm endpoints is simplified in Table 4. Notably, earthworm survival agreed for all but one soil. As the disagreement was a false positive (i.e., the rapid bioassay flagged a toxic result when it should not have), the rapid bioassay results would still be protective from the standpoint that they would not result in conducting chronic earthworm tests with soils that fail this endpoint. The presence of false positives could become a greater concern, however, if they resulted in digging and dumping soils that would otherwise pass the chronic earthworm endpoints.

Results for the juvenile reproduction endpoint were much less positive, as six of the nine soils yielded false positive or negative results. As five of the six disagreements were false negatives (i.e., the rapid bioassay results would recommend conducting the full 63-day earthworm test), the rapid bioassay does not appear reliable for safely predicting this endpoint.

Results for the juvenile weight endpoint were mixed, with agreement for six of the nine soils. Two of the three disagreements, however, were false negatives, indicating that while the rapid bioassay may be generally predictive of this endpoint, the results may not be completely protective/reliable.

Table 4. Agreement between the rapid bioassay and chronic earthworm endpoints for the nine donated soils.

Sample ID	Description	Earthworm	Juvenile	Juvenile Weight	
		Survival	Production	IC ₅₀ (%)	IC ₂₅ (%)
		LC ₂₅ (%)	IC ₅₀ (%)	IC ₅₀ (%)	IC ₂₅ (%)
13-1258-1	Greenfill	Agree	Agree	Agree	Agree
13-1258-2	sample 1 11-31-69-13W6	Agree	FN	Agree	Agree
13-1259-1	sample 1	Agree	FN	Agree	Agree
13-1259-2	sample 2	Agree	FN	Agree	Agree
13-1361	sample 2	FP	FP	FP	FP
13-1362-1	Gregg River 4-6-49-24W5	Agree	FN	FN	FN
13-1362-2	McLeod River 4-13-50-23W5	Agree	Agree	Agree	Agree
13-1362-3	OBED 8-31-52-22W5	Agree	FN	FN	FN
13-1637	2-31-88-8W5	Agree	Agree	Agree	Agree

Note: FN = False Negative; FP = False Positive

Stantec-donated soils. Of the six Stantec-donated soils, three had solvent-extractable PHC (F2-F4) in excess of Tier 1 criteria. Four of these soils had F2 and/or F3 fractions in excess of Tier 1 criteria (130 mg/kg and 300 mg/kg, respectively).

Results of the rapid bioassay and chronic earthworm test for the nine donated soils are shown in Table 5. Notably, results of the duplicate rapid bioassays conducted at HydroQual and ALS agreed for all five soils tested.

Table 5. Rapid bioassay and chronic earthworm test results for the six Stantec-donated soils.

Sample ID	Sample Description	Rapid Bioassay EC ₅₀ (15min) (%)		Earthworm Survival	Juvenile Production	Juvenile Weight
		HQ data	ALS data	%	#	mg/worm
13-1162-1	ON Ref. (Control)	>82	-	100	54	0.2
13-1162-2	ON Plot 1 (Contaminated)	<10	<10	0	-	-
13-1162-5	MB TP11-1 (Control)	>82	>82	80	15	1.65
13-1162-6	MB TP2-1 (Contaminated)	>82	>82	100	11	1.45
13-1162-7	MB TP5-1 (Contaminated)	>82	>82	90	7	3.26
13-1162-8	MB TP9-1 (Contaminated)	>82	>82	70	3	3.18

Note: Earthworm test data were not available for soils 13-1162-3 and 13-1362-4.

Agreement between the rapid bioassay and the chronic earthworm endpoints is simplified in Table 6. Notably, and similar to results for the nine donated soils, earthworm survival agreed for all Stantec soils tested. The rapid bioassay therefore appears to be predictive for the earthworm survival endpoint.

Results for the juvenile reproduction endpoint were similar to those obtained for the nine donated soils, with agreement for only half (2 of four) soils tested. Additionally, the two disagreements were false negatives (i.e., the rapid bioassay results would recommend conducting the full 63-day earthworm test). Again, the rapid bioassay does not appear reliable for safely predicting this endpoint.

Results for the juvenile weight endpoint were positive, with agreement for all four soils tested. These results underscore those obtained for the donated soils, in that the rapid bioassay generally appears reliable for predicting this endpoint.

Table 6. Agreement between the rapid bioassay and chronic earthworm endpoints for the six Stantec-donated soils.

Sample ID	Description	Earthworm	Juvenile	Juvenile
		Survival	Production	Weight
		25% Effect	50% Effect	25/50% Effect
13-1162-1	ON Ref. (Control Soil)	Agree	n/a	n/a
13-1162-2	ON Plot 1 (Contaminated)	Agree	Agree	Agree
13-1162-5	MB TP11-1 (Control)	Agree	n/a	n/a
13-1162-6	MB TP2-1 (Contaminated)	Agree	Agree	Agree
13-1162-7	MB TP5-1 (Contaminated)	Agree	FN	Agree
13-1162-8	MB TP9-1 (Contaminated)	Agree	FN	Agree

Note: FN = False Negative. Effects for contaminated soils were determined by comparing mean endpoint value relative to the corresponding control. Survival effects for control soils were evaluated relative to 100% survival.

Bioassay results and PHC values. There was little correspondence between solvent-extractable PHC content of the soils as tested and the rapid bioassay and chronic earthworm test results (Table 7). PHC content of one or more fractions above Tier 1 criteria corresponded to a confirmed toxic result (toxic to both the rapid bioassay and chronic earthworm endpoints) in only one instance. The other confirmed toxic result was for soil 13-1258-1, which had low PHC content. Conversely, soils with one or more elevated PHC fractions yielded confirmed non-toxic results for one or more endpoints in six instances. No trends of false positive or negative results relative to PHC content were observed. Observed toxicity may have been altered by one or more physicochemical characteristics (e.g., soil texture, Ba content; see Tables 1, 2), however no obvious trends were observed. Alternatively, observed toxicity may have varied as a function of site age (and therefore degree of PHC weathering). As site ages for the soils tested in this study are not known, it is possible that the non-toxic results for soils with elevated solvent-extracted PHC values may be partially due to low concentrations of bioavailable PHC.

Table 7. Comparison of rapid bioassay and chronic earthworm endpoint results with solvent-extractable PHC content

Sample ID	Description	Earthworm Survival	Juvenile Production	Juvenile Weight	PHC Fraction		
		25% Effect	50% Effect	25/50% Effect	F2 (mg/kg)	F3 (mg/kg)	F4 (mg/kg)
13-1162-1	ON Ref. (Control)	Non-Toxic	n/a	n/a	5	5	5
13-1162-2	ON Plot 1 (Contaminated)	Toxic	Toxic	Toxic	3620	2500	748
13-1162-5	MB TP11-1 (Control)	Non-Toxic	n/a	n/a	5	110	18
13-1162-6	MB TP2-1 (Contaminated)	Non-Toxic	Non-Toxic	Non-Toxic	31	2500	500
13-1162-7	MB TP5-1 (Contaminated)	Non-Toxic	FN	Non-Toxic	120	5400	1000
13-1162-8	MB TP9-1 (Contaminated)	Non-Toxic	FN	Non-Toxic	130	8100	1600
13-1258-1	Greenfill	Toxic	Toxic	Toxic	70.4	121	14.5
13-1258-2	sample 1 11-31-69-13W6	Non-Toxic	FN	Non-Toxic	13.2	32.3	<5.0
13-1259-1	sample 1	Non-Toxic	FN	Non-Toxic	39.8	78.8	5.7
13-1259-2	sample 2	Non-Toxic	FN	Non-Toxic	9.8	269	109
13-1361	sample 2	FP	FP	FP	36.9	47.5	10.4
13-1362-1	Gregg River 4-6-49-24W5	Non-Toxic	FN	FN	18.2	694	30.5
13-1362-2	McLeod River 4-13-50-23W5	Non-Toxic	Non-Toxic	Non-Toxic	8	119	85
13-1362-3	OBED 8-31-52-22W5	Non-Toxic	FN	FN	29	744	35
13-1637	2-31-88-8W5	Non-Toxic	Non-Toxic	Non-Toxic	31.4	389	130

Note: FN = False Negative; FP = False Positive. Red values indicate PHC levels above Tier 1 criteria (Alberta Environment 2010).

Conclusions

Overall, results for the rapid bioassay were mixed. While this test was quite robust in predicting earthworm survival results (agreement for 14 of fifteen soils, with one soil yielding a false positive), predictive value for the juvenile reproductive endpoint was negligible (agreement for 5 of thirteen soils) with a concerning number (from the standpoint of the ability of the test to identify soils likely to fail the chronic earthworm endpoints) of false negative results (7 of thirteen soils). Results for the juvenile weight endpoint were generally positive (agreement for 10 of thirteen soils), however the presence of two false negative outcomes tempers the predictive ability of the rapid bioassay for this endpoint as these results indicate the likely success of a given soil in passing the chronic earthworm test in instances where it would fail.

The lack of an obvious relationship between both rapid bioassay and chronic earthworm test results, and the measured soil physical and chemical characteristics (including PHC content), speaks to the larger issue of the complexity of soil physicochemical interactions and observed toxicity in mandatory tests. For example, soil texture (in regard to clay-rich sub-soils) is acknowledged in the Tier 2 guidelines as being potentially problematic when conducting earthworm bioassays (Alberta Environment 2007, section 4.6). This phenomenon was further demonstrated during the peat-addition trials conducted as part of Phase 2 of this project. It is possible that rather than being a discrepancy between the rapid bioassay and PHC-related earthworm toxicity, the frequency of false negative results for the juvenile reproduction endpoint may instead indicate earthworm toxicity from non-PHC related factors and/or cumulative effects of PHC with other soil characteristics.

While the results obtained in Phase 3 of this project do not support the blanket applicability of the rapid bioassay for screening Tier 2 candidate soils, the strong agreement between the rapid bioassay results and the chronic earthworm survival endpoint suggest that this test may serve as a useful decision-making tool in conjunction with additional analytical and/or experimental information.

Literature References

Alberta Environment, 2007. Tier 2 Eco-Contact Guideline Derivation Protocol – Draft.

Alberta Environment, 2010. Alberta Tier 1 Soil and Groundwater Remediation Guidelines.

Ashworth and Bullecer, 2012. A rapid bioassay to screen soils for toxicity of residual petroleum hydrocarbons. *Canadian Journal of Soil Science* 92:901-904.

Axiom, 2005. Environmentally acceptable endpoints for weathered/aged PHC fraction F3 in soil – development of a bio-availability index. Axiom Environmental Inc. for PTAC, Calgary.

CCME, 2008. Canada-wide standard for PHC in soil: scientific rationale. Supporting Technical Document PN1399.

Environment Canada, 2004. Biological Test Method: Tests for Toxicity of Contaminated Soil to Earthworms (*Eisenia andrei*, *Eisenia fetida*, or *Lumbricus terrestris*). EPS 1/RM/43 (with June 2007 amendments).

Jodál, Kandra, Harangi, Nánási and Szejtli, 1984. Hydrolysis of cyclodextrin by *Aspergillus oryzae* α -amylase. *Starch* 36:140-143.

Reid, Stokes, Jones and Semple, 2000. Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. *Environmental Science and Technology* 34:3174-3179.