

WESTERN CANADA MICROTOX USERS COMMITTEE MEETING

November 16, 2007

BodyCote Testing Group, Edmonton, AB

Call to Order – John Ashworth called meeting to order at 10:08am

1. Introductions – Gordon with Osprey, Teleconference/weblink with SDI – reps are Tracy Weidman and Michael Jones
2. Review/Modify Agenda – Ansar Q. moved to accept the additions listed below and Sharon L. 2nd. All were in favour.
3. Approve Minutes of Previous Meeting, May 11, 2007 – Ansar Q. moved to accept the previous minutes as corrected within the report, Irene G. 2nd and all were in favour
 - 3.1. CAEAL Collaboration – table until May 2008 meeting when a representative from CAEAL can be present (Ken Middlebrook?). Anthony will extend the invitation based on the fact that WCMUC would like to be designated an alternate provider for PT when a CAEAL participant fails a PT round (fast track – proviso ii)
 - 3.2. Sample Preparation SOP – Irene prepared as per document used by ARC for sample preparation. Some items were omitted from the original document (volume size – homogeneity and sub-sampling requirements). This will help in effort to achieve CAEAL targets. Irene would like a tentative approval as is written (does include solids preparation) so it can be in place for May 2008 meeting. Motion to approve made by Anthony N, 2nd by Esther E. and all were in favour. ([pdf document](#))
 - 3.3. No new matters arising.
4. New Business
 - 4.2. D-50 (is now listed as a directive instead of a guideline) – In relation to WCMUC the guideline for MTX has been recycled into the D50.

Centrifugation vs pH adjustment – when pH adjusting first it appears to result in a clearer sample as opposed to a coloured sample (dk brown). The debate is of the legitimacy of the order between centrifugation and pH adjustment. There is no specified statement as to the order, however clarification is written ahead of pH adjustment in the document, which leads to an understanding of one before the other. The pH change could have an apparent change in toxicity if performed prior to centrifugation. Ansar feels that pH should be done post sample clarification. He agrees that it will provide a clearer sample but has the greater potential to remove the toxicity of the sample. Anthony – pH isn't always performed prior to analysis (as it could already be within the accepted limits of 6.0-8.0), but will be pH adjusted when the waste hits the soil, either by spread m/b/c. This could potentially mask the presence of toxicity or the lack of toxicity. The usefulness of Microtox comes into question when dealing with high turbidity samples (which pH prior to could minimize). The greater question is: Is pH really removing toxicity, or is it providing a clearer sample, which is providing

tighter confidence intervals. When samples are highly toxic then the order really has no effect, but as they approach 50-65%, is the practice representative of good science. John – feels that pH should be performed prior to centrifugation. Samples aren't centrifuged in the field prior to disposal – it is the toxicity of the fluid phase that is introduced to the soil, therefore it shouldn't be limiting the order of clarification (pH vs Centrifugation). The greater issue is colour correction procedure and it's supporting math. The double walled method mathematically could prove colour issues vs true toxicity whereas absorbance method mathematically breaks down.

WCMUC would like to see a footnote added to the client report when pH is performed **prior** to centrifugation. John is to put forth a letter to be circulated amongst the membership as to be proposed to Sue Halla with EUB.

Break at 11:17am – returned at 11:30am

- 4.1. Report from SDI – Teleconference/web link with Tracy Weidman – PowerPoint presentation. SDI has invested \$125,000 into a new lyophilizer. Have been running batch performance along side the older model to ensure quality control is maintained. The Certificate of Analysis will be modified to include Confidence Intervals; as well will include light loss specifications beginning with 1Q of 2008. Maxxam Analytics presented with a “Run time error ‘6’” in software and was wondering if SDI had been able to solve this error. Because IT support at SDI was able to generate the same error it is not a compatibility issue with the software.

Break for lunch at 11:53am (disconnected with SDI) – returned at 12:39pm (reconnected with SDI)

5. Round Robin #37

- 5.1. Review Data – Samples were sent out on September 13 with testing September 20/21. Data was due back on October 5. There were 13 labs sent samples with 9 reporting back.

Sample 1 – Zinc Sulfate 56mg/L (13mg/L Zn²⁺)

Sample 2 – Same Diesel contaminated soil from RR#36

Sample 3 – Desco CF made at 2kg/m³ (max working conc. in the field)

- 5.1.1. Sample 1 – Lab 2 had data rejected EC50(5), Lab 8 had data rejected EC50(15) and Lab 5 reported extrapolated data.

The last two columns in table the EC50(5/15) data was converted back to Zn.

Lab 1 (both days), Lab 3 (2nd day) and Lab 5 (1st day) all had values below the long-term data of 0.6-2.2mg/L. Long-term data was added to the graph as dashed lines.

- 5.1.2. Sample 2 – should not have required colour or pH adjustment. Lab 7 (EC20(5) data rejected both days, EC20(15) data rejected 1st day). All labs used their own water for RR#37 and this proved that the solid sample was stable from RR#36. The mean for RR#36 was 2.3 and #37 was 2.03. There was good repeatability – Lab 2 had a wide CI (0.8-4.0)

- 5.1.3. Sample 3 – Sample was highly coloured with slight particulate. Lab 9 had EC20(5) rejected, Lab 5 and 8 had EC50(15) day 2/1 rejected. Results based on graph – are fairly tight within 1SD.
Colour correction – Lab 1/5 reported EC50(5/15) non toxic with colour correction while remaining labs reported ~23%.
- 5.2. Open Discussion – Labs were required to report before and after data for colour correction. Data should have been submitted with correction factor calculated out if dilution was made. Also the instructions asked for reporting to one decimal. Page 5 of round robin should state #37. Lab 8 did a colour correction for sample 2.
Colour correction – labs that did colour correction submitted original data to John Ashworth to determine why variance in results. The first two pages of Data Reduction Formulas – the data reduction formulas were taken from the Azur's manual on colour correction. The formula includes R (ratio of initial control) is on the first page but not in formula on the 2nd page, which causes some question as to the validity of the results. Lab 8 - raw data readings for It as well as absorbance corrected readings for It. When calculating gamma and log gamma vs log of conc. one gets a higher reading for EC50(15). Lab 7 (data submitted after RR#37 data) – they recognized an issue with the plot of log gamma vs log conc and so redid analysis. Lab 7 diluted sample 2x and then achieved the 25% mark which the original two labs achieved. The two labs that reported >100% reported data where the y-intercept was in the upper left hemisphere of graph. When absorbance is really high – have a breakdown in math. Need to look at diluting down colour and re-read on spectrometer when extrapolation is out of control
- 4.1. (Revisited) – Michael Jones has been asked to help with mathematics in regards to double walled cuvette vs absorbance. Phenol vs light loss is improving and there has been significant activity in reagent production stability. SDI highly values WCMUC's input and therefore if possible to reschedule meeting for earlier in week, they would be able to attend meetings in person. Diluent issue should not recur going forward from here – a change had been made in how conc. was measured for salt. New model M550 and beta-testing almost complete. Software to launch at end of year – working out some tweaks in help files and test before sending out as final software. WCMUC should get a copy prior to final release to help with complete review of software
- 5.3. Sample Type – Colour correction – submit actual absorbance readings and will re-analyze Desco CF. Standard will be phenol. Soil and Oil – presieved (1kg) from John Ashworth. April 3 & 4 for analysis, ship Mar 27 and data by April 18.
6. Treasurer's Report - \$802 in bank with \$1300 in fees to come in. There are three labs with outstanding dues, two are from Environment Canada (full fees). Have been approached by a potential member in Brazil – Irene to check into shipping and regulations before a decision is made to allow them to become participants. Motion to accept treasurer's report (with corrections) by Irene G., 2nd by Genevieve P., all were in favour.

7. Vitrak Creative Services – Western Canada Microtox – Google, Yahoo & Ask - #1/2; Canada Microtox - Google #1, Yahoo 1/3 and Ask 1/2; Microtox – Google pg. 4 #39, Yahoo #10 and Ask #7. Irene to set up a questionnaire on website to determine where the hits are coming from. \$336 submitted for expenses for hosting domain and maintenance.
8. Any Other Business - Gordon Nelson with Osprey Scientific joined us at the table today. He is new with the Business Development division of Osprey Scientific.
9. Next Meeting
 - 9.1. Time and Place – May 8, 2008 at Epcor Water Treatment Plant. 10am start
 - 9.2. Topics for Discussion – SDI: Quality Improvements, New developments with Mutatox, New Developments; Colour Correction; CAEAL

Thank you to SDI for linking by web for presentation and conference.

10. Motion to adjourn meeting at 2:05pm made by Anthony N., Sharon L. 2nd and all were in favour.
11. Attached: Document Information Sheet (as written and approved), Data Reduction Formula, Treasurer's Report and Attendance Sheet

Attendance Sheet - November 16, 2007

<u>Name</u>	<u>Company</u>	<u>Email Address</u>
Genevieve Payeur	Peace Analytical Laboratories	genevievep@palgrandeprairie.com
Esther Enders	Maxxam	estherenders@maxxamanalytics.com
Barbara Bomersback	Osprey	barbara@ospreyscientific.com
Sharon Lu	Epcor Water Services	slu@epcor.ca
Irene Gaudet	AB Research Council Vittrak Creative	irene@arc.ab.ca idgaudet@telusplanet.net
John Ashworth	ALS	john.ashworth@alsenviro.com
Ansar Qureshi	AQSS	ansar@shaw.ca
Anthony Neumann	BTG	anthony.neumann@norwestlabs.com
Charles MacDonald	ALS	charles.macdonald@alsenviro.com
Pearl Poon	Epcor	ppoon@epcor.ca
Maria Mejia	Maxxam Analytics	maria.mejia@maxxamanalytics.com
Gordon Nelson	Osprey Scientific	gordon@ospreyscientific.ca

WCMUC Register Report
1/05/2007-9/11/2007

DATE	CHK#		6452.53
BALANCE 30/04/07			
1/5/07		INTEREST	INCOME 0.22
16/5/07		Round Robin 35	Expense 2744.87
22/5/07		WCMUC meeting cost	Expense 138.32
25/5/07		Bank management fee	Expense 3.50
01/06/07		Interest	INCOME 0.82
25/06/07		Bank Management Fee	Expense 2.00
03/07/07		Interest	INCOME 0.36
25/07/07		Bank Management Fee	Expense 2.00
01/08/07		Interest	INCOME 0.37
24/08/07		Bank Management Fee	Expense 2.00
04/09/07		Interest	INCOME 0.37
25/09/07		Bank Management Fee	Expense 2.00
01/10/07		Interest	INCOME 0.36
03/10/07		Round Robin 36	Expense 2756.00
25/10/07		Bank Management Fee	Expense 2.00
01/11/07		Interest	INCOME 0.09
TOTAL INFLOWS			2.59
TOTAL OUTFLOWS			5652.69

NET TOTAL	802.43
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Accounts Receivable	Memberships 2006-2007	2400.00 ²⁹⁰⁰
Accounts Receivable	Memberships 2007-2008	6000.00
Small Account		0.00
Large Account		802.43

Accounts Payable	Round Robin #37	-2954.75
Accounts Payable	Website Services	-336.00
Accounts Payable	Round Robin #38	-2954.75

Total Equity	2956.93 ^{3456.93}
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For 2007:
 10 Full Memberships – 1 new member and loss of one old member (\$5000)
 3 Subsequent Memberships (\$900)
 1 Associate Membership (\$100)
 2 Honorary Memberships

Reviewed by: _____

Reviewed by: _____

Data Reduction Formulas

Calculation of Gamma for Microtox Acute Toxicity Protocols: Basic Test, Comparison Test, Basic Solid-Phase Test & 45% Screen

Correction Factor

The Correction Factor (R_t) is the fraction obtained when the light output of the Control (Blank) remaining after time t is divided by the initial light output of the untreated Control (Blank):

$$R_t = I_t \div I_0$$

Gamma

The gamma (G) value is the ratio of the light lost at time t to the light remaining at time (t) for a given sample concentration:

$$G_t = [(R_t \times I_0) \div I_t] - 1$$

The second form of this equation stresses the fact that gamma is the ratio of light expected for a nontoxic sample to that observed, minus (-) 1.

% Effect at time (t)

$$\% \text{ Effect}_t = [G_t \div (1 + G_t)] \times 100$$

Absorbance (Colour) Correction

The Absorbance Correction procedure determines the light seen by the Analyzer photodetector at each sample concentration using a spectrophotometer.

The contribution to absorbance (A) at concentration (x) is determined using the absorbance (490 nm, 10 mm path width) reading (ABS_x) taken for each sample concentration:

$$A_x = 1.75 \text{ ABS}_x$$

The A_x is then used to calculate the Transmittance (T_x) for each sample concentration using:

$$T_x = (1 - e^{-A_x}) \div A_x$$

The Absorbance Corrected I_0 (ACI_0) is then calculated using the equation:

$$ACI_0 = I_0 \times T_x$$

The Absorbance Corrected (AC) Gamma (G) at time (t) or ACG_t is then calculated using the equation:

$$ACG_t = [(R_t \times ACI_0) \div I_t] - 1$$

Data Reduction Formulas

Calculation of Gamma for: Microtox Chronic Test
Calculation of Gamma for Microtox Acute Toxicity
Protocols: Inhibition Test, Solid-Phase Test,
Whole Effluent Toxicity Test & 2% Screen

GAMMA

The gamma (G) value is the ratio of the light output of the
Control (c) at time (t) to the light output for a given sample
(s) concentration at the same test time, minus (-) 1:

$$G_t = (I_{tc} \div I_{ts}) - 1$$

% Effect at time (t)

$$\% \text{ Effect} = [G_t \div (1 + G_t)] \times 100$$

Absorbance (Colour) Correction

The Absorbance Correction procedure determines the
light seen by the Analyzer photodetector at each
sample concentration using a spectrophotometer.

The contribution to absorbance (A) at concentration (x) is
determined using the absorbance (490 nm, 10 mm path
width) reading (ABS_x) taken for each sample
concentration:

$$A_x = 1.75 \text{ ABS}_x$$

The A_x is then used to calculate the Transmittance
(T_x) for each sample concentration using:

$$T_x = (1 - e^{-A_x}) \div A_x$$

The Absorbance Corrected (AC) Control (c) at time
(t) or ACI_{tc} is then calculated using the equation:

$$ACI_{tc} = I_{tc} \times T_x$$

The Absorbance Corrected (AC) Gamma (G) at time
(t) or ACG_t is then calculated using the equation:

$$ACG_t = (ACI_{tc} \div I_{ts}) - 1$$

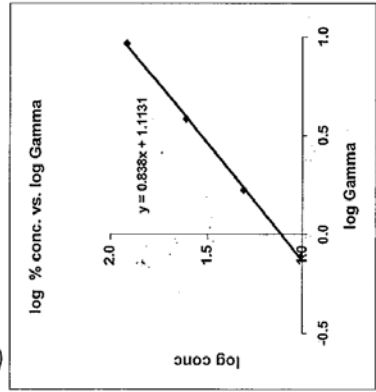
This uses data for WCMC RR # 37 -3, and a log/log plot but regresses x on y

Lab 8 data

Raw light output readings		Colour Correction	
Initial	15 min	Absorbance	Transmittance
Blank	89	10.2	0.8355
Conc.	91	20.4	0.417
	90	40.9	0.830
	86	81.8	1.647
	91		0.3275

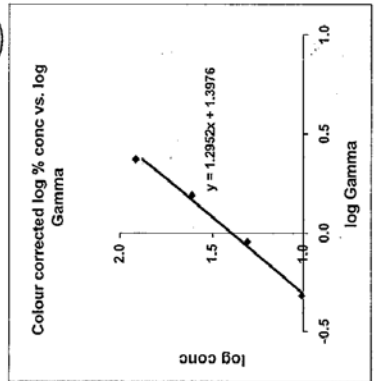
Absorbance corrected

Sample concentration (%)	A/C gamma	A/C log	Corrected log gamma	log conc
10.2	0.212	76	-0.320	1.009
20.4	0.417	64	-0.046	1.310
40.9	0.830	45	0.180	1.612
81.8	1.647	30	0.371	1.913



Interpolation
log % conc log gamma
1,113 0.00
Dln factor EC50(15)
1 13.0 %

Uncorrected EC50(15)



Interpolation
log % conc log gamma
1,296 0.00
Dln factor EC50(15)
1 25.0 %

Colour corrected EC50(15)

This uses data for WCMUC RR # 37 -3, and a log/log plot but regresses x on y

Lab 7 data

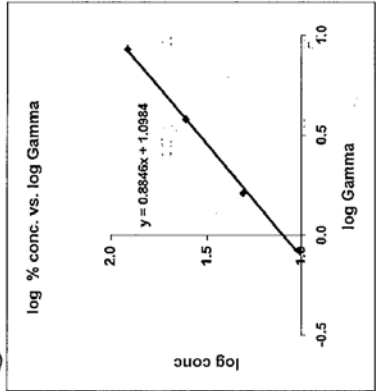
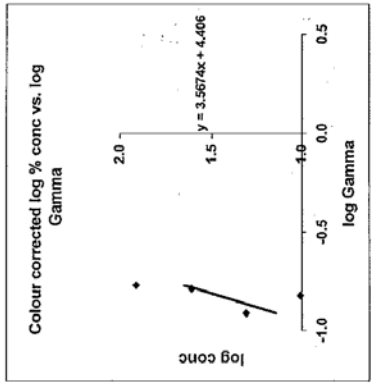
Absorbance corrected

Raw light output readings		15 min		30 min		45 min		60 min		75 min		90 min		105 min	
Blank	Conc.	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
10.2	20.4	95	95	95	95	95	95	95	95	95	95	95	95	95	95
20.4	40.8	95	95	95	95	95	95	95	95	95	95	95	95	95	95
40.8	81.6	95	95	95	95	95	95	95	95	95	95	95	95	95	95
81.6		95	95	95	95	95	95	95	95	95	95	95	95	95	95

Sample concentration (%)	Absorbance	Transmittance	A/C to
10.2	0.58	0.6382	60
20.4	1.16	0.4279	41
40.8	2.32	0.2176	23
81.6	4.64	0.1231	11

gamma	log gamma	log conc
0.830	-0.081	1.009
1.652	0.210	1.310
3.268	0.514	1.612
6.486	0.829	1.915

corr factor	gamma	log gamma	log conc
1.02	0.830	-0.081	1.009
1.02	1.652	0.210	1.310
1.02	3.268	0.514	1.612
1.02	6.486	0.829	1.915



Interpolation
log % conc log gamma
1 1.098 0.00

Interpolation
log % conc log gamma
1 25118.9 0.00

Uncorrected EC50(15)

Colour-corrected EC50(15) > 100% !!

Diln fact EC50(15)
1 12.5 %

Diln fact EC50(15)
1 25118.9 %