

# ANTHRAQUINONE

**FIELD TRIAL : CORN TREATED WITH FLIGHT CONTROL**  
**WISCONSIN SITE, 2001 SEASON**  
**Owen B. Mathre, Ph.D.**

This is the analytical report on the samples received from the field site in Wisconsin. The attached spreadsheets provide the laboratory data related to sample preparation and analysis by high performance liquid chromatography, HPLC. The work in the laboratory was done by our analytical chemists, John R. Hargrove and Michele Santer. The spreadsheet results are expressed in micrograms anthraquinone per corn kernel. Information provided to me stated that the corn seed used in the trial had a count of about 2419 kernels per pound (or 5333 kernels per kilogram). The sample kernels supplied to us had absorbed variable amounts of water so that a report based on the weight of the kernels in the sample would not be related to the original weight of the seed before planting.

The field samples received in a refrigerated container were placed in a freezer until they were to be prepared for analysis. The corn kernels were separated from the roots and the plant stalks and then were extracted in a capped vial with acetonitrile. Ultrasonic agitation was used to aid the dissolution and extraction of the anthraquinone. The sample extracts were filtered through a 0.45 micron syringe filter and diluted with acetonitrile to the appropriate concentration with acetonitrile. The solutions were analyzed by reverse phase HPLC using water : methanol mobile phase and a Zorbax C8 column. A Hewlett Packard Model 1050 with a UV detector and autosampler were used. The instrument signal was collected and processed by a Hewlett Packard Chem Station. The instrument response was calibrated with solutions of anthraquinone purchased from a chemical supply house. When sample extracts showed an instrument signal that was above the calibration range a new aliquot was diluted with acetonitrile and analyzed. They are designated in the spreadsheet table.

This procedure is one that has been used routinely in this lab for grain samples and has been validated by use of spike recovery tests.

This report is prepared for transmission by e-mail and by surface mail. The surface mail will also contain a copy of the standard procedure used for the analysis of this type of sample.

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**DETERMINATION OF ANTHRAQUINONE ON GRAIN AND DERIVED PRODUCTS  
REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD**

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**I. Principle**

Anthraquinone deposited on grains or derived products such as bran, hulls, milled seeds and formulated animal feeds is extracted with a suitable solvent such as acetonitrile. The extract is analyzed by reverse phase high performance liquid chromatography using an UV detector to measure the anthraquinone in the column effluent. The signal data is collected and processed with a Hewlett Packard Chem Station.

**II. Applicability and Interferences**

This method has been developed and validated for the measurement of anthraquinone in samples of barley, corn, rice, sorghum, derived milling products from rice, and formulated animal feed. Compounds present in some matrices that are extracted by the chosen solvent and are not separated by the chromatographic conditions will interfere and must be dealt with. Also, it must be established with new matrices that the extraction of the anthraquinone is satisfactory. The recovery of anthraquinone from fortified samples is required to validate the method for new sample matrices.

**III. Special Safety Considerations**

Standard laboratory safety regulations must be adhered to. Acetonitrile and methanol used in this procedure are flammable solvents. The chromatographic column effluent is flammable. Acetonitrile may also cause dermatitis in some people so that care should be taken to avoid skin contact with it or with mixtures that contain it.

**IV. Precision and Accuracy**

Four rice samples with hulls on were analyzed in triplicate. The percent RSD values were 1.05, 5.5, 7.9, and 3.8 for average values in the range of 381 to 583 ppm. Recovery of added anthraquinone ranged from 95 – 105 %.

Ten standard solutions prepared from reference anthraquinone were analyzed by the method. The concentration ranged from 5 to 212 micrograms anthraquinone per gram of solution. The integrated area response had a RSD of 1.4%.

**V. Apparatus and Instruments (Equivalent apparatus may be substituted)**

1. High performance liquid chromatograph equipped with a dual pumping system and an UV detector. The Hewlett Packard model 1050 equipped with a variable wavelength ultraviolet / visible detector and a 10-mm path photometric cell has been found satisfactory.
2. Autosampler for the HPLC is preferred but is not essential to generating satisfactory results.
3. Hewlett Packard Chem Station, rev. B.02.06 has been found to be satisfactory,
4. The HPLC column, 4.6 x 250-mm, packed with Zorbax SB, C8, 5 micrometer particles has been found satisfactory.
5. A water bath equipped with an ultrasonic generator operated at 55 C has been found satisfactory for the extraction of anthraquinone from these samples using acetonitrile.
6. Screw cap vials, 20 – 30 ml. capacities, equipped with polyethylene cone caps are needed for the extraction. Standard 20 ml. scintillation vials have been found to be satisfactory.
7. Acrodisc syringe filters, 13 mm diameter, and 0.2-micron pore size nylon membrane.

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REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD**

**VI. Reagents (Equivalent quality reagents may be substituted)**

1. Acetonitrile, HPLC grade, Aldrich Cat. No. 27,071-7
2. 9,10-Anthraquinone, 97 % or better
3. Methanol, HPLC grade. Aldrich Cat. No. 27,047 - 4
4. Deionized water, HPLC grade or equivalent.
5. HPLC Mobile Phase Solvent, 65 / 35 volume percent Methanol / Water (mixed before use).  
Used only if a binary pumping system is not used.
6. Calibration Standards. Set of five within the range of 0.50 to 250 micrograms 9,10-anthraquinone per gm. in acetonitrile. If the expected concentration in the extract from the samples is not above 50 micrograms per gm. The top standard should be in the range of 75 - 100 micrograms per gm. of gm. Correct the anthraquinone concentration of the standards for the purity of the standard material used.
7. Matrix Samples. It is preferable to have matrix samples such as rice or chicken feed that are representative of the samples to be analyzed to validate the method. They are used in analyte spike recovery experiments.

**VII. Procedure**

1. Instrument Operating Parameters (Hewlett Packard Chem Station)

Run Time Check List

Pre - Run CMD / Macro	Off
Data Acquisition	On
Standard Data Analysis	On
Customized Data Analysis	Off
Save GLP Data	Off
Post - Run CMD / Macro	Off
Save Method with Data	Off

2. PUMP Quaternary  
Settings

Stop Time	17 minutes
Post Time	Off
Store Flow Curve	Yes
Max. Pressure Limit	400 bar
Min. Pressure Limit	0 bar
Store Pressure Curve	Yes
Solvent A	0.0 %
Store Solvent A Curve	No
Solvent B (Deionized water)	35 %
Store Solvent B Curve	Yes
Solvent C	Off
Store Solvent C Curve	No
Solvent D (Methanol)	65 %
Store Solvent D Curve	No

**DETERMINATION OF ANTHRAQUINONE ON GRAIN AND DERIVED PRODUCTS  
REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD**

3. PUMP Quaternary

Contacts

Contact 1 Off  
Contact 2 Off

Heater

Temperature 30.0 C.  
Store Temperature Curve Yes

Auxiliary Settings

Primary Channel Auto  
Pump Stroke Auto  
Compressibility 40 E – 6 bar

4. AUTOMATIC LIQUID SAMPLER

Settings

Injection volume 10 microliters (20 microliters for trace analysis)  
Stop Time as Pump, 17 min.  
Post Time Off  
Draw Speed 200 microliters / min.  
Eject Speed 200 microliters / min.  
Draw Position 0.00 mm  
Mix in BCR Off

Contacts

Contact 1 Off  
Contact 2 Off

5. MULTIPLE WAVE LENGTH DETECTOR

Settings

Stop Time as Pump, 17 min.  
Post Time Off  
Response Time 1.0 sec  
Peak Width >0.05 min.  
Pre Run Autobalance On  
Post Run Autobalance Off

6. SEQUENCE CALIBRATION TABLE

Cal. Line	Cal. Level	Update Response Factor	Update Retention Times
1	1	Average	No Update
2	2	Average	No Update
3	3	Average	No Update
4	4	Average	No Update
5	5	Average	No Update
6	6	Average	No Update

**DETERMINATION OF ANTHRAQUINONE ON GRAIN AND DERIVED PRODUCTS  
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7. INTEGRATION EVENT TABLE

Event	Value	Time
Initial Area Reject	0.000	Initial
Initial Threshold	-1.000	Initial
Initial Peak Width	0.450	Initial
Initial Shoulders	Off	Initial
Integrator Off		0.698
Integrator On		3.500

8. INTEGRATION EVENT TABLE "Event DAD1A"

Event	Value	Time
Initial Area Reject	5.00	Initial
Initial Area Threshold	1.00	Initial
Initial Peak Width	0.040	Initial
Initial Shoulders	Off	Initial

9. INTEGRATION EVENT TABLE "Event MWD1B"

Event	Value	Time
Initial Area	0.000	Initial
Initial Threshold	-2.000	Initial
Initial Peak Width	0.200	Initial
Initial Shoulders	Off	Initial
Integrator Off		0.928
Integrator On		3.495

10. INTEGRATION EVENT TABLE "Event MWD1A"

Event	Value	Time
Initial Area	0.000	Initial
Initial Threshold	-2.000	Initial
Initial Peak Width	0.200	Initial
Initial Shoulders	Off	Initial
Integrator Off		0.928
Integrator On		3.690

11. SPECIFY REPORT

Destination	Printer
Quantitative Results Sorted By:	Retention Time
Report Style	Short
Sample Info on each Page	No
Add Chromatogram Output	Yes
Chromatogram Output	Portrait
Size in Time Direction	90 % of page
Size in Response Direction	35 % of page

**DETERMINATION OF ANTHRAQUINONE ON GRAIN AND DERIVED PRODUCTS  
REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD**

12. SIGNAL OPTIONS

Include: Axes, Retention Times, Baselines, Tick Marks  
Font: Arial, Size 8  
Ranges: Full  
Multi Chromatograms Overlaid, all the same scale

13. CALIBRATION TABLE

Default Calibration  
Calibration Data Modified (Date and time printed)  
Calculate External Standard  
Based on: Peak Areas  
Relative Reference Window 10.0 %  
Abs.Reference Window 11 min.  
Relative Non-Reference Window 4.00 %  
Abs. Non Reference Window 0.000 min  
Default Multiplier 1.000 (if not set in the sample table)  
Default Dilution 1.000 (if not set in the sample table)  
Default Sample Count 1.000 (if not set in the sample table)  
Calculate Uncalibrated Peaks No  
Partial Calibration Yes, identified peaks are recalibrated  
Correct all Retention Times No, only for identified peaks  
Curve Type Linear  
Origin Forced  
Weight Equal  
Recalibration Settings  
Average Response Average of all calibrations  
Average Retention Time Floating average, New 75 %  
Calibration Report Options  
Printout of recalibrations within a sequence  
Calibration Table after Recalibration  
Normal Report after Calibration  
If Sequence is done with bracketing  
Results of first cycle (ending previous bracket)  
Calibration Table Example  
Signal 1: MWD1 A, Sig = 254, 4 Ref = 550, 80  
Signal 2: New

Ret. Time Min.	Signal	Level	Amount Mcg/gm.	Area	Amount / Area	Name
11.246	1	1	0.49	60.599	8.086 x exp-3	AQ
		2	12.24	1494.08	8.182 x exp-3	AQ
		3	53.0	6433.11	8.239 x exp-3	AQ
		4	102.0	12,120.3	8.416 x exp-3	AQ
		5	204.0	24,102.9	8.464 x exp-3	AQ
		5	204.0	24,471.1	8.336 x exp-3	AQ
		5	204.0	24,550.0	8.310 x exp-3	AQ
Average				8.290 x exp-3 , Std. Dev. = 0.132 X exp-3		

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**14. OTHER INSTRUMENT OPERATING PARAMETERS**

Column Temperature	30.0 C.
Mobile Phase Flow Rate	1.00 ml. / min.
Adjust to meet separation needs and to keep the 9,10-anthraquinone within the relative retention time required.	
Sample Concentration:	0.5 to 2.5 mg dissolved solids per ml.
Detector settings	Monitor on 2 channels 254 nm for anthraquinone 210 nm to aid QC for separation assessment

**15. INSTRUMENT RESPONSE CALIBRATION**

Prepare new standards once per month or when there is evidence that they have deteriorated due to failure of the QC tests.

- Record the tare weight of a 50 ml. volumetric flask with stopper to 0.1 mg. Transfer 10 – 11 mg. 9,10-anthraquinone of known purity directly into the 50 ml. volumetric flask. Record the weight of the anthraquinone to 0.01 mg. Add about 40 ml. acetonitrile, cap and place on a shaker and shake until the solids are dissolved, usually about 30 min. at 25 C. Dilute to 50 ml. with acetonitrile, mix thoroughly and record the total weight. Calculate the concentration of the anthraquinone, mg. AQ / gm. solution. Record the value to 0.0001 mg AQ / gm. stock solution.
- Tare three 25 ml. volumetric flasks and record the weights to 0.001 gm. Add 0.625 ml., 1.50 and 2.50 ml. of the stock solution to flasks numbered 1,2 and 3 respectively. Record the weight of stock solution to 0.001 gm. for each of the flasks. Tare two 10 ml. volumetric flasks numbered 4 and 5 and record the weights to 0.001-g. Add 2.50 and 5.00 ml. of anthraquinone stock solution to the flasks respectively. Record the weight of the stock solution added to each flask. Dilute all of the flasks to volume with acetonitrile and mix thoroughly. Record the total weight of each flask. Calculate the micrograms of anthraquinone per ml. for each standard. These calibration standards will be satisfactory for analysis of sample extract solutions from 2 – 150 micrograms anthraquinone per ml. If all samples have concentrations in the range 0.5 – 30 micrograms per ml. are to be analyzed replace the highest standard with made up by adding 0.200 ml. of stock solution to a 50 ml. volumetric flask.  
Calculate the concentration of the anthraquinone in each standard. Record the value to 0.01 micrograms anthraquinone per gm. of solution.  
Note: If desired 40 ml. glass vials may be used in place of the volumetric flasks for the diluted calibration standards. The stock and the acetonitrile are added by weight. The density of acetonitrile is near 0.78 gm per ml. at 25 C.
- Load three autosampler vials with each standard and place them in the autosampler. Enter their identification in the sample table in the Chem Station.
- Check the flow rate, the column temperature and the mobile phase level in the reservoirs to confirm that they are ready for the calibration run.

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**16. INSTRUMENT RESPONSE CALIBRATION (CONTINUED)**

- e. Start the autosampler and let the calibration run begin. Inspect the first chromatogram and determine if the component separation and the peak elution times are satisfactory. If they are not the calibration run must be started again. Check the parameters to confirm that they are satisfactory. Adjust the flow rate and column temperature if necessary to maintain separation quality.
- f. At the end of the run examine the data and the response factors for the different concentrations of AQ taken. The average of the values of the response factors should show a RSD of less than 3.1 %. If not, determine the reason for the deviation, correct the problem and calibrate again.

Note: This full calibration is needed at the beginning of a campaign or once per month unless major maintenance is conducted on the instrument or the QC standard samples are outside of control limits. It will be satisfactory to run two prepared standards in duplicate with each daily set of samples. They should "bracket" the sample range within reasonable limits. If the results are within 3 % relative of the specified values the current calibration is acceptable. If not, correct the operating problem and do a full calibration before any sample results are generated for reporting.

- g. Retain a copy of the calibration set and the QC calibration check set with the sample set in the file records. Copies of this information are to be included with any report to a GLP compliant system.

**17. SAMPLE PREPARATION AND ANALYSIS OF THE EXTRACT**

- a. Weigh an appropriate size sample into a 30 ml. tared screw cap vial. The vial should have a polyethylene cone cap or a Teflon lined cap (or septum) to avoid contamination of the sample with components from the cap. Label the vials before they are weighed. Record the weight to 0.01 gm. For each set of 10 samples prepare a duplicate and a spiked duplicate. Use the same sample if possible for these extra samples. It is necessary to have these duplicates for a fraction of a set beyond the set of 10 samples.
- b. Add 10.0-gm. acetonitrile to each sample. Record the weight of acetonitrile added to 0.01 gm. Cap the vials and place them in the ultrasonic bath containing water maintained at 50 – 55 deg. C for 45 +/- 5 minutes. Remove the vials from the ultrasonic bath and cool them to room temperature.
- c. Filter the extract through the 0.2 micron nylon Acrodisc filter attached to a syringe (a 2 or 5 ml. Luer Lock syringe is adequate.) into the Hewlett Packard autosampler vial. Use 2 vials per sample extract. Retain one vial for reanalysis if needed. Label the vials so that they may be identified.
- d. Enter the sample weight data into the sample information table in the Hewlett Packard Chem Station. Include the duplicate and spiked sample extracts. Include one each of two QC standard solutions at the beginning of the sample run and after each 20 sample vials. Include a set of QC vials at the end of all sample runs.

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- e. Start the autosampler run. Examine the first three chromatograms to verify that the performance of the instrument is acceptable. The QC calibration check samples must be within 3.1 % relative of the specified value to be acceptable. The separation of the AQ in the first chromatogram must meet the requirements to give acceptable integrated peak areas, proper retention times and normal peak shapes.
- f. Examine the data at the end of the autosampler run and determine if the QC calibration check samples are within 3.1 % relative of the specified value. Duplicate samples must be within 10 % relative of each other and spike recovery must be consistent with the validation tests for the matrix. The usual requirement is that the spike recovery must be between 85 % and 120 % of the expected value. The Hewlett Packard Chem Station does the calculations for each chromatographic run. Averages of results and external calculations can be done with a conventional hand held calculator or with a spreadsheet program. The choice must be documented.

Note: If the QC fails in mid run it will be necessary to repeat the analysis of all sample solutions since the last set of acceptable QC set of data. If this is necessary the problem must be identified and the steps taken to resolve it must be documented in the report summary that is attached to the run data and report.

- g. Compile the data from the chromatographic printer output and attach to it the set of chromatograms. Note any exceptions in the data set.

### **VIII. Quality Control**

The quality control procedures are incorporated in the sample analysis section. The basic guidelines for the practice are derived from ASTM and US EPA SW-846 documents. It has been found satisfactory to follow the steps in this document to achieve data that is acceptable as long as the validation procedure gives data that reproducibility and spike recovery within the limits given in the procedure.

### **IX. Validation**

Obtain sufficient sample of the base material that has not been treated with 9,10 anthraquinone and weigh out samples according to the regular analysis procedure. Add AQ calibration standard solution or stock standard by weight to give values on the AQ at 3 different concentrations within the range for the regular samples. Prepare a minimum of 3 replicates at each of the three concentrations chosen. They need not be duplicates but should be within 5 % relative of each other. Carry out the sample extraction, filtration and HPLC analysis as described above. Determine the average percent recovery and the standard deviation. If the data meets the requirements of the analysis proceed with the analysis of samples. If the results are not satisfactory develop the necessary modifications in the analysis procedure, including sample preparation conditions necessary to obtain satisfactory data. Then repeat the validation of the procedure with the revised procedure.

# HPLC DETERMINATION OF 9,10 ANTHRAQUINONE IN CORN SAMPLES

Analysis Date	Sample	Sample Prep Data				Extract Dilution		9,10 Anthraquinone	
		Weight of ACN extract	Volume of extract, mL	Number of kernels	Extract Aliquot, uL	Dilution Volume, uL	Peak Area, mAU	Extract Conc, ug/mL	ug AQ/ kernel
7/25/01	5/15 untreated post plant	7.8090	9.9351	6	1	1	27.1	0.049	0.1
	5/15 AQ preplant	8.1434	10.3606	6	1	1	51169.2	over range	
	5/15 AQ preplant	8.1434	10.3606	6	200	1000	10634.2	85.040	734.2
	5/16 muck	7.7184	9.8198	6	1	1	17659.6	141.330	231.3
	5/16 sandy	8.0971	10.3017	6	1	1	36543.5	over range	
	5/16 sandy	8.0971	10.3017	6	250	1000	9145.9	73.120	502.2
	5/17 muck	8.0169	10.1996	6	1	1	188513.4	150.880	256.5
	5/17 muck	8.0169	10.1996	6	500	1000	9406.5	75.200	255.7
	5/17 sandy	7.7753	9.8922	6	1	1	22846.2	over range	
	5/17 sandy	7.7753	9.8922	6	500	1000	11467.9	91.720	302.4
	5/18 east	3.9258	4.9947	3	1	1	38798.4	over range	
	5/18 east	3.9258	4.9947	3	250	1000	9688.2	77.460	515.8
	5/18 middle	3.8973	4.9584	3	1	1	22131.2	over range	
	5/18 middle	3.8973	4.9584	3	500	500	10953.4	87.600	144.8
	5/18 west	3.6005	4.5808	3	1	1	56555.2	over range	
	5/18 west	3.6005	4.5808	3	200	1000	11854.4	94.820	723.9
	5/19 east end	7.7898	9.9107	6	1	1	35725.4	over range	
	5/19 east end	7.7898	9.9107	6	250	1000	8791.5	70.280	464.3
	5/19 west end	7.7898	9.9107	6	1	1	26375.2	over range	
	5/19 west end	7.7898	9.9107	6	500	1000	13383.6	107.070	353.7
	5/20 sandy	7.8025	9.9268	6	1	1	51347.7	over range	
	5/20 sandy	7.8025	9.9268	6	200	1000	10100.1	80.760	668.1
	5/20 muck	7.6117	9.6841	6	1	1	37411.4	over range	
	5/20 muck	7.6117	9.6841	6	250	1000	9439.9	75.470	487.2
	5/21 sandy	7.8032	9.9277	6	1	1	9584.3	76.630	126.8
	5/21 muck	8.0406	10.2298	6	1	1	11867.4	94.920	161.8
7/26/01	5/22 east	7.8066	9.9321	6	1	1	29308.4	234.670	388.5

Analysis Date	Sample Prep Data				Extract Dilution			9,10 Anthraquinone		
	Sample	Weight of ACN extract	Volume of extract, mL	Number of kernels	Extract Aliquot, uL	Dilution Volume, uL	Peak Area, mAU	Extract Conc, ug/mL	ug AQ/ kernel	
	5/22 west	7.7135	9.8136	6	1	1	18526.1	148.280	242.5	
	5/23 east	7.5174	9.5641	6	1	1	22333.9	177.940	283.6	
	5/23 west	7.7215	9.8238	6	1	1	42897.4	over range		
	5/23 west	7.7215	9.8238	6	500	1000	20545.7	163.692	536.0	
	5/24 east	7.8266	9.9575	6	1	1	24537.7	195.490	324.4	
	5/24 west	7.6717	9.7604	6	1	1	50573.8	over range		
	5/24 west	7.6717	9.7604	6	500	1000	26212.9	208.829	679.4	
	5/25 muck	8.0232	10.2076	6	1	1	12457.2	99.270	168.9	
	5/25 sandy	7.5476	9.6025	6	1	1	29792.3	237.330	379.8	
	5/26 east	7.6201	9.6948	6	1	1	27828.9	221.700	358.2	
	5/26 west	8.0102	10.1911	6	1	1	33904.3	270.090	458.8	
	5/27 east	7.9021	10.0536	6	1	1	36114.3	287.690	482.1	
	5/27west	7.4965	9.5375	6	1	1	40815.5	over range		
	5/27west	7.4965	9.5375	6	500	1000	21821.7	173.855	552.7	
	5/28 muck	7.7655	9.8798	6	1	1	12093.9	96.377	158.7	
	5/28 sandy	8.2152	10.4519	5	1	1	33692.9	268.404	561.1	
	5/29 muck	7.8101	9.9365	6	1	1	14005.1	111.599	184.8	
	5/29 sandy	7.8207	9.9500	6	1	1	25068.3	199.713	331.2	
	5/30 muck	7.9852	10.1593	6	1	1	21528.6	171.521	290.4	
	5/30 sandy	7.8272	9.9583	5	1	1	31799.9	253.328	504.5	
	5/31 east	7.8192	9.9481	6	1	1	21719.0	173.038	286.9	
	5/13 west	7.7167	9.8177	6	1	1	33918.9	270.204	442.1	
	6/1 east	7.7886	9.9092	6	1	1	10895.2	86.830	143.4	
	6/1 west	8.1623	10.3846	6	1	1	25838.2	205.845	356.3	
	6/2 east	7.7397	9.8469	6	1	1	28389.3	226.163	371.2	
	6/2 west	7.4997	9.5416	5	1	1	15428.9	122.939	234.6	
	6/3 muck	7.4949	9.5355	6	1	1	10780.5	85.917	136.5	
	6/3 sandy	7.8203	9.9495	6	1	1	25111.6	200.058	331.7	
	6/4 east	7.6087	9.6803	6	1	1	42257.1	over range		
	6/4 east	7.6087	9.6803	6	500	1000	21637.9	172.392	556.3	
	6/4 west	8.0364	10.2244	6	1	1	21906.9	174.533	297.4	
	6/5 muck	7.8025	9.9268	6	1	1	7780.1	62.020	102.6	
	6/5 sandy	7.7893	9.9101	6	1	1	21784.6	173.560	286.7	

Analysis Date	Sample Prep Data				Extract Dilution			9,10 Anthraquinone	
	Sample	Weight of ACN extract	Volume of extract, mL	Number of kernels	Extract Aliquot, uL	Dilution Volume, uL	Peak Area, mAU	Extract Conc, ug/mL	ug AQ/ kernel
	6/6 muck	7.7824	9.9013	6	1	1	49731.0	over range	
	6/6 muck	7.7824	9.9013	6	500	1000	25677.8	204.569	675.2
	6/6 sandy	7.7964	9.9191	6	1	1	41712.6	over range	
	6/6 sandy	7.7964	9.9191	6	250	1000	10593.1	83.462	551.9
	6/7	7.7455	9.8543	6	1	1	17762.8	141.528	232.4
	6/7 sandy	7.7762	9.8934	6	1	1	30080.5	239.633	395.1
	6/8 muck	7.6930	9.7875	6	1	1	22268.0	177.410	289.4
	6/8 sandy	7.8600	10.0000	6	1	1	20645.6	164.489	274.1
7/30/01	6/9 muck	7.9301	10.0892	6	1	1	17466.5	139.168	234.0
	6/9 sandy	7.7070	9.8053	6	1	1	21659.2	172.561	282.0
	6/10 east	7.8449	9.9808	6	1	1	12980.3	103.437	172.1
	6/10 west	7.8745	10.0184	4	1	1	43354.8	over range	
	6/10 west	7.8745	10.0184	4	250	1000	10738.2	84.604	847.6
	6/11 east	7.7020	9.7990	6	1	1	7096.1	56.572	92.4
	6/11 west	7.7931	9.9149	6	1	1	41713.8	over range	
	6/11 west	7.7931	9.9149	6	250	1000	10324.4	81.346	537.7
	6/12 muck	7.7031	9.8004	5	1	1	5829.5	46.484	91.1
	6/12 sandy	7.8212	9.9506	6	1	1	25043.9	199.519	330.9
	6/13 east	7.7916	9.9130	5	1	1	23026.5	183.451	363.7
	6/13 west	7.5746	9.6369	6	1	1	51962.6	over range	
	6/13 west	7.5746	9.6369	6	250	1000	12921.5	101.794	654.0
	6/14 muck	7.7184	9.8198	4	1	1	12202.4	97.241	238.7
	6/14 sandy	7.7939	9.9159	6	1	1	16921.1	134.824	222.8
7/31/01	6/26 east	7.7293	9.8337	6	1	1	37727.1	297.092	486.9
7/31/01	6/26 west	7.8312	9.9634	6	1	1	37639.9	296.406	492.2
7/31/01	6/27 east	7.8182	9.9468	6	1	1	30953.3	243.760	404.1
7/31/01	6/27 west	7.9388	10.1003	6	1	1	45306.7	over range	
	6/27 west	7.9388	10.1003	6	250	1000	11352.0	89.436	602.2
	6/28 east	7.8080	9.9338	6	1	1	44105.7	over range	
	6/28 east	7.8080	9.9338	6	250	1000	11002.9	86.688	574.1
	6/28 west	7.8520	9.9898	6	1	1	29022.8	231.209	385.0
	6/29 east	7.4732	9.5079	6	1	1	20797.2	165.695	262.6
	6/29 west	7.4341	9.4561	6	1	1	21993.0	175.219	276.2

Analysis Date	Sample Prep Data					Extract Dilution			9,10 Anthraquinone		
	Sample	Weight of ACN extract	Volume of extract, mL	Number of kernels	Extract Aliquot, uL	Dilution Volume, uL	Peak Area, mAU	Extract Conc, ug/mL	ug AQ/ kernel		
	6/30 east	7.8062	9.9316	6	1	1	2468.4	196.658	325.5		
	6/30 west	7.5206	9.5682	6	1	1	38228.3	304.527	485.6		
	7/1 east	8.0694	10.2664	6	1	1	48422.7	over range			
	7/1 east	8.0694	10.2664	6	250	1000	12216.5	96.243	658.7		
	7/1 west	7.8189	9.9477	6	1	1	44970.9	over range			
	7/1 west	7.8189	9.9477	6	250	1000	11291.8	88.963	590.0		
	7/2 east	7.7204	9.8224	6	1	1	40814.6	over range			
	7/2 east	7.7204	9.8224	6	250	1000	10158.2	80.038	524.1		
	7/2 west	7.8305	9.9625	6	1	1	28077.0	223.676	371.4		
	7/3 muck	5.3909	6.8587	6	1	1	33747.9	268.842	307.3		
	7/3 sandy	8.0327	10.2197	6	1	1	16170.5	128.846	219.5		
	7/4 east	7.8942	10.0435	6	1	1	37241.5	296.668	496.6		
	7/4 west	7.8163	9.9444	6	1	1	54456.1	over range			
	7/4 west	7.8163	9.9444	6	250	1000	13943.8	109.842	728.2		
	7/6 muck	7.7954	9.9178	6	1	1	22908.8	182.514	301.7		
7/31/01-	7/6 sandy	7.8232	9.9532	5	1	1	30973.2	246.743	491.2		
8/1/01	7/7 muck	7.8354	9.9687	6	1	1	40198.3	316.548	525.9		
	7/7 sandy	8.0082	10.1885	6	1	1	39440.9	310.585	527.4		
	7/8 east	7.6091	9.6808	6	1	1	17537.0	138.132	222.9		
	7/8 west	7.8001	9.9238	6	1	1	13505.1	106.388	176.0		
	7/9 east	8.1059	10.3128	6	1	1	10643.5	126.373	217.2		
	7/9 west	7.7977	9.9207	6	1	1	32106.6	252.841	418.1		

NOTE: For the analyses performed 7/25/01, method AQ5.M was calibrated to 110 ug AQ/mL. Sample extracts exceeding the calibration range were diluted volumetrically with acetonitrile and reanalyzed.

For analyses performed 7/26-8/1/01, the calibration range was extended to 300 ug AQ/mL.