

**STUDY TITLE**

GWN-10235: Aquatic Field Dissipation of the Herbicide Benzobicyclon at  
Two Rice Production Locations (Louisiana and California)

**REPORT TYPE**

Final Report

**DATA REQUIREMENT**

OPPTS 835.6200

## II. OBJECTIVE

Waterborne Environmental, Inc. (WEI) contracted Golden Pacific Laboratories, LLC (GPL) in Fresno, California, to conduct the analytical phase of an aquatic field dissipation of benzobicyclon and its major degradation products in soil (sediment) and water samples. The objective of this study was to determine the persistence of benzobicyclon and its major degradation products in soil (sediment) and paddy water when applied under field conditions to a rice paddy. This study was conducted to fulfill the aquatic field dissipation requirements as outlined in US EPA Fate, Transport and Transformation Test Guideline 835.6200: Aquatic Field Dissipation and in accordance with EPA's Good Laboratory Practice Standards (GLPs) 40 CFR Part 160.

## III. MATERIALS AND METHODS

### A. Reference Substances

The analytical reference substances were received in good condition on March 26, 2014, July 21, 2014, and June 29, 2016, along with Certificates of Analysis and a MSDS for benzobicyclon. The following table contains detailed information on each analytical standard used in this study.

Analytical Standard	Description	Batch/Lot #	CAS Number	Purity (%)	Expiration Date
Benzobicyclon	Light colored granulated powder	1A0110	156963-66-5	99.9	05/20/15 and 04/23/17
1315P-070	Light colored powder	95Z25	126656-88-0	99.7	05/20/15 and 04/23/17
1315P-570	Light colored, granulated powder	TNA-9-186 H/M-13-52-1	Not Available	99.8 99.65	07/10/16 02/22/18
1315P-683	Light colored, small solid pieces	TM-8-198	Not Available	99.1 99.77	07/10/16 02/22/18
1315P-960	Light colored, powder with solid pieces	MB-16-023 H/M-13-57-2	Not Available	99.2 99.48	07/10/16 02/22/18
1315P-076	Light colored, small solid pieces	TNA-10-074 TT-4-196	Not Available	99.5 99.39	07/10/16 02/22/18
1315P-962	Light colored, granulated powder	TNA20130717	Not Available	88.3	07/31/16 12/31/16
1315P-966	Light colored powder	CB01-08-0037	Not Available	99.5	07/10/16 12/31/16

Upon receipt, the reference substances benzobicyclon and 1315P-070 were stored in a freezer set to maintain at  $\leq -10$  °C per the certificates of analysis. The certificates of analysis for the other reference substances required a cool dark place, therefore the reference substances were stored at room temperature. When the second shipment of reference substance was received

for 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966, the new certificates of analysis required a cool, dry, and dark conditions, so to better suit the condition, the new subsample was stored refrigerated. Solutions of benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966 were prepared in acetonitrile to serve as stock solutions. Subsequent dilutions of the stock solutions were prepared in acetonitrile for use as laboratory spiking solutions. Initially, calibration standards for the analysis of soil were prepared in acetonitrile/water (30:70, v/v) and contained benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076. After the method was established the calibration standards for soil were prepared in acetonitrile/water (40:60, v/v). For water two sets of calibration standards had to be prepared. The first set of water calibration standards contained benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 and were prepared in acetonitrile/water (30:70, v/v). The second set of water calibration standards contained 1315P-962 and 1315P-966 and were prepared in acetonitrile/water (10:90, v/v).

Preparation and dilution data forms pertaining to the stock solutions, working solutions, spiking solutions, and calibration standards are located in the raw data. All solutions were stored frozen when not in use. Solutions were prepared every three months or as needed.

The storage units that stored the reference substances, stock, and working solutions were temperature monitored. Temperature records showing weekly temperature ranges are located in the raw data package.

Characterizations and stability of the reference standards is the responsibility of Gowan Corporation.

### C. Determination of Percent Moisture

Percent moistures were determined for the soil samples according to GPL SOPs and are reported in Tables 90 and 91. A subsample of approximately five grams was taken from the homogenized sample and transferred to an aluminum weigh boat. The exact weight was documented. The sample was placed in a Fisher Isotemp 600 oven set at ~135° C overnight. The following day the sample was removed from the oven and allowed to cool to room temperature. The sample was weighed and the weight recorded. The sample was placed back into the oven and weighed once more the following day as previously described. Using the wet weight and the dry weight from the second weighing, percent moisture was calculated using Microsoft® Excel. Field samples with measured residues were corrected for percent moisture and residues were reported using both wet weight and dry weight (correction for the percent moisture). LOQ and LOD are defined based on wet weight.

The soil moisture content (percent moisture) for the sample was then calculated according to the following equation:

$$\% \text{ Moisture} = \frac{(\text{Wet Weight (g)} - \text{Dry Weight (g)})}{\text{Wet Weight (g)}} \times 100$$

### D. Methods of Analysis

#### 1. Scope of Method Validations

Methods were developed and validated at GPL for the analysis of water and soil samples. Method validations were conducted prior to study sample analysis to establish the performance of each method.

The method validation results were approved by the Study Director prior to sample analysis.

For water, the method validation fortification levels were ~0.001 and ~0.01 µg/mL for benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966. For purposes of reporting final data, the Limit of Quantitation (LOQ) was established at 0.001 µg/mL as the lowest validated concentration.

For soil, the method validation fortification levels were ~0.005 ppm and ~0.05 ppm for benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076. For purposes of reporting final data, the Limit of Quantitation (LOQ) was established at 0.005 ppm (wet weight) as the lowest validated concentration.

### 3. Method Summary - Water

The fortification levels for the concurrent laboratory fortification samples were conducted at ~0.001 and ~0.01 µg/mL for

benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966. After analysis of the water samples, the high fortification level was raised for benzobicyclon and 1315P-070 to bracket the residues found in the field samples.

Water samples were analyzed using GPL method (document number GPL-MTH-087 Revision 2) entitled “Analytical Method for the Determination of Benzobicyclon and its Metabolites 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966 in Water by LC-MS/MS.” The method flow chart is provided in Appendix A of this report.

For all water samples being analyzed for benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076, after shaking the sample a 7 mL aliquot of sample was added to a 3 mL aliquot of acetonitrile and mixed well. The samples were then submitted for analysis by LC-MS/MS. No further clean-up of the samples was needed. Depending on the amount of sample available, aliquot volumes can be adjusted as long as the 7:3 ratio of sample to acetonitrile is maintained.

Samples requiring further dilutions were diluted with acetonitrile/water (30:70, v/v) so that the concentration fell within the range of the calibration curve.

For all water samples being analyzed for 1315P-962 and 1315P-966, after shaking the sample a 9 mL aliquot of sample was added to a 1 mL aliquot of acetonitrile and mixed well. The samples were then submitted for analysis by LC-MS/MS. No further clean-up of the samples was needed. Depending on the amount of sample available, aliquot volumes can be adjusted as long as the 9:1 ratio of sample to acetonitrile is maintained.

Samples requiring further dilutions were diluted with acetonitrile/water (10:90, v/v) so that the concentration fell within the range of the calibration curve.

#### 4. Method Summary - Soil

The fortification levels for the concurrent laboratory fortification samples were conducted at ~0.005 and ~0.05 µg/mL for benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076.

Soil samples were analyzed using GPL method (document number GPL-MTH-088 Revision 1) entitled “Analytical Method for the Determination of Benzobicyclon and its Metabolites 1315P-070,

1315P-570, 1315P-683, 1315P-960, and 1315P-076 in Soil by LC-MS/MS.” The method flow chart is provided in Appendix A of this report.

For soil samples, approximately 10 grams of homogenous sample was weighed into individual 250-mL Nalgene® bottles. Samples were extracted by adding 90 mL of acetonitrile/0.55 M Citric Acid (80:20, v/v) and shaking on a platform shaker for 30 minutes. The entire sample was filtered by vacuum using a 70-mm Whatman #5 filter placed in a Buchner funnel with an approximate 1 cm thick layer of celite on the filter. The Buchner funnel was attached to a 100-mL graduated cylinder fitted with a vacuum side arm. The filter cake was rinsed with 10 mL of acetonitrile/0.55 M Citric Acid (80:20, v/v) and the extract was brought to a final volume of 100 mL using acetonitrile/0.55 M Citric Acid (80:20, v/v) and hand mixed. An aliquot of sample extract was filtered through a 0.45- $\mu$ m PTFE filter attached to a plastic syringe and viald 1:1 with water for analysis by LC-MS/MS. No further clean-up of the samples was needed.

Samples requiring further dilutions were diluted with acetonitrile/water (40:60, v/v) so that the concentration fell within the range of the calibration curve.

5. Instrument Parameters: LC-MS/MS

Instrument: Sciex API5000 LC/MS/MS with Shimadzu LC-20AD XR HPLC Pumps, Shimadzu CBM-20A Controller, Shimadzu SIL-20AC XR Autosampler

HPLC Column: Phenomenex Luna 3 $\mu$  C18 100Å  
(30 x 2.00 mm), Catalog #00A-4114-B0

Data System: Analyst Chromatography Data System version 1.5 and 1.6, AB Sciex.

**Parameters for Benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076**

Mobile Phase (Gradient Program):

Time	A, %	B, %
0.0	10	90
7.00	95	5
8.00	95	5
8.10	10	90
9.50	10	90
A = 0.2% formic acid in acetonitrile		
B = 0.2% formic acid in water		

Flow Rate: 500  $\mu$ L/minute

Run Time: 9.50 minutes

Injection Volume: 10  $\mu$ L

Mass Spectrometer Parameters: (operated in LC-MS/MS mode)

Interface: TurboIonSpray<sup>®</sup> (ESI)

Polarity: Positive

Scan Type: MRM Monitoring with Unit/Unit resolution

Ions Monitored:	Q1	Q3
Benzobicyclon	447.1	257.1
1315P-070	355.0	165.0
1315P-570	354.0	164.0
1315P-683	319.1	240.1
1315P-960	412.1	176.0
1315P-076	398.1	208.1

Retention Times:

Benzobicyclon	~4.78	minutes
1315P-070	~3.75	minutes
1315P-570	~2.56	minutes
1315P-683	~2.38	minutes
1315P-960	~2.61	minutes
1315P-076	~2.40	minutes



**Parameters for 1315P-962 and 1315P-966**

Mobile Phase (Gradient Program):

<b>Time</b>	<b>A, %</b>	<b>B, %</b>
0.0	0	100
7.00	95	5
8.00	95	5
8.10	0	100
9.50	0	100
A = 0.2% formic acid in acetonitrile B = 0.2% formic acid in water		

Flow Rate: 500  $\mu$ L/minute

Run Time: 9.50 minutes

Injection Volume: 20 or 50  $\mu$ L

Mass Spectrometer Parameters: (operated in LC-MS/MS mode)

Interface: TurboIonSpray<sup>®</sup> (ESI)

Polarity: Negative

Scan Type: MRM Monitoring with Unit/Unit resolution

Ions Monitored:	Q1	Q3
1315P-962	157.0	113.0
1315P-966	235.0	191.0

Retention Times:		
1315P-962	~2.32	minutes
1315P-966	~2.65	minutes

LC-MS/MS conditions varied slightly from run to run when instrument and column maintenance were performed. The instrument parameters were checked for analyte sensitivity and resolution prior to each chromatographic run and the exact parameters were documented with each data set.

**E. Analysis of Study Samples**

Study sample analysis was initiated on January 12, 2015 and completed on October 20, 2016. A total of 533 field samples were received and 247 samples were analyzed: 96 water samples, 121 soil samples, and 30 application rate filter paper samples. Field samples and transit stability samples were analyzed in 35 sets. Each set consisted of a control (bulk source for water, a field sample for soil, and bulk source for filter paper), two

laboratory fortification samples and six to seventeen field samples. Prior to extraction, a unique laboratory code designation was assigned by GPL to each sample extract. The laboratory code consisted of the last three digits of the GPL study number, the sample set and a sample number (e.g., 544SET01-1).

Laboratory fortifications were prepared by using Wiretrol<sup>®</sup> micropipettes and directly fortifying the measured samples as follows:

Fortification Level	Amount of Spiking Solution Used
<b>Filter Paper Samples</b> (Benzobicyclon)	
328 µg/filter paper	250 µL of a 1.31 mg/mL solution
642 µg/filter paper	0.49 mL of a 1.31 mg/mL solution
917 µg/filter paper	0.7 mL of a 1.31 mg/mL solution
1048 µg/filter paper	0.8 mL of a 1.31 mg/mL solution
1585 µg/filter paper	0.5 mL of a 3.17 mg/mL solution

Fortification Level	Amount of Spiking Solution Used
<b>Water Samples</b> (Benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, 1315P-966)	
0.001 µg/mL	100 µL of a 101/100/101/101/101/100/101/101 ng/mL solution
0.01 µg/mL	100 µL of a 1.01/1.00/1.01/1.01/1.01/1.00/1.01/1.01 µg/mL solution or 100 µL of a 1.01/1.00/1.01/1.01/1.01/1.00/1.00/1.00 µg/mL solution or 100 µL of a 0.983/1.01/1.01/1.01/1.01/1.01/1.01/1.01 µg/mL solution
0.4 µg/mL	40 µL of 101/101/101/101/101/101/101/101 µg /mL solution
0.8 µg/mL	80 µL of 101/101/101/101/101/101/101/101 µg /mL solution
<b>Soil Samples</b> (Benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076)	
0.005 ppm	100 µL of 0.500/0.500/0.500/0.500/0.505/0.505 µg/mL solution or 50 µL of 1.01/1.00/1.01/1.01/1.01/1.00 µg /mL solution
0.05 ppm	100 µL of 5.00/5.00/5.00/5.00/5.05/5.05 µg /mL solution or 50 µL of 10.1/10.0/10.1/10.1/10.1/10.0 µg /mL solution or 50 µL of a 9.83/10.1/10.1/10.1/10.1/10.1 µg/mL solution
0.1 ppm	100 µL of 10.1/10.1/10.1/10.1/10.1/10.1 µg /mL solution or 100 µL of a 9.83/10.1/10.1/10.1/10.1/10.1 µg/mL solution

## F. Quantitation Procedures

Analyst Chromatography Data System version 1.5 and 1.6, a product of AB Sciex, was used to acquire, integrate, and calculate the concentrations of

benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966 as ng/mL using the linear regression function with 1/x weighting. For the regression calculations, concentration was designated as the independent variable and plotted on the x-axis. Peak response was designated as the dependent variable and plotted on the y-axis. From this regression curve, a slope, a correlation coefficient (r), and other parameters of the standard curve were calculated. The correlation coefficients were equal to or greater than 0.9973. Calibration standards were injected every two to six sample injections as well as at the beginning and end of the injection sequence. Six different standard concentrations were injected within each analytical set. The concentrations (ng/mL) of benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966 detected in sample extracts were interpolated from the standard calibration curve. The concentration as µg/mL or ppm (µg/g) of residue found in the samples was then calculated with Microsoft® Excel using the following equations:

For water:

$$\mu\text{g/mL} = \frac{(\text{ng/mL from curve}) \times (\text{dilution factor}) \times 1 \mu\text{g}}{(1000 \text{ ng})}$$

For soil:

$$\text{ppm} = \frac{(\text{ng/mL from curve}) \times (\text{Final Vol. in mL}) \times 1 \mu\text{g}}{(\text{sample amount in grams} \times 1000 \text{ ng})}$$

Recovery of the analyte from fortified samples was calculated as follows:

$$\% \text{ Recovery} = \frac{(\text{Measured Concentration, } \mu\text{g/mL or ppm}) \times 100}{(\text{Theoretical Concentration, } \mu\text{g/mL or ppm added})}$$

An example calculation for a benzobicyclon laboratory fortification of water in set 544SET04, sample 544SET04-2 Low Spike at 0.00101 µg/mL, is as follows:

$$\text{standard curve equation: } y = 1.18 \times 10^5 (x) + 519$$

where  $x$  = benzobicyclon concentration in ng/mL and

$$y = \text{peak response} = 89308.5$$

$$\text{benzobicyclon concentration from the curve} = 0.749 \text{ ng/mL}$$

$$\text{benzobicyclon residue } (\mu\text{g/mL}) = \frac{(0.749 \text{ ng/mL}) (1.43) (1 \mu\text{g})}{(1000 \text{ ng})}$$

$$\text{Benzobicyclon residue} = 0.00107 \mu\text{g/mL}$$

$$\% \text{ recovery} = \frac{0.00107 \mu\text{g/mL} \times 100}{0.00101 \mu\text{g/mL}} = 106\%$$

An example calculation for a benzobicyclon laboratory fortification of soil in set 544SET15, sample 544SET15-2 Low Spike at 0.00504 ppm, is as follows:

$$\text{standard curve equation: } y = 1.2 \times 10^5 (x) + 1.18 \times 10^3$$

where  $x$  = benzobicyclon concentration in ng/mL and

$$y = \text{peak response} = 26043.0$$

$$\text{benzobicyclon concentration from the curve} = 0.207 \text{ ng/mL}$$

$$\text{benzobicyclon residue } (\mu\text{g/mL}) = \frac{(0.207 \text{ ng/mL}) (200 \text{ mL}) (1 \mu\text{g})}{(10.01 \text{ grams}) (1000 \text{ ng})}$$

$$\text{Benzobicyclon residue} = 0.00414 \text{ ppm}$$

$$\% \text{ recovery} = \frac{0.00414 \text{ ppm}}{0.00504 \text{ ppm}} \times 100 = 82.1\%$$

## **G. Statistical Procedures**

Laboratory statistical procedures included calculation of arithmetic mean, the corresponding standard deviation (where  $n \geq 3$ ) and coefficient of variation for analyte recovery data. Linear regression analysis with  $1/x$  weighting was applied to LC-MS/MS calibration curves for the determination of slope, y-intercept and correlation coefficient values.

2. Laboratory Fortification for Water

Concurrent laboratory fortification recoveries of water samples, which were analyzed with each set of samples from California, are tabulated in Tables 29 through 36. Concurrent laboratory fortification recoveries of water samples, which were analyzed with each set of samples from Louisiana, are tabulated in Tables 37 through 44.

Laboratory fortification samples were prepared using bulk untreated control water obtained from each site. Each sample set consisted of a laboratory UTC sample and two fortification samples fortified with a mixed fortification solution that contained benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966. The laboratory fortification samples were fortified at  $\sim 0.001 \mu\text{g/mL}$  and  $\sim 0.01 \mu\text{g/mL}$  for water. After analysis of the water samples, the high fortification level was raised to  $\sim 0.8 \mu\text{g/mL}$  for benzobicyclon and to  $\sim 0.4 \mu\text{g/mL}$  for 1315P-070 to bracket the residues found in the field samples.

### 3. Laboratory Fortification for Soil

Concurrent laboratory fortification recoveries of soil samples, which were analyzed with each set of samples from California, are tabulated in Tables 45 through 50. Concurrent laboratory fortification recoveries of soil samples, which were analyzed with each set of samples from Louisiana, are tabulated in Tables 51 through 56.

The laboratory fortification samples were prepared using an untreated sample obtained from the field site. Each sample set consisted of a UTC sample and two fortification samples fortified with a mixed fortification solution that contained benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076. The laboratory fortification samples were fortified at ~0.005 ppm and ~0.05 ppm for soil.

:

## C. Freezer Storage Stability

A storage stability component was implemented on samples of water and soil from each site.

### 1. Freezer Storage Stability on Water

The storage stability component for water evaluated the stability of benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966 residues in water after 60, 120, 185, 360, and 443 days in storage, starting with a Day 0 to establish

the percent of residue each stored sample began with. All samples (field and frozen storage) were stored in amber glass jars with no impact on the data.

On August 3, 2015, thirty 10 mL subsamples of California bulk water samples and thirty 10 mL subsamples of Louisiana bulk water samples were aliquoted into 30-mL amber glass jars. The samples designated as the storage stability samples were fortified at approximately 0.01 µg/mL with benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966 and then placed into frozen storage, along with the samples designated for fresh concurrent QC samples for each time point. On the same day, one control and four samples freshly fortified were extracted and analyzed serving as the zero-time analysis (Day 0). At 60, 120, 185, 360, and 443 days after initial storage, a control, two fresh concurrent quality control (QC) samples, and two storage stability samples were extracted and analyzed for benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966. On each day, there were no residues detected in the control sample.

## 2. Freezer Storage Stability on Soil

On April 21, 2015, thirty  $10.0 \text{ g} \pm 0.04 \text{ g}$  subsamples of homogenized soil from Louisiana were weighed into 250-mL Nalgene® bottles and stored in the freezer. On April 23, 2015, thirty  $10.0 \text{ g} \pm 0.04 \text{ g}$  subsamples of homogenized soil from California were weighed into 250-mL Nalgene® bottles and stored in the freezer. On April 28, 2015, samples designated as the storage stability samples were fortified at approximately 0.05 ppm benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076 and then placed into frozen storage. On the same day, one control and four samples freshly fortified were extracted and analyzed serving as the zero-time analysis (Day 0). At 120, 180, 360, and 531 days after initial storage, a control, two fresh concurrent quality control (QC) samples, and two storage stability samples were extracted and analyzed for benzobicyclon, 1315P-070, 1315P-570, 1315P-683, 1315P-960, and 1315P-076. On each day, there were no residues detected in the control sample.



## **V. CHANGES TO THE PROTOCOL**

Seven amendment/deviations to the study protocol were issued by the Study Director and approved by a representative of the Sponsor. Amendment 1 added an additional water sampling event to the study to allow for better characterize the persistence of benzobicyclon and its degradation products in paddy water. Amendment 2 detailed the analytical phase of the study to the protocol since all information was not available when the protocol was issued. Amendment 3 changed the Gowan Sponsor and Monitor, providing new contact information, added a freezer storage stability component to the protocol, and eliminated the analyte 1315P-168 from being analyzed in water and soil samples. Amendment 4 changed the Gowan Sponsor and Monitor, the Study Director and the Principal Field Investigator at the California site,

providing new contact information for all. Amendment 5 changed the methods for both water and soil to be used for analysis to GPL methods that included all the degradation products to be monitored. Amendment 5 also added a component to lower the LOQ for water which was then amended out of the protocol in Amendment 6. Amendment 5 had a deviation part to it in which it was documented that the transit stability spikes were not completed as required in Amendment 2 and the new detail of how the transit stability samples were to be handled was issued. Amendment 6 detailed that due to time limitations an analytical report was to be issued without the analysis and results of the transit stability and additional freezer storage stability time points remaining, however those samples still pending would be completed and the results reported in an amended report at a future date, which were retracted in Amendment 7. Amendment 7 also added instructions to send an additional transit stability set of water and soil to the field sites to re-evaluate and help determine the cause of low recovery values in the original samples.

**Analysis of Benzobicyclon and its Degradation Products 1315P-070,  
1315P-570, 1315P-683, 1315P-960, 1315P-076, 1315P-962, and 1315P-966 in  
Water by LC-MS/MS**

To prepare laboratory QC samples

Measure 10 mL of the matrix water into a 15-mL wide mouth amber packer

↓  
Fortify as necessary

↓  
(Procedure for QC and field samples)

**For Benzobicyclon, 1315P-070, 1315P-570,  
1315P-683, 1315P-960, 1315P-076**

↓  
Aliquot 7 parts of sample into an appropriate vessel  
Aliquot Volume \_\_\_\_\_ mL

↓  
Add 3 parts Acetonitrile for a dilution factor of 1.43x  
Dilution Volume \_\_\_\_\_ mL

↓  
Mix well, vial, and analyze using LC-MS/MS

*If further dilution is necessary, dilute with  
Acetonitrile/HPLC grade water (30:70, v/v)*

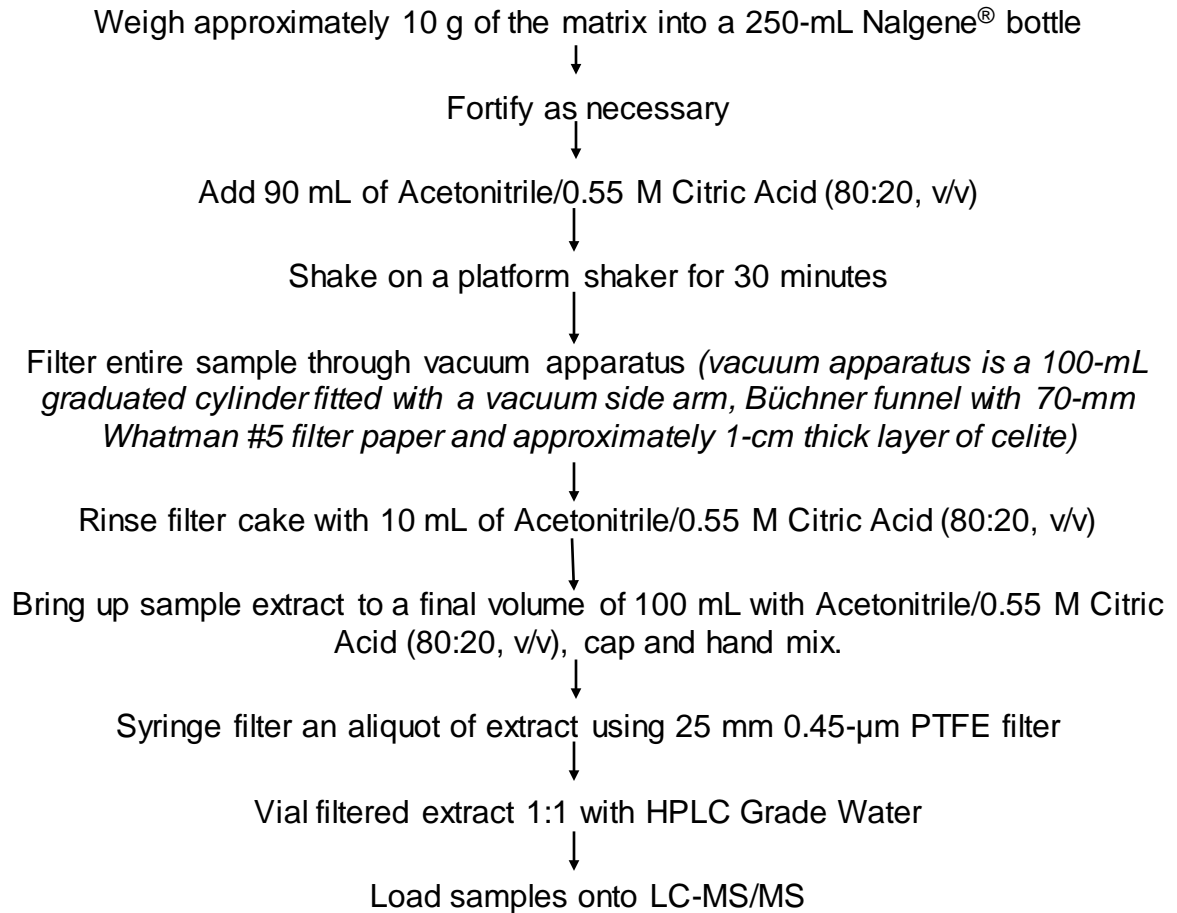
**For 1315P-962 and 1315P-966**

↓  
Aliquot 9 parts of sample into an appropriate vessel  
Aliquot Volume \_\_\_\_\_ mL

↓  
Add 1 part Acetonitrile for a dilution factor of 1.11x  
Dilution Volume \_\_\_\_\_ mL

↓  
Mix well, vial, and analyze using LC-MS/MS

*If further dilution is necessary, dilute with  
Acetonitrile/HPLC grade water (10:90, v/v)*

**Analysis of Benzobicyclon and its Degradation Products 1315P-070, 1315P-076, 1315P-570, 1315P-683, and 1315P-960 in Soil by LC-MS/MS**

*If further dilution is necessary, dilute with Acetonitrile/HPLC grade water (40:60, v/v)*

## 7.8 Freezer Storage Stability

Freezer storage stability of benzobicyclon and its metabolites in water and soil will be conducted concurrently with the analysis of the study samples. The longest storage interval will be at least as long as the maximum storage interval of the study samples. The details of the freezer storage stability assessment will be added by amendment.

## 7.10 Data Evaluation

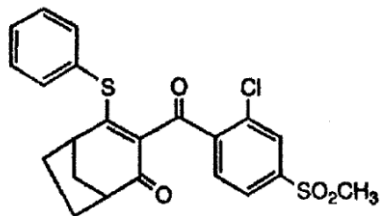
In general, calculations will be performed as described in the analytical method. Results, however, will not be corrected for procedural recovery, unless specifically requested by the Study Director. Accuracy and precision of the method, measured by evaluation of procedural recovery data, will be determined by calculating the mean percent recovery, standard deviation (SD) and relative standard deviation (RSD) for each matrix and fortification level.

## 7.11 Data Retention

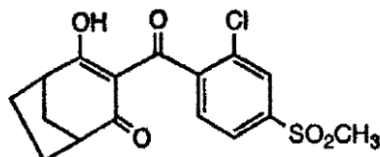
All original raw data generated during the analytical phase of the study will be organized and maintained by the analytical laboratory. Following completion of the study the raw data will be transferred to Waterborne Environmental, Inc.'s archives. This raw data will be subsequently transferred to Gowan Company.

## 7.12 Analytical Reference Standard Descriptions

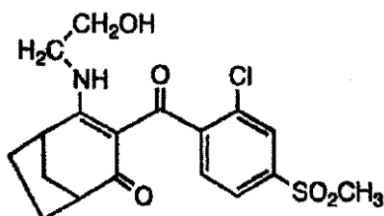
<b>Name</b>	: Benzobicyclon
<b>Lot Number</b>	: 1A0110
<b>Purity</b>	: 99.9%
<b>CAS Number</b>	: 156963-66-5
<b>Chemical Name</b>	: 3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylthio) bicyclo[3.2.1]oct-3-en-2-one
<b>Molecular Formula</b>	: C <sub>22</sub> H <sub>19</sub> ClO <sub>4</sub> S <sub>2</sub>
<b>Molecular Weight</b>	: 447.0 g/mole
<b>Expiration Date</b>	: May 20, 2015
<b>Structure</b>	:



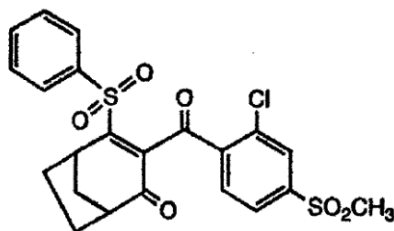
**Name** : 1315P-070  
**Lot Number** : 95Z25  
**Purity** : 99.7%  
**CAS Number** : 126656-88-0  
**Chemical Name** : 3-[2-chloro-4-(methylsulfonyl)benzoyl]-bicyclo[3.2.1]octane-2,4-dione  
**Molecular Formula** : C<sub>16</sub>H<sub>15</sub>ClO<sub>5</sub>S  
**Molecular Weight** : 354.8 g/mole  
**Expiration Date** : May 20, 2015  
**Structure** :



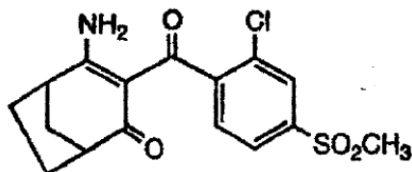
**Name** : 1315P-076  
**Lot Number** : TNA-10-074  
**Purity** : 99.5%  
**CAS Number** : NA  
**Chemical Name** : (3-(2-chloro-4-(methylsulfonyl)benzoyl)-4-(2-hydroxyethylamino))bicyclo[3.2.1]oct-3-en-2-one  
**Molecular Formula** : NA  
**Molecular Weight** : NA  
**Expiration Date** : July 10, 2016  
**Structure** :



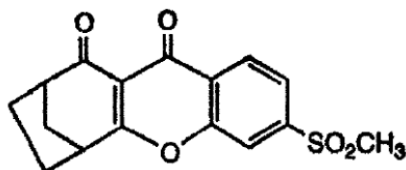
**Name** : 1315P-168  
**Lot Number** : TNA-10-158  
**Purity** : 98.4%  
**CAS Number** : NA  
**Chemical Name** : 3-[2-chloro-4-(methylsulfonyl)benzoyl]-4-(phenylsulfonyl)bicyclo[3.2.1]oct-3-en-2-one  
**Molecular Formula** : NA  
**Molecular Weight** : NA  
**Expiration Date** : September 24, 2016  
**Structure** :



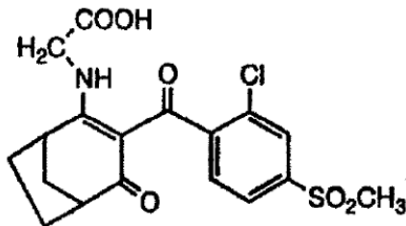
**Name** : 1315P-570  
**Lot Number** : TNA-9-186  
**Purity** : 99.8%  
**CAS Number** : NA  
**Chemical Name** : (3-(2-chloro-4-(methylsulfonyl)benzoyl)-4-amino)bicyclo[3.2.1]oct-3-en-2-one  
**Molecular Formula** : NA  
**Molecular Weight** : NA  
**Expiration Date** : July 10, 2016  
**Structure** :



**Name** : 1315P-683  
**Lot Number** : TM-8-198  
**Purity** : 99.1%  
**CAS Number** : NA  
**Chemical Name** : 3,4-dihydro-2,4-ethylene-6-methylsulfonyl-1,9(2H)-xanthendione  
**Molecular Formula** : NA  
**Molecular Weight** : NA  
**Expiration Date** : July 10, 2016  
**Structure** :

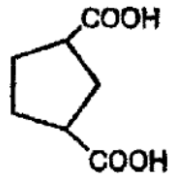


**Name** : 1315P-960  
**Lot Number** : MB-16-023  
**Purity** : 99.2%  
**CAS Number** : NA  
**Chemical Name** : (4-(carboxymethyl)amino-3-(2-chloro-4-methylsulfonylbenzoyl))bicyclo[3.2.1]oct-3-en-2-one  
**Molecular Formula** : NA  
**Molecular Weight** : NA  
**Expiration Date** : July 10, 2016  
**Structure** :





**Name** : 1315P-962  
**Lot Number** : TNA20130717  
**Purity** : 88.3%  
**CAS Number** : NA  
**Chemical Name** : 1,3-*cis*-cyclopentanedicarboxylic acid  
**Molecular Formula** : NA  
**Molecular Weight** : NA  
**Expiration Date** : July 31, 2016  
**Structure** :



**Name** : 1315P-966  
**Lot Number** : CB01-08-0037  
**Purity** : 99.5%  
**CAS Number** : NA  
**Chemical Name** : 2-chloro-4-methylsulfonylbenzoic acid  
**Molecular Formula** : NA  
**Molecular Weight** : NA  
**Expiration Date** : July 10, 2016  
**Structure** :

