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# Determination of benzotriazole and analog compounds by liquid chromatography-mass spectrometry in surface runoff water samples from Wilmington Air Park

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# DETERMINATION OF BENZOTRIAZOLE AND ANALOG COMPOUNDS BY LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY IN SURFACE RUNOFF WATER SAMPLES FROM WILMINGTON AIR PARK

A thesis submitted in partial fulfillment of the
requirements for the degree of
Master of Science

By

JESSICA A. WIESE

B.S., Rensselaer Polytechnic Institute, 2015

2019

Wright State University

WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

December 5, 2019

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Jessica A. Wiese ENTITLED Determination of Benzotriazole and Analog Compounds by Liquid Chromatography-Mass Spectrometry in Surface Runoff Water Samples from Wilmington Air Park BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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# ABSTRACT

Wiese, Jessica A. M.S., Department of Chemistry, Wright State University, 2019.
Determination of Benzotriazole and Analog Compounds by Liquid Chromatography-Mass Spectrometry in Surface Runoff Water Samples from Wilmington Air Park.

Benzotriazole (BTri) and its analog compounds are widely used in aircraft de-icing and anti-icing solutions, however their high solubility in water and resistance to biodegradation results in only their partial removal in wastewater treatment, and therefore makes them potential contaminants in watershed systems. The Wilmington Air Park in Wilmington, Ohio currently has two surface-runoff lagoons to pre-treat water containing these de-icing compounds before releasing it into nearby streams. Because the Wilmington Air Park has recently made an agreement with a major online retailer to lease over two dozen planes and open a package-sorting facility beginning in late 2019, the goal of this project was to determine the quantity of BTri compounds that may be present in runoff from the air park's water treatment beds and establish a water quality baseline before the retailer begins their operations in Wilmington. Water samples were collected from three sites—Lytle Creek, Indian Run, and Cowan Creek—throughout a six-week period during the winter (January and February) of 2019 and isolated via solid-phase extraction, then analyzed for the presence of BTris using a quadrupole LC-MS in Single Ion Monitoring detection mode. No BTri compounds were detected in the control site (Cowan Creek) samples, and BTri was only detected in trace amounts (less than 0.100

µg/L) in the Lytle Creek and Indian Run sites. However, tolytriazole (TTri, collective name for the isomers of 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole) was found in the concentration ranges of 0.111-0.869 µg/L for the Indian Run site and 0.822-2.724 µg/L for the Lytle Creek site. Because the extraction process did not yield 100 percent elution of the analytes, a surrogate standard (5,6-dimethyl-benzotriazole) was used to calculate the maximum theoretical concentration ranges of TTri to correct for the percent recovery: 0.167-1.248 µg/L for the Indian Run site and 1.201-3.435 µg/L for the Lytle Creek site. These TTri concentrations corresponded well with the type of weather and precipitation conditions that would require the application of de-icing and anti-icing fluids to aircraft—the highest concentrations were detected when the temperature was the lowest and there was both freezing rain and snowfall, while the lowest concentrations were detected when the temperature was above 10 °C (50 °F).

# Table of Contents

# List of Figures

# List of Tables

# 1. Introduction

## 1.1 Water Quality Studies of the Wilmington Watershed Area

Since 2007, the southern Little Miami watershed has been the subject of much investigation by the Ohio Environmental Protection Agency (OEPA), who issued a technical report in 2009 on the "Biological and Water Quality Study of the Lower Little Miami River and Selected Tributaries 2007 Including the Todd Fork Subwatershed." This report provided an assessment of the overall water quality and biodiversity in the watershed area, and—with regards to the scope of this thesis—the report specifically stated that the macroinvertebrate populations of Lytle Creek and Indian Run in Wilmington, Ohio have been compromised. Building upon previous research on the possible pollutant sources (i.e., heavy metals and *E. Coli* levels) that have caused this reduction in biodiversity (Alsenbel, 2017), this thesis is an investigation of the presence of a class of chemicals known as benzotriazoles, which may be entering the streams due to de-icing/anti-icing runoff from Wilmington Air Park.

As part of the Wilmington watershed area, Lytle Creek was an important research site for municipal wastewater treatment processes from the 1940s through the 1960s, when that kind of technology was being rapidly developed. During this period of time, the US Public Health Service and Department of Health Education and Welfare conducted analyses on the effects of municipal waste pollution; while there are many records documenting a lack of fish and invertebrate diversity coupled with the existence of fungi due to polluted water in Lytle Creek (Gaufin, 1956), no such records exist for

Indian Run. More recently, the aforementioned OEPA technical report designated the fish community status of Lytle Creek as “Poor” with only six species found in the stream. In 2015, the Clinton Country Streamkeepers published their annual assessment of the various streams in Clinton County in the Wilmington News Journal, where the quality of Lytle Creek was again rated “Poor” with “very few living things found”; in contrast, several of the other Clinton County streams were rated “Excellent” or “Good.” Interestingly, all of these reports and studies do not take into account the presence and operations of the nearby Wilmington Air Park, which has likely contributed to the declining health of these creeks for over 80 years.

### 1.2 Wilmington Air Park History

Wilmington Air Park was originally opened in 1929, and has been under the ownership of various parties throughout the last 80 years, including the United States Air Force and the Airborne Freight Corporation. DHL acquired Wilmington Air Park in 2003 in order to expand its DHL Express operations. In 2009, DHL Express made the decision to relocate their operations to the Cincinnati/Northern Kentucky International Airport and transferred ownership of Wilmington Air Park to the Clinton County Port Authority (CCPA).

In 2015, a major online retail company started negotiations to establish an air cargo operation in 2017 at Wilmington Air Park (Salomone, 2017) in partnership with the Air Transport Services Group (ATSG). This agreement laid out the plans to lease 20 freighter aircraft from the ATSG and was going to serve as an initial “test run” of the retailer’s capabilities to operate their own air cargo hub, as the company typically relies on third-parties like UPS or FedEx for air transportation of their packages. These plans ultimately fell through, and the retailer established its air cargo hub at the Cincinnati/Northern Kentucky International Airport instead. However, in late 2018, it was announced that a revived agreement between the retailer and Wilmington Air Park had come to fruition (Gnau, 2018), and these plans will not only establish air operations for several of the company's planes, but a brand-new package sorting facility as well. In addition to extending the leases of the 20 Boeing 767 aircraft the retailer already has in operation through 2023, the new deal outlines the lease of 10 more planes (provided in increments of five in 2019 and 2020) plus the potential to acquire up to 17 more cargo planes. The company's air operations officially began at the air park on June 26, 2019 (Huffenberger, 2019).

### *1.2.1 History of Water Treatment Facilities and Pollution*

Back in 2001, two water treatment beds were constructed at the air park for the purpose of treating the runoff water generated when planes are de-iced before takeoff—one on the northwest side of the park for runoff entering Lytle Creek and one on the southeast side for runoff entering Indian Run. These structures consist of a storage lagoon that collects the initial runoff from de-icing activities, which is then diverted from the lagoon to the treatment facility before it is allowed to enter the watershed.

In May 2008, the OEPA and Ohio Department of Natural Resources (ODNR) investigated a fish and wildlife kill and water quality standards violations in Cowan Creek. These agencies determined that these violations were a result of inadequately treated runoff from the air park—the treatment beds at the air park were not the correct size relative to the amount and concentration of de-icing runoff and other wastewater they received, and so the lagoons overflowed and runoff ran directly into Lytle Creek and

Indian Run, which violated the air park's discharge permit in 2007 and 2008. To resolve these water pollution discharges, DHL Express was ordered to pay an $80,000 fine to the OEPA (Bennish, 2011). This pollution incident also served as a factor for DHL transferring ownership of the air park to the CCPA in 2009.

As a result of the air park's history of pollution, the most recent discharge permit issued by the OEPA (Permit 1II00031*HD, effective December 2015) requires the CCPA to monitor chemical oxygen demand (COD), dissolved oxygen (DO), ammonia concentrations, total suspended solids, flow rate, and dissolved solids in outfalls from the runway runoff while de-icing chemicals are in use (typically throughout the winter and early spring). Specifically, the air park's effluent (post-treatment) runoff cannot be less than 4.0 mg/L per month in DO during all months of the year; the COD is not allowed to exceed 106 mg/L per month from June-October, 637 mg/L per three weeks from November-February, 714 mg/L per three weeks from March-April or 262 mg/L per month in May.

However, if the storage lagoons overflow due to the events of winter storm precipitation or heavy rainfall, the air park is not considered culpable and the extra flow is still routed directly into the creeks. With this permit still in place, it is unclear what kind and how much of these de-icing/anti-icing and other wastewater chemicals have entered the streams in the past four years, let alone the six years DHL Express was operating out of the air park. And although Lytle Creek and Indian Run have been recently assessed for overall quality and the presence of heavy metals and *E. Coli* (Alsenbel, 2017), there has been no investigation with regards to whether or not chemicals and additives in jet fuel and aircraft de-icing and anti-icing fluids have contaminated these streams due to overflow from the air park’s water treatment beds.

### 1.3 Emerging Contaminants of Interest

Aircraft de-icing and anti-icing fluids (ADAFs) are primarily composed of glycols and water (typically 98-99% of the solution), with the other 1-2% of the solution consisting of various additives such as alkylphenol ethoxylates (detergents) and benzotriazoles (BTris), which have recently emerged as a contaminant of interest. Benzotriazoles are heterocyclic compounds containing three nitrogen atoms (base formula $C_6H_5N_3$). While these compounds only make up 0.2-1.7% of most ADAFs (Kiss, 2009), they are readily soluble in water, have a limited sorption tendency, and are highly resistant to biodegradation. This presents a significant concern for the environment because benzotriazole (BTri) and its derivative tolytriazole (TTri, collective term for two isomers containing a methyl group bonded to either C4 or C5 of the benzene ring) are widely used as corrosion inhibitor and flame-retardant additives in these de-icing and anti-icing fluids, as well as in dishwashing detergents. See Figure 1 for the structures of these compounds.

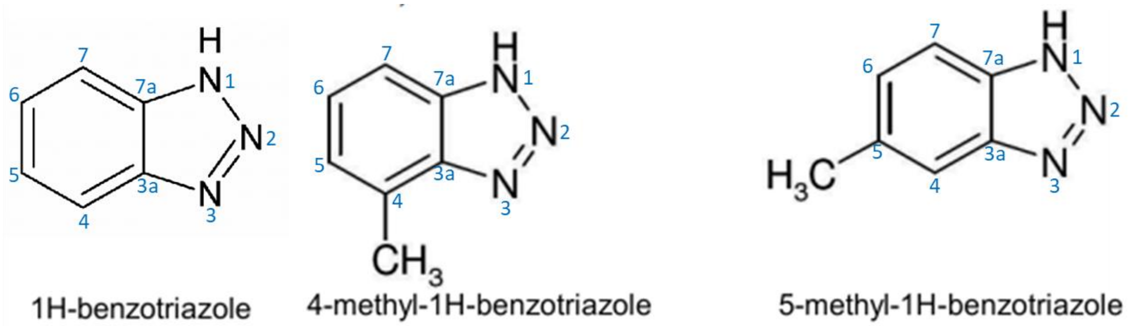


**Figure 1:** Chemical structures of 1H-benzotriazole, 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole; ring labeling scheme is in blue text.

Due to their high solubilities in water (20 g/L for BTri and 4.05 g/L for TTri), BTris are very difficult to remove from municipal water or airport runoff, and so a significant fraction reaches surface water systems like streams and lakes. There is also the potential for TTri to readily be removed from the runoff water and stick to the soil or sediment, as TTri has both a higher soil adsorption coefficient value ($K_{oc}$) and octanol-water partition coefficient value ($\log K_{ow}$) than BTri. Hart, et al. (2004) reported $K_{oc}$ ranges of 31.7-79.2 $mg^{-1}$ for BTri and 39.3-110 $mg^{-1}$ for TTri, while another study (Giger, 2006) reported $\log K_{ow}$ values of 1.23 and 1.89 for BTri and TTri, respectively. Both of these measurements indicate that TTri is more likely to adsorb to a non-aqueous phase like soil—especially soil high in organic matter—than BTri.

Relatively little is known about the effects of these compounds on the environment and humans, but there have been a few studies to determine the toxicity levels of BTris in common aquatic organisms using acute toxicity assays. Cancilla, et al. (1997) reported $EC_{50}$ (half-maximal effective concentration) values of 41.13 mg/L for BTri, 21 mg/L for the 4-methyl isomer of TTri, and 4.25 mg/L for the 5-methyl isomer of TTri; these Microtox bioassays showed that the 5-methyl isomer of TTri was the most toxic type of benzotriazole compound to *Allivibrio fischeri* (a common aquatic bioluminescent bacteria). Another study (Damalas, 2018) reported $LC_{50}$ (minimum lethal concentration required to kill 50% of a population) values of 170 mg/L for BTri, 59 mg/L for the 4-methyl isomer of TTri, and 128 mg/L for the 5-methyl isomer of TTri; these assessments showed that the 4-methyl isomer of TTri has the highest acute toxicity towards zebrafish larvae (a common aquatic vertebrate). It has also been shown that high concentrations of BTris exhibited anti-androgenic activity in zebrafish embryos (Fent, 2014). While there are no studies investigating the effects of BTri compounds on humans directly, Wang, et.al (2017) found that BTri could induce the invasion and migration of common endometrial carcinoma cells found in humans (i.e., Ishikawa and HEC-1-B cells) at a concentration of 120 ng/L *in vitro*. These results seem to indicate that BTri and its derivative compounds could act as a carcinogen that promotes the development of endometrial carcinoma. This increased awareness of BTri compounds has resulted in two countries setting drinking water standards for these chemicals (Dummer, 2014); Australia placed a limit on the 5-methyl isomer of TTri (< 2.4 µg/L) and Denmark placed a limit on BTri (<0.02 µg/L). However, even though more research efforts are being devoted to the study of BTris, there is still a clear deficiency in the understanding of the chronic effects of these compounds relative to how persistent they are in the aquatic environment.

Within the scope of this project, the potential for BTris to survive the air park's water treatment process and contaminate Lytle Creek and Indian Run is about to increase now that the air park is expanding its operations with the retail company. Therefore, before the air traffic in and out of Wilmington Air Park increases significantly, the goal of this project was to determine the concentration of BTris that are currently transported into Lytle Creek and Indian Run due to winter storm runoff from the air park's water treatment beds. In order to achieve this goal, water samples were collected from these two sites plus a control site on Cowan Creek over a six-week period from January 23, 2019 to February 28, 2019 (since winter is the time of year when ADAFs are most likely to be used on aircraft). The BTri and TTri analytes were then isolated from the water samples via solid-phase extraction and their detection was performed using a quadrupole LC-MS run in Single Ion Monitoring mode. The results of this analysis could then be used to establish a water quality baseline of Lytle Creek and Indian Run, which means further monitoring of the water quality once the retailer’s air operations begin could help determine if the heightened activity has any impact on the health of those streams.

# 2. Experimental

## 2.1 List of Chemical Reagents

The following is a list of the chemical reagents used throughout this project, detailing their CAS Registry Numbers, sources, and purities:

1. 1H-benzotriazole (CAS #95-14-7, Sigma-Aldrich, ≥ 98.0% purity)
2. 4-methyl-1H-benzotriazole (CAS #29878-31-7, Sigma-Aldrich, ≥ 90.0% purity)
3. 5-methyl-1H-benzotriazole (CAS #136-85-6, Sigma-Aldrich, ≥ 98.0% purity)
4. 5,6-dimethyl-1H-benzotriazole (CAS #4184-79-6, Chem Bridge, 100% purity)
5. Methanol (CAS #67-56-1, Fisher Scientific, HPLC-Grade, 99.9% purity)
6. Water (CAS #7732-18-5, ASTM Type I Water, 17.5-MΩ resistance)
7. Dichloromethane (DCM, CAS #75-09-2, Fisher Scientific, 99.9% purity)
8. Hydrochloric acid (HCl, CAS #7647-01-0, Fisher Scientific, TraceMetal Grade, 34-37% HCl)
9. Formic acid (CAS #64-18-6, Fisher Scientific, LC/MS-Grade, ≥ 99.0% purity)
10. Nitrogen gas (CAS #7727-37-9, Airgas Operations, Ultra High Purity 5.0-Grade)

## 2.2 Sampling Procedures

Sampling was conducted once a week over the six-week period of January 23, 2019 to February 28, 2019. At each of the three sampling sites described below, duplicate water samples were collected in 500-mL amber glass bottles with Teflon (R) closures, and then stored at -20 °C. The sampling area included two different watersheds. Indian Run merges with Cowan Creek, which runs south of the city of Wilmington and ultimately drains into Cowan Lake. Lytle Creek runs through the city of Wilmington before merging with Todd Fork, which eventually drains into the Little Miami River. A map of the area near Wilmington Air Park detailing the investigated creek locations is presented in Figure 2.

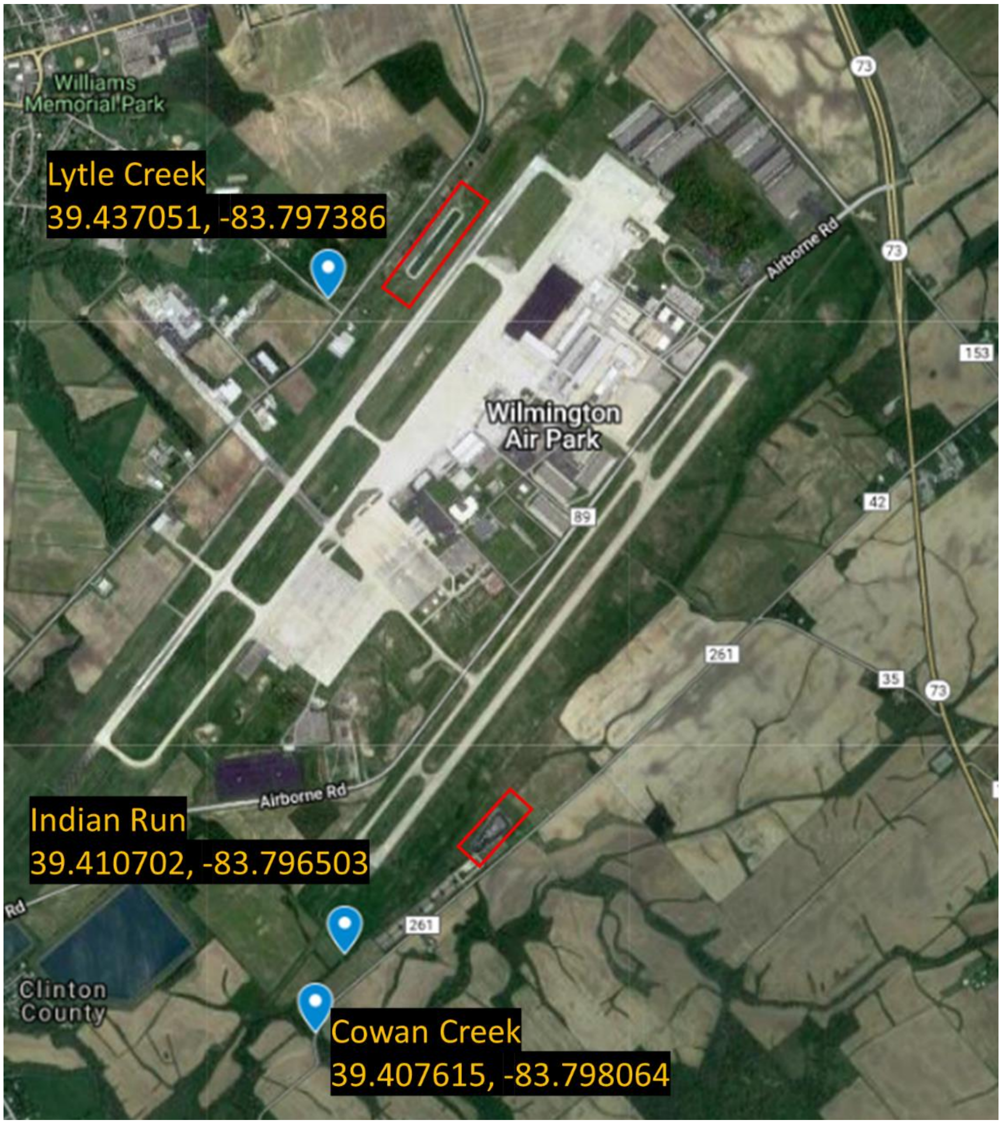


**Figure 2:** Satellite map detailing the locations of the two water treatment beds used by Wilmington Air Park, and the geographic coordinates of each sample site.

The sampling site areas depicted in the satellite map can be further enhanced to show exactly where runoff water from the air park's filtration beds is being channeled into Lytle Creek and Indian Run (see Figure 3 below). It has been reported (Travis Luncan, personal communication, January 23, 2019) that the filtration bed that drains into Lytle Creek is the only one that is actively in use, whereas the one that drains into Indian Run is not currently in use.

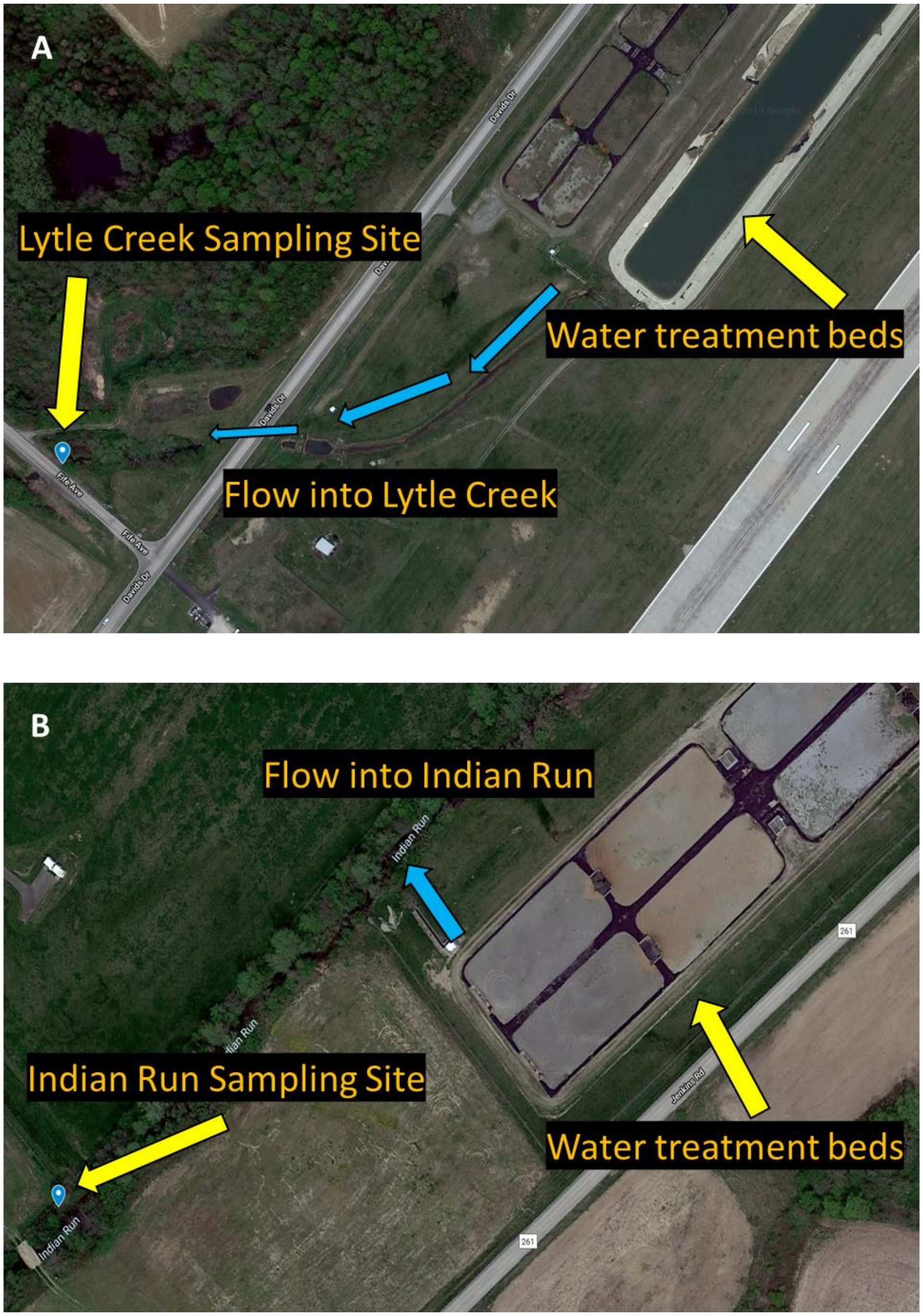


**Figure 3:** Satellite maps detailing the flow of water from Wilmington Air Park's treatment beds into Lytle Creek (A) and Indian Run (B).

The site at Lytle Creek is located just outside of the air park and is downstream of the filtration beds on the northwest side of the air park. It can be accessed easily from the side of the road at the corner of Fife Road and David's Drive. Samples were taken directly at the surface of the water.

**Figure 4:** Photos of the Lytle Creek sampling site taken on 2/01/2019 (A) and 2/06/2019 (B).

The site at Indian Run is located 0.2 miles upstream from where Indian Run joins with Cowan Creek and it is just downstream of the filtration beds on the southeast side of the air park. It can be accessed from a road pull-off on Jenkins Road. Although there is a slight drop-off to get down to the edge of the creek, samples can be taken directly at the surface of the water.

**Figure 5:** Photos of the Indian Run sampling site taken on 2/06/2019; (A) depicts a source of air park runoff into the creek, and (B) depicts where sampling was performed.

The site at Cowan Creek was used as the control site, as it is located about 0.1 miles upstream of where Indian Run joins with Cowan Creek. It can be accessed from a bridge on Jenkins Road. Since there is no safe way to get to the edge of the creek from the bridge, samples were collected using a bucket lowered down into the creek with a rope, and then transferred to the amber glass bottles.

**Figure 6:** Photos of the Cowan Creek sampling site taken on 2/01/2019 (A) and 2/06/2019 (B).

The following water quality parameters were measured at each site using a calibrated YSI Multi-meter: water temperature, pH, dissolved oxygen (DO, in % and mg/L), concentration of nitrogen in $NH_4^+$, conductivity, and pressure. The Standard Operating Procedure (SOP) outlining the complete details of the sampling process can be found in Appendix A.

## 2.3 Solid-Phase Extraction

### *2.3.1 Materials*

The following is a list of materials used throughout the solid-phase extraction (SPE) procedure, detailing their sources and relevant product specifications:

1. 0.7-µm glass fiber filters (Whatman, GF/F 47 mm)
2. OASIS HLB cartridges (Waters Inc., 500 mg, 6 mL)
3. 15.00-mL graduated centrifuge tubes (Kimax, Kimble-Chase Glassware)

### *2.3.2 Optimized Solid-Phase Extraction Method*

The water samples were allowed to thaw at room temperature for 16 hours prior to filtration through the glass fiber filters; each filtrate was then divided into three 100-mL replicates. Each filtrate was acidified to a pH of 2.5-3.0 using three drops of HCl and then spiked with 54.0 ng (10.0 µL of a 5.40-ppm solution) of 5,6-dimethyl-1H-benzotriazole (5,6-dimethyl-BTri) as the surrogate standard.

SPE was performed using the OASIS HLB cartridges and a 12-port vacuum extraction manifold. Each cartridge was sequentially conditioned with 3 x 2 mL of methanol and 3 x 2 mL of water by applying a slight vacuum. Sample extraction was performed at a flow rate of 5 mL/min; the cartridges were then dried under vacuum for 2 hours and 30 minutes. The analytes were eluted into the graduated centrifuge tubes with 5 mL of a solution of 3% methanol in DCM, and then evaporated to dryness under a stream of nitrogen gas. The dry residues were redissolved in 1 mL of methanol and stored at -20 °C overnight, then transferred to 2-mL autosampler vials prior to LC-MS analysis. The SOP outlining the complete details of the SPE process can be found in Appendix B.

### *2.3.3 Solid-Phase Extraction Method Development*

Before the optimized SPE method was fully developed, the water samples that were taken on January 23, 2019 were filtered, acidified, and spiked with the surrogate standard according to the procedures outlined above. However, 200-mL replicates of each sample were run through the SPE cartridges as opposed to the 100-mL replicates in the optimized method. This yielded very low percent recoveries of the surrogate standard, and so the analyte concentrations could not be calculated with high confidence in their accuracy. As a result, the samples from January 23, 2019 have been omitted from the data discussed in the results section.

In order to modify the SPE method to generate better percent recoveries of the surrogate standard, a water sample taken at the Lytle Creek site on February 9, 2018 was filtered, acidified, and spiked with the surrogate standard according to the procedures outlined above. The replicates were then run through the SPE cartridges under four different parameters: 200 mL of sample through a cartridge; 200 mL of sample through a cartridge with a 1.000-g layer of Florisil on top of the solid phase; 100 mL of sample through a cartridge; and 100 mL of sample through a cartridge with a 1.000-g layer of Florisil on top of the solid phase. The following table outlines the percent recovery data for each condition:

**Table 1:** Percent recovery of the surrogate standard for each of the four SPE optimization parameters.

| SPE Optimization Parameter | Percent Recovery of Surrogate Standard |
|---|---|
| 200 mL | 35.00% |
| 200 mL with Florisil layer | 18.10% |
| 100 mL | 58.90% |
| 100 mL with Florisil layer | N/A (below LOD) |

It is clear from the above results that using Florisil to try and clean out possible interfering compounds (i.e., inorganic or sulfur-containing compounds) does not help in the extraction of the analytes of interest; instead, the surrogate standard was likely retained in the Florisil layer. However, using 100 mL of sample as opposed to 200 mL of sample almost doubled the percent recovery of the surrogate standard. Therefore, all other water samples were run through the SPE cartridges in 100-mL replicates.

## 2.4 LC-MS Analysis

### *2.4.1 Optimized LC-MS Method*

An Agilent Technologies 1220 Infinity LC equipped with an Agilent Eclipse Plus C18 (1.8-µm I.D. 2.1 x 100-mm) column, autosampler, variable wavelength detector (VWD), column thermostat, and a quadrupole mass spectrometer was used for the separation and analysis of the BTri and TTri analytes dissolved in the methanol. The samples were eluted isocratically using a mobile phase consisting of a 60:40 methanol to water ratio with 0.1% formic acid using an injection volume of 2.0 µL and flow rate of 0.140 mL/min, with a total scan time of six minutes. The column temperature was maintained at 25.0 °C, the internal pressure of the instrument was set with a minimum of 0 bar and maximum of 370 bar, and the VWD was programmed to scan within the range of 190-400 nm.

Analyte detection was performed using electrospray ionization in positive polarity and in single-ion monitoring mode (SIM). Due to the instrument's lack of sensitivity to fragmented ions at *m/z* values below 100, only the $[M+1]^+$ ions of each analyte were chosen for SIM detection: *m/z* 120 for BTri, *m/z* 134 for TTri, and *m/z* 148 for 5,6-dimethyl-BTri (surrogate standard). The two isomers of TTri could not be separated, so the concentration results are reported as the sum of the two isomers.

Calibration of each compound was performed over the concentration range 25 μg/L - 1000 μg/L; all calibration curves were linear with $R^2$ values ranging from 0.9999 - 1.0000 for BTri, 0.9998 - 1.0000 for TTri, and 0.9990 - 1.0000 for 5,6-dimethyl-BTri. A complete list of calibration curves can be found in Appendix C.

In order to determine the concentration, each analyte peak was integrated manually using the OpenLAB CDS ChemStation Software. The peak areas were then incorporated into their respective calibration curve equations to generate the calculated concentrations, which were then divided by 100 to account for the concentration factor (100 mL of sample concentrated down to 1 mL of extracted analyte).

The SOP outlining the complete details of the LC-MS analysis process can be found in Appendix D.

### *2.4.2 LC-MS Method Development*

Three different combinations of elution solvents were tested during the development of the LC-MS procedure to determine the appropriate mobile phase: a 70:30 methanol to water solution, a 50:50 methanol to water solution, and a 60:40 methanol to water solution (all of which contained 0.1% formic acid). The resulting elution times for each analyte of interest (BTri, 4-Me-BTri, and 5-Me-BTri) are presented in the following table:

**Table 2:** Mean retention time (RT) plus standard deviation (SD) for each analyte of interest under the three different elution solution ratios.

| Analyte | 70:30 Methanol:Water | 50:50 Methanol:Water | 60:40 Methanol:Water |
|---|---|---|---|
| **BTri** | 2.544 ± 0.017 min | 3.555 ± 0.013 min | 2.921 ± 0.016 min |
| **4-Me-BTri** | 2.832 ± 0.011 min | 5.120 ± 0.018 min | 3.577 ± 0.037 min |
| **5-Me-BTri** | 2.786 ± 0.011 min | 4.931 ± 0.070 min | 3.519 ± 0.021 min |

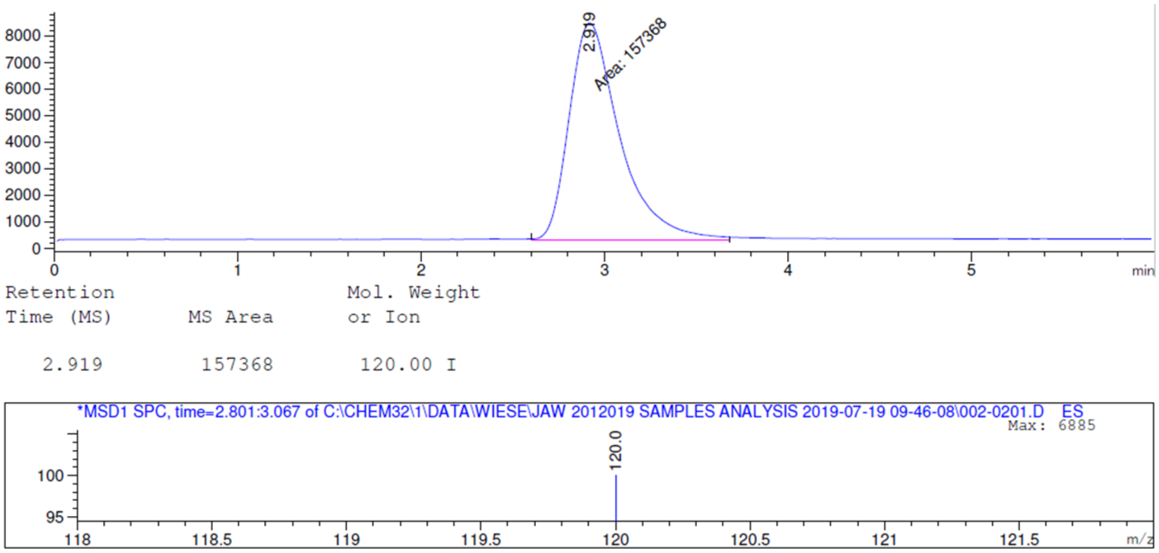
The mobile phase consisting of the 60:40 methanol to water ratio was chosen for the method of analysis because it achieved sufficient separation of BTri from the TTri isomers without exceeding the pressure limits of the instrument’s pumping system (maximum pressure of 370 bar). The 70:30 mixture did not separate the analytes as well as the 60:40 mixture, while the 50:50 mixture caused the pressure within the instrument to exceed 370 bar.

Because the two TTri isomers could not be resolved from each other, analytical standards were prepared as a mixture of 4-Me-BTri and 5-Me-BTri. The elution times of this mixed TTri standard and the surrogate standard (5,6-dimethyl-BTri) were then determined using the 60:40 methanol to water mobile phase.

**Table 3:** Mean retention time (RT) plus standard deviation (SD) for BTri, TTri, and 5,6-dimethyl-BTri under the 60:40 methanol to water solvent ratio.

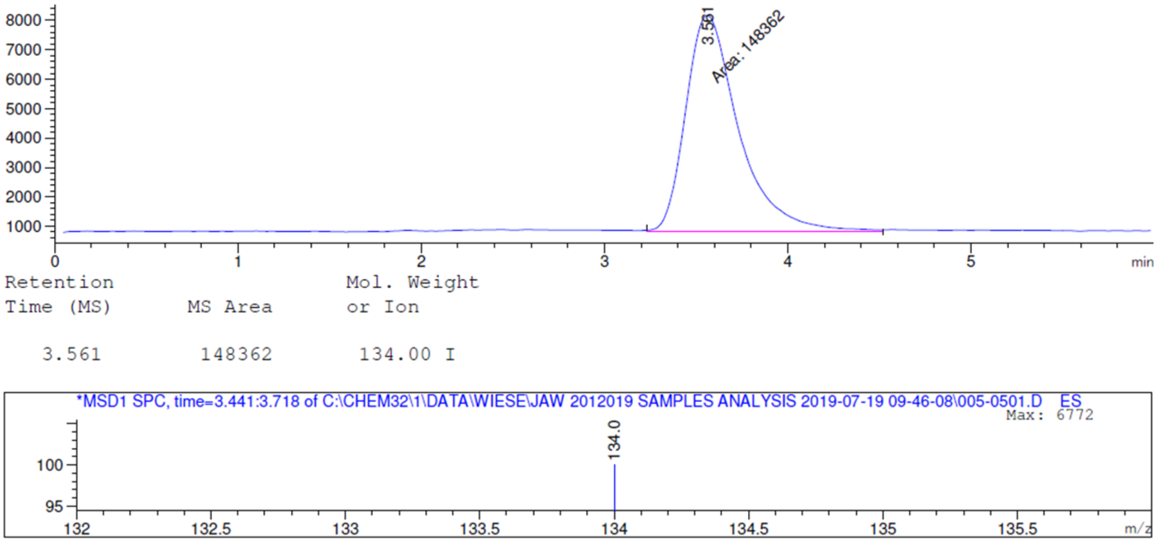
| Analyte | Mean RT plus SD in the 60:40 Methanol:Water solution |
|---|---|
| **BTri** | 2.921 ± 0.016 min |
| **TTri** | 3.556 ± 0.012 min |
| **5,6-dimethyl-BTri** | 4.290 ± 0.016 min |

The following three figures are examples of the chromatograms, retention times, and mass spectra that were obtained for the BTri, TTri, and 5,6-dimethyl-BTri standards when run through the LC-MS using the 60:40 methanol to water mobile phase:



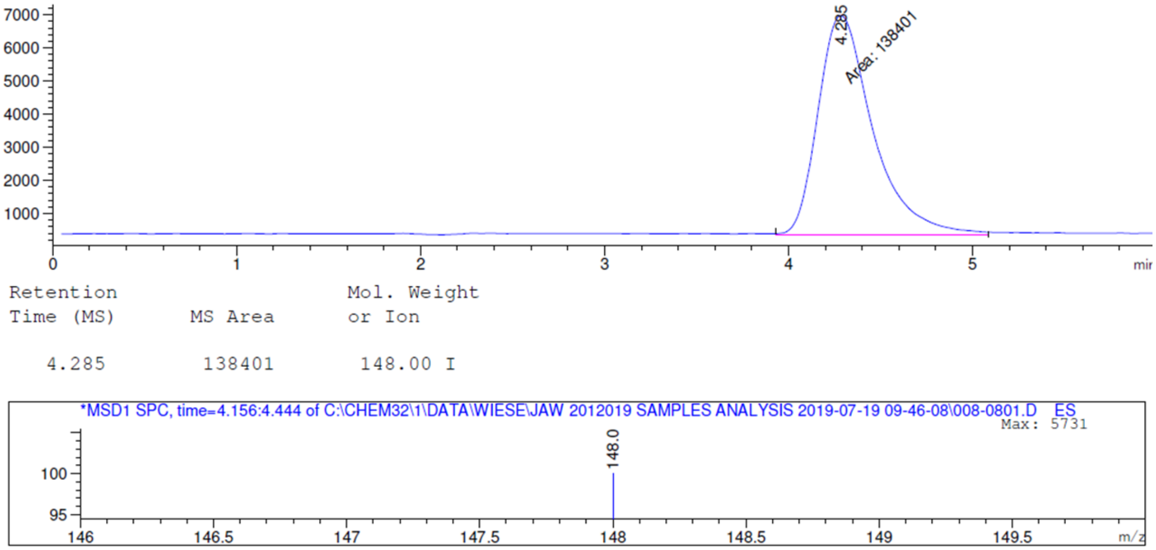
```
Retention
Time (MS)       MS Area       Mol. Weight
                              or Ion

  2.919          157368        120.00 I
```

**Figure 7:** Example of a typical chromatogram and mass spectrum obtained during the detection of BTri (concentration of BTri in this figure is 100 µg/L).



```
Retention
Time (MS)       MS Area       Mol. Weight
                              or Ion

  3.561          148362        134.00 I
```

**Figure 8:** Example of a typical chromatogram and mass spectrum obtained during the detection of TTri (concentration of the TTri mixed standard in this figure is 100 µg/L).



| Retention Time (MS) | MS Area | Mol. Weight or Ion |
|---|---|---|
| 4.285 | 138401 | 148.00 I |

**Figure 9:** Example of a typical chromatogram and mass spectrum obtained during the detection of 5,6-dimethyl-BTri (concentration of the 5,6-dimethyl-BTri in this figure is 100 µg/L).

# 3. Results and Discussion

## 3.1 Cowan Creek Results and Discussion

Because the sampling site on Cowan Creek is located upstream of where runoff from the air park would drain into Indian Run, BTri and TTri were not expected to be present in any of the water samples taken at this control site. The results of the LC-MS analysis proved this conjecture to be correct. See Figure 10 for an example of typical chromatograms for a Cowan Creek water sample.

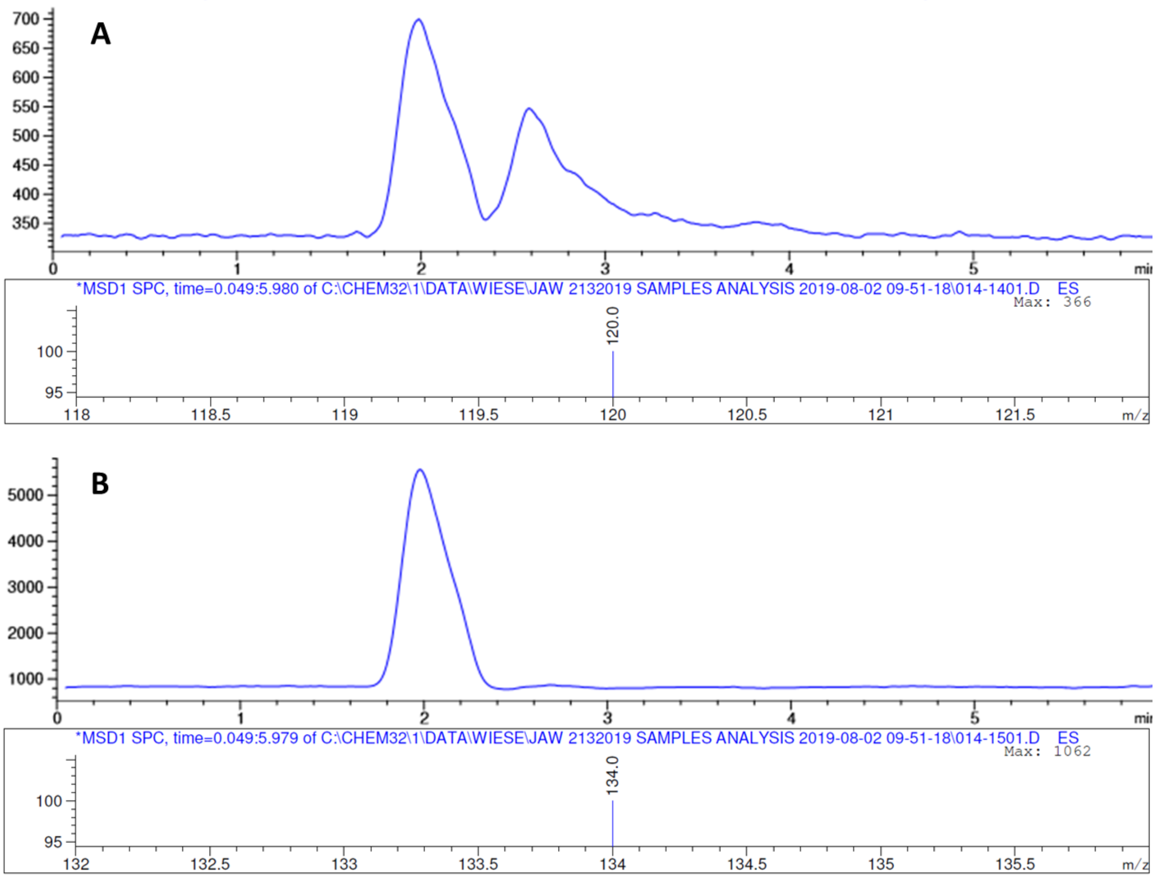
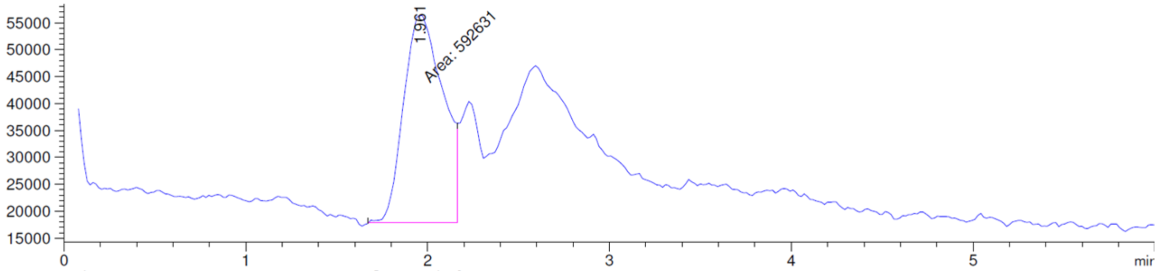


**Figure 10:** Chromatograms and mass spectra of a Cowan Creek sample taken on 2/13/2019, run in SIM mode for *m/z* values of 120 (A) and 134 (B).

As shown in Figure 10, the chromatograms for each Cowan Creek sample displayed no peaks within the standard deviation of the average elution times (see Table 3) of BTri or TTri. However, the scans displayed an unknown compound that eluted at approximately 2 minutes, and the scans at *m/z* 120 showed an additional peak that eluted at approximately 2.6 minutes. Both of these unknown compound peaks were present in the samples from Indian Run and Lytle Creek as well.

In order to determine the complete molecular ion fragmentation of the unknown compounds, a sample taken the previous year at the Lytle Creek site (LCFIF-T-02232018) was filtered and extracted using the optimized solid-phase extraction process (without addition of the surrogate standard), then run through the LC-MS under the optimized conditions and in “Full Scan” (total ion) mode. The following chromatogram and mass spectrum were obtained:



```
Retention                 Mol. Weight
Time (MS)      MS Area    or Ion

   1.961        592631      163.00 I
                            149.00 I
                            135.00 I
                            133.00 I
                            131.00 I
                            117.00 I
                            103.00 I
                            101.00 I
```

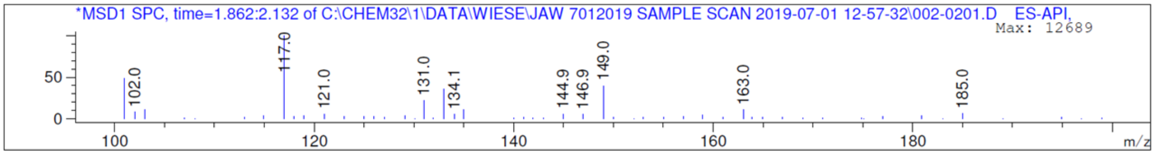
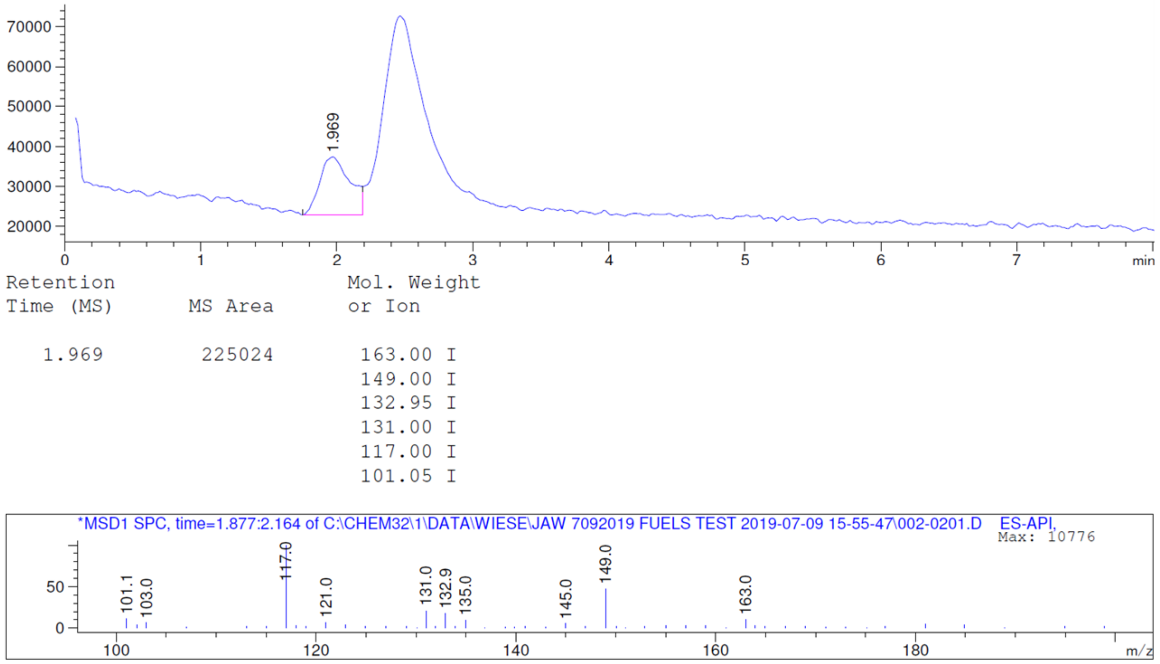


**Figure 11:** Full scan chromatogram and mass spectrum of sample taken at Lytle Creek on 2/23/2018 that exhibits characteristics of the unknown compound.

This spectrum displays several fragment ions that are 14 *m/z* units apart from each other, which indicates that the unknown compound likely contains a hydrocarbon chain (i.e., the –$CH_2$ groups are being fragmented off of the parent molecule). While the unknown compound could be an organic molecule that is naturally present in these watershed areas, there is also the possibility that it is a different type of environmental contaminant. Due to the apparent presence of a hydrocarbon chain, the unknown compound could be some sort of fuel, as fuels can contain any number of straight-chain hydrocarbons, branched hydrocarbons, and even polycyclic aromatic hydrocarbons.

To examine this possibility, a drop of JP-8 fuel was added to 1 mL of methanol and run through the LC-MS under the optimized conditions and in “Full Scan” (total ion) mode. The following chromatogram and mass spectrum were obtained:



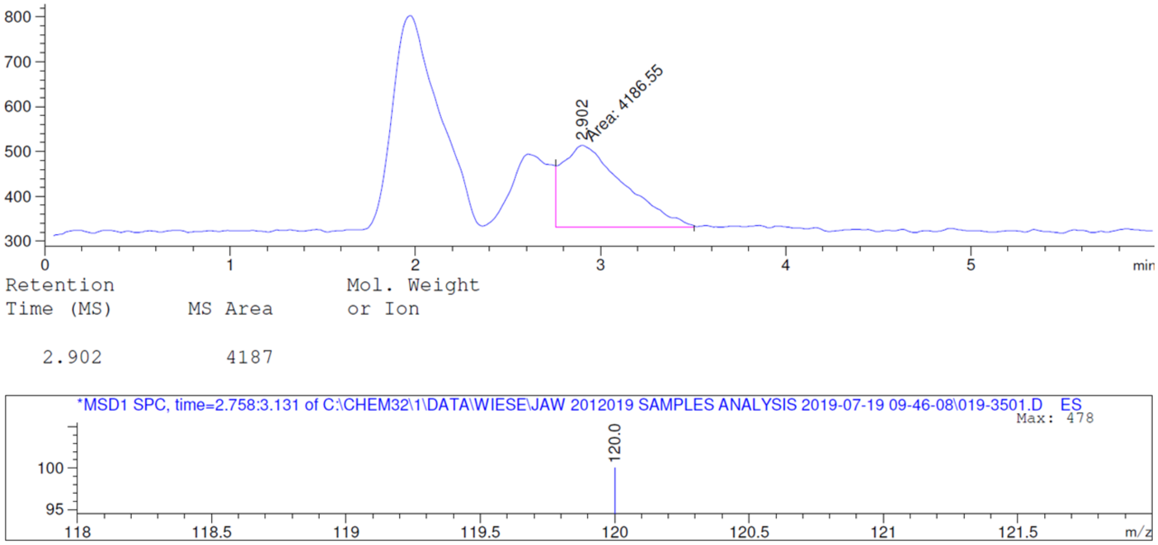
```
Retention                  Mol. Weight
Time (MS)      MS Area     or Ion

   1.969        225024       163.00 I
                             149.00 I
                             132.95 I
                             131.00 I
                             117.00 I
                             101.05 I
```

**Figure 12:** Full scan chromatogram and mass spectrum of the JP-8 fuel in methanol.

In addition to the near-identical elution times, there are a lot of similarities between the fragmentation patterns of the unknown compound and the JP-8 fuel, including many of the same *m/z* values. If the unknown compound is in fact a fuel component, because it is present in Cowan Creek and not just in Indian Run and Lytle Creek, it is more likely that the fuel is from cars as opposed to planes at the air park. There are several roads that cross over each stream, so it is possible that fuel leaking from cars could run off into the creeks. While the exact determination of the unknown compound's identity and source is beyond the scope of this project, it certainly warrants further studies as another potential contaminant of the Wilmington watershed area.

### 3.2 Indian Run and Lytle Creek Results and Discussion

The LC-MS scans indicated the presence of BTri in the Indian Run water samples from 2/01/2019, but only in trace concentrations that were too far below the detection limit (10.0 µg/L) to yield a BTri peak (elution time 2.9 minutes) that was completely resolvable from the unknown interference peak (elution time 2.6 minutes). Therefore, any partial peak area that could be integrated would not yield accurate or reliable concentration calculations. See Figure 13 for an example of the chromatogram and mass spectrum obtained for BTri detection in the 2/01/2019 Indian Run samples.



```
Retention                 Mol. Weight
Time (MS)      MS Area    or Ion

   2.902         4187
```

**Figure 13:** Chromatogram and mass spectrum of an Indian Run sample taken on 2/01/2019, run in SIM mode for *m/z* value of 120.

On the other four sample days, no distinct BTri peak could be detected in the Indian Run samples. As shown in the following figure, the interference peak is shifted to about 2.75 minutes and it tails off considerably close to 2.9 minutes; this indicates that it is likely a trace amount of BTri was present in the sample, but its concentration was much lower than the interfering substance's concentration to even generate a partial peak.

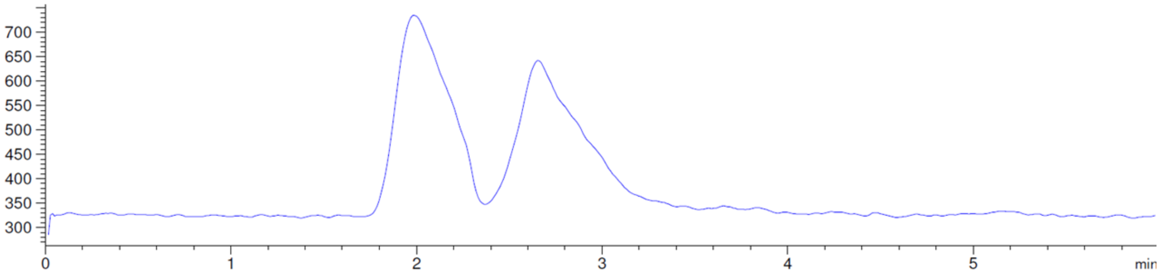
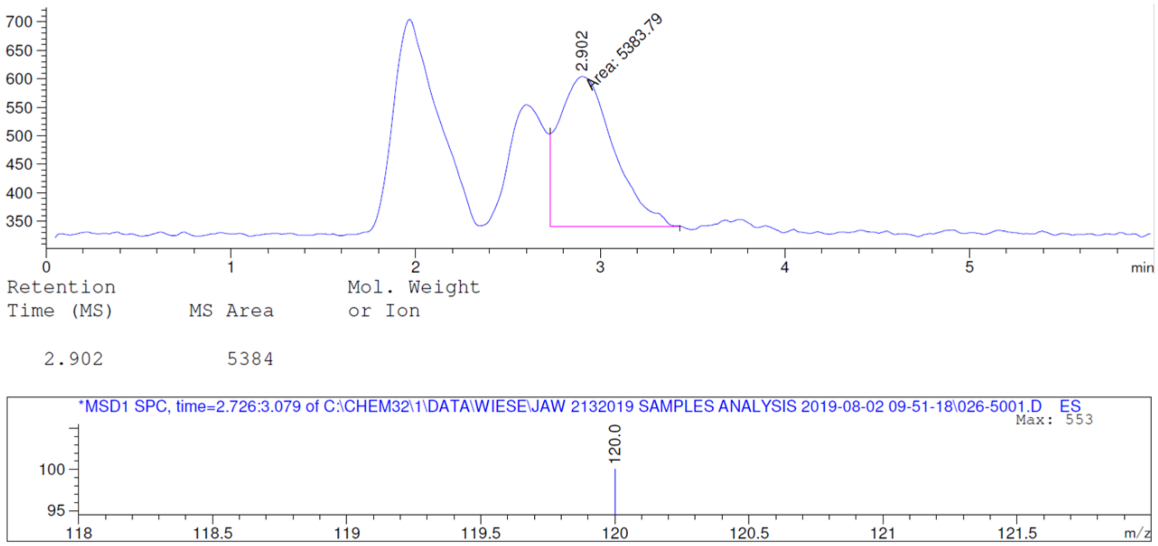


**Figure 14:** Chromatogram of an Indian Run sample taken on 2/22/2019, run in SIM mode for *m/z* value of 120.

With regards to the presence of BTri in the Lytle Creek samples, trace concentrations were detected on each sample day except 2/06/2019 (no BTri was detected on that day). The following figure displays a typical chromatogram and mass spectrum obtained for BTri detection in the Lytle Creek samples; these trace BTri concentrations were too far below the detection limit to yield a BTri peak that was completely resolvable from the unknown interference peak. As a result, any partial peak area that could be integrated would not yield accurate or reliable concentration calculations.

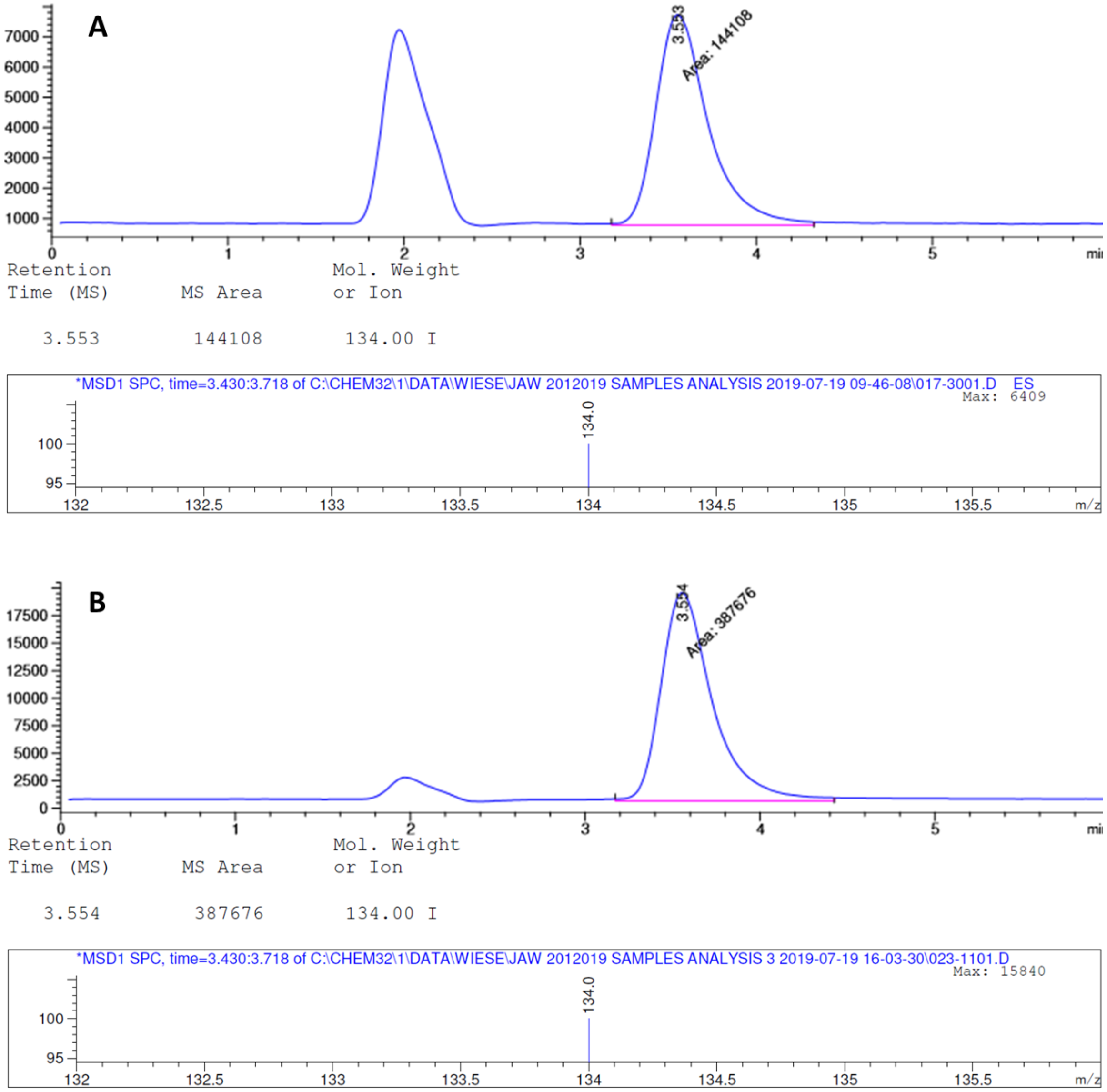


```
Retention                 Mol. Weight
Time (MS)      MS Area    or Ion

   2.902         5384
```

**Figure 15:** Chromatogram and mass spectrum of a Lytle Creek sample taken on 2/13/2019, run in SIM mode for *m/z* value of 120.

Even though the concentrations of BTri in the Indian Run and Lytle Creek samples could not be accurately quantified, the LC-MS chromatograms clearly display trace amounts of BTri in both streams. Therefore, one of the goals for the continuation of this research should be to identify and isolate the interfering substance from the water samples during the solid-phase extraction process or further optimize the LC-MS method to achieve better separation of the BTri peak from the interference peak.

In contrast to the detection of BTri, TTri was present at measurable concentrations on all sampling days in both Indian Run and Lytle Creek. See Figure 16 for an example of typical chromatograms and mass spectra for TTri detection in the Indian Run and Lytle Creek samples.



```
Retention
Time (MS)      MS Area      Mol. Weight
                            or Ion

   3.553        144108       134.00 I
```

```
Retention
Time (MS)      MS Area      Mol. Weight
                            or Ion

   3.554        387676       134.00 I
```

**Figure 16:** Chromatograms and mass spectra of an Indian Run sample (A) and a Lytle Creek sample (B) taken on 2/01/2019, run in SIM mode for *m/z* value of 134.

As shown in both Figure 16 (A) and (B), TTri was shown to be present in each sample as a highly resolved peak within the standard deviation of the average elution time for TTri. Each Indian Run and Lytle Creek sample contained TTri in a high enough concentration above the detection limit, so the integrals for all TTri peaks yielded reliable concentration calculations. The complete set of TTri concentration data (and 95% confidence interval) for both streams is presented in the following table and graph:

**Table 4:** Mean concentration and 95% confidence interval of TTri calculated for all samples collected at Indian Run and Lytle Creek.

| Sample Date | Sample Site | Mean Concentration Plus 95% Confidence Interval (µg/L) | Number of Samples |
|---|---|---|---|
| **2/1/2019** | Indian Run | 0.869 ± 0.10 | 6 |
| | Lytle Creek | 2.724 ± 0.13 | 6 |
| **2/6/2019** | Indian Run | 0.111 ± 0.04 | 3* |
| | Lytle Creek | 0.822 ± 0.18 | 6 |
| **2/13/2019** | Indian Run | 0.112 ± 0.04 | 6 |
| | Lytle Creek | 1.731 ± 0.13 | 6 |
| **2/22/2019** | Indian Run | 0.204 ± 0.04 | 6 |
| | Lytle Creek | 1.714 ± 0.13 | 6 |
| **2/28/2019** | Indian Run | 0.596 ± 0.04 | 6 |
| | Lytle Creek | 1.660 ± 0.12 | 6 |

* One sample bottle cracked and leaked during thawing process

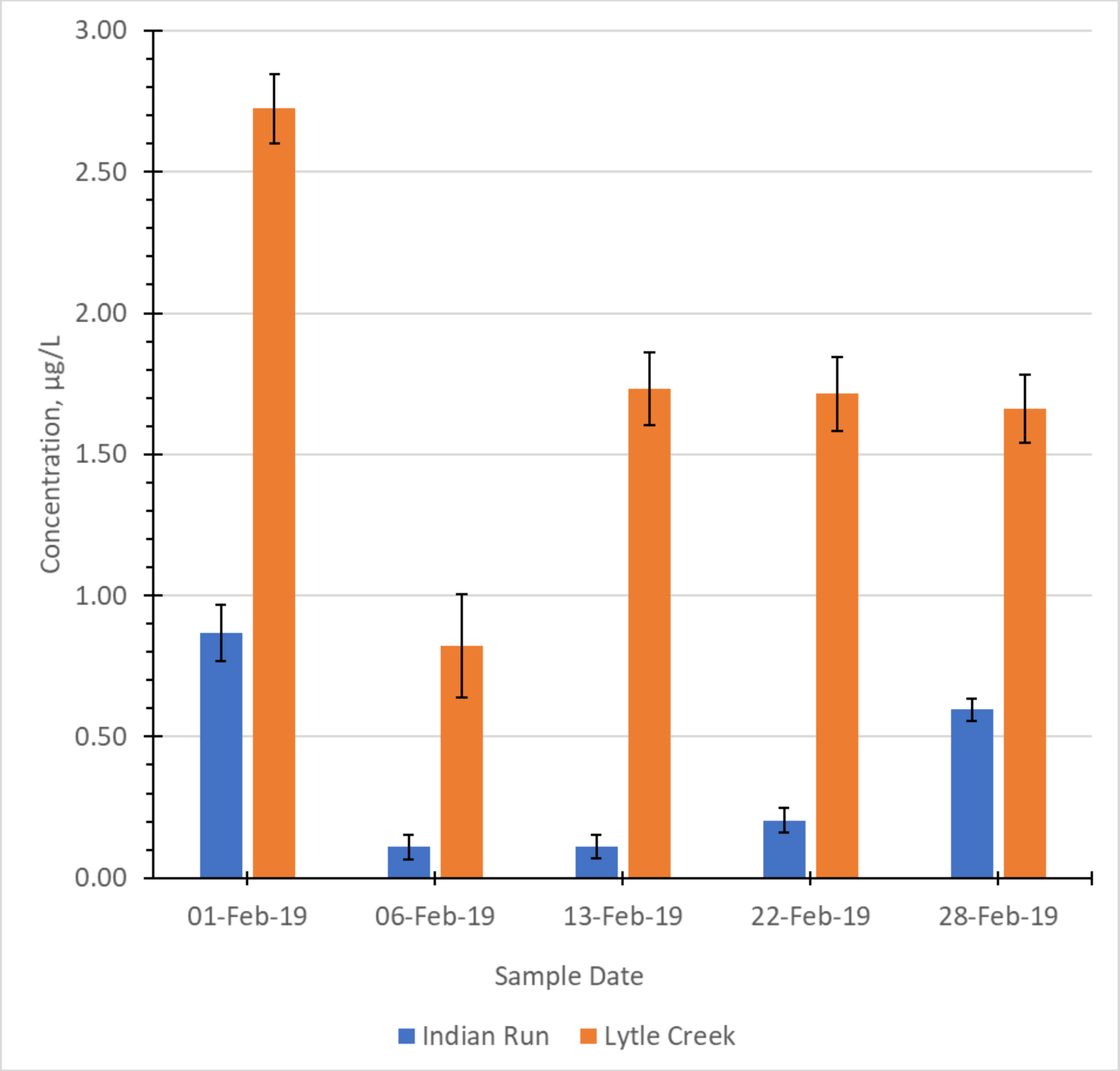


**Figure 17:** Mean concentration of TTri in Indian Run and Lytle Creek over the five-week sampling period; the error bars represent the calculated 95% confidence interval. This data set has not been corrected for percent recovery of the analyte.

As expected, the TTri concentrations in Lytle Creek were significantly higher than those in Indian Run since the water treatment facility on the northwest side of the air park is the only one actively in use at this time. In addition, the levels of TTri concentrations in the two streams corresponded with the temperature and precipitation conditions observed on each sampling day (see Table 5).

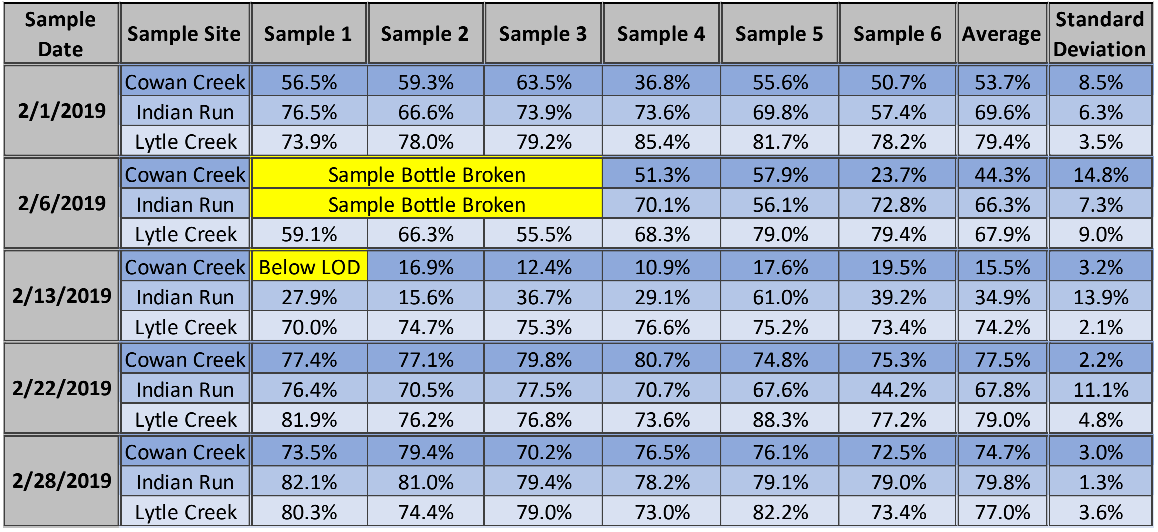
**Table 5:** Air temperature (low and high), precipitation, and snowfall data for each sampling day. All data was obtained from the National Weather Service.

| Sample Date | Air Temperature (°C , Low to High) | Precipitation (in) | Snowfall (in) |
|---|---|---|---|
| **2/1/2019** | -12.2 to -7.2 | 0.4 | 4 |
| **2/6/2019** | 4.4 to 14.4 | 1.52 | 0 |
| **2/13/2019** | -3.9 to 1.1 | 0 | 0 |
| **2/22/2019** | -0.6 to 4.4 | 0 | 0 |
| **2/28/2019** | -2.2 to 1.7 | 0 | 0 |

According to FAA regulations, ground icing conditions exist any time the temperature is less than 10 °C and there is precipitation, and so planes must be de-iced before takeoff; if it is below 10 °C but no precipitation, anti-icing fluids are applied so that icing does not occur during flight at higher altitudes. As can be seen in Figure 17, the TTri concentrations were the highest on 2/01/2019, when the temperature was the lowest and there was both freezing rain and snowfall. The TTri concentrations were the lowest on 2/06/2019 when it was above 10 °C, but there was significant precipitation that could still cause icing to occur at higher altitudes. On the next three sampling days, the temperature remained below 10 °C with no precipitation, and so the TTri concentrations did not vary appreciably. These results indicate that the amount of TTri running off into Indian Run and Lytle Creek on a given day depends greatly on the temperature and precipitation conditions that determine whether or not de-icing/anti-icing solutions are applied to the planes at the air park.

Because the SPE process does not yield 100 percent recovery of the BTri and TTri compounds present in the original water samples, a known amount (54.0 ng) of 5,6-dimethyl-BTri was added to each sample replicate before extraction. Its structural similarity to BTri and TTri and distinct elution time under the optimized LC-MS conditions (4.2 minutes) make 5,6-dimethyl-BTri an ideal surrogate standard to utilize in calculating the actual percent recovery of the analytes. The full percent recovery data is summarized in the following table:

**Table 6:** Percent recovery of 5,6-dimethyl-BTri calculated for each sample; the average percent recovery and standard deviation of the surrogate standard was then calculated for each sample site on each sample day.



| Sample Date | Sample Site | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Average | Standard Deviation |
|---|---|---|---|---|---|---|---|---|---|
| 2/1/2019 | Cowan Creek | 56.5% | 59.3% | 63.5% | 36.8% | 55.6% | 50.7% | 53.7% | 8.5% |
| | Indian Run | 76.5% | 66.6% | 73.9% | 73.6% | 69.8% | 57.4% | 69.6% | 6.3% |
| | Lytle Creek | 73.9% | 78.0% | 79.2% | 85.4% | 81.7% | 78.2% | 79.4% | 3.5% |
| 2/6/2019 | Cowan Creek | Sample Bottle Broken | | | 51.3% | 57.9% | 23.7% | 44.3% | 14.8% |
| | Indian Run | Sample Bottle Broken | | | 70.1% | 56.1% | 72.8% | 66.3% | 7.3% |
| | Lytle Creek | 59.1% | 66.3% | 55.5% | 68.3% | 79.0% | 79.4% | 67.9% | 9.0% |
| 2/13/2019 | Cowan Creek | Below LOD | 16.9% | 12.4% | 10.9% | 17.6% | 19.5% | 15.5% | 3.2% |
| | Indian Run | 27.9% | 15.6% | 36.7% | 29.1% | 61.0% | 39.2% | 34.9% | 13.9% |
| | Lytle Creek | 70.0% | 74.7% | 75.3% | 76.6% | 75.2% | 73.4% | 74.2% | 2.1% |
| 2/22/2019 | Cowan Creek | 77.4% | 77.1% | 79.8% | 80.7% | 74.8% | 75.3% | 77.5% | 2.2% |
| | Indian Run | 76.4% | 70.5% | 77.5% | 70.7% | 67.6% | 44.2% | 67.8% | 11.1% |
| | Lytle Creek | 81.9% | 76.2% | 76.8% | 73.6% | 88.3% | 77.2% | 79.0% | 4.8% |
| 2/28/2019 | Cowan Creek | 73.5% | 79.4% | 70.2% | 76.5% | 76.1% | 72.5% | 74.7% | 3.0% |
| | Indian Run | 82.1% | 81.0% | 79.4% | 78.2% | 79.1% | 79.0% | 79.8% | 1.3% |
| | Lytle Creek | 80.3% | 74.4% | 79.0% | 73.0% | 82.2% | 73.4% | 77.0% | 3.6% |

Although the percent recovery values of the surrogate standard were generally very good (the majority falling within the range of 70-80%), very low percent recoveries were calculated for the Indian Run and Cowan Creek samples from the sample date 2/13/2019. It was observed during the final steps of the SPE process that these specific samples took between 1 and 1.5 hours to dry with nitrogen, as opposed to the 20-30 minutes it took to dry the Lytle Creek samples taken on the same day and every sample from the other four days. Additionally, as these eluate solutions were close to being fully dried, they appeared to be more viscous than the eluate solutions in the other samples. These abnormalities in the SPE process and low percent recovery values indicate that there was some compound present in both Indian Run and Cowan Creek that was interfering with the separation of the BTri and TTri analytes. Because Lytle Creek exists in a separate watershed from the other two streams, it makes sense that its samples from the same day were not affected by this interference. While the determination of what this compound may be is outside the scope of this project, if similar findings appear in future samples taken at these sites, an investigation as to whether this compound is naturally occurring or the result of human activities (i.e., car fluid leaks or crop fertilizers) should be conducted.

This percent recovery data was then used to calculate the maximum theoretical concentrations of TTri present in the water samples. The complete set of corrected TTri concentration data (and 95% confidence interval) for Indian Run and Lytle Creek is presented in the following table and graph:

**Table 7:** Mean concentration and 95% confidence interval of TTri calculated for all samples collected at Indian Run and Lytle Creek, corrected for percent recovery.

| Sample Date | Sample Site | Mean Concentration Plus 95% Confidence Interval (µg/L) | Number of Samples |
|---|---|---|---|
| **2/1/2019** | Indian Run | 1.248 ± 0.06 | 6 |
| | Lytle Creek | 3.435 ± 0.19 | 6 |
| **2/6/2019** | Indian Run | 0.167 ± 0.01 | 3* |
| | Lytle Creek | 1.201 ± 0.11 | 6 |
| **2/13/2019** | Indian Run | 0.335 ± 0.06 | 6 |
| | Lytle Creek | 2.333 ± 0.15 | 6 |
| **2/22/2019** | Indian Run | 0.302 ± 0.04 | 6 |
| | Lytle Creek | 2.175 ± 0.20 | 6 |
| **2/28/2019** | Indian Run | 0.757 ± 0.04 | 6 |
| | Lytle Creek | 2.157 ± 0.17 | 6 |

* One sample bottle cracked and leaked during thaw process

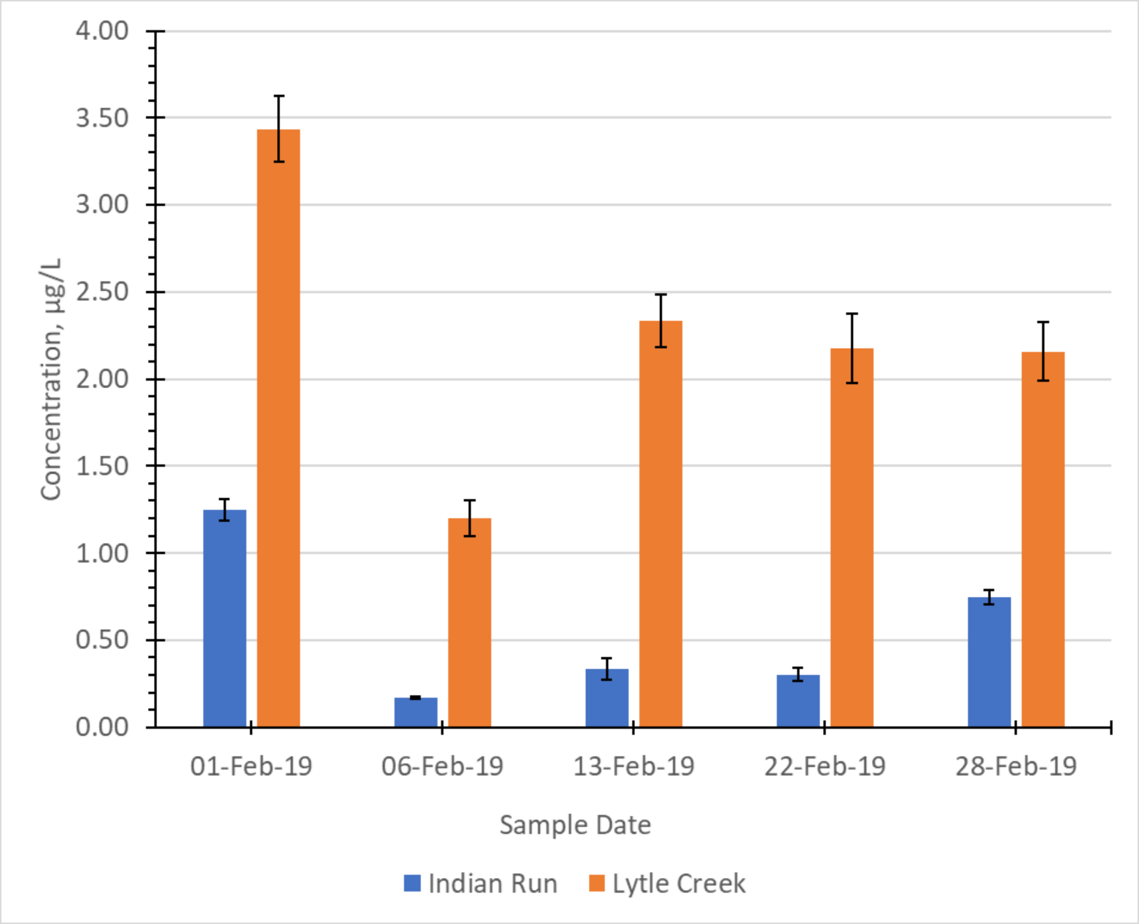


**Figure 18:** Mean concentration of TTri in Indian Run and Lytle Creek over the five-week sampling period; the error bars represent the calculated 95% confidence interval. This data set has been corrected for percent recovery of the analyte.

Upon calculating the TTri concentrations corrected for percent recovery, no significant changes in the overall trends were observed—the concentration values still corresponded as expected with the weather and precipitation data and the usage of the air park's water treatment beds. However, this corrected data now provides a more accurate estimate of the potential range of the TTri concentrations in Indian Run and Lytle Creek, with the uncorrected values being the lower end of the concentration range and the corrected values being the higher end. Thus, the concentration of TTri detected in Indian Run could be as low as 0.111-0.869 µg/L or as high as 0.167-1.248 µg/L, and the concentration of TTri detected in Lytle Creek could be as low as 0.822-2.724 µg/L or as high as 1.201-3.435 µg/L. At this low of a level, however, TTri and BTri will not be detected in the air park's monthly assessments of the COD levels in their effluent discharge. As stated previously, OHEPA Permit 1II00031*HD requires the airport to not exceed monthly COD levels of 637 mg/L from November-February and 714 mg/L in March-April, as these time periods are when temperature and precipitation conditions are most likely to necessitate the use of ADAFs. These COD levels are five orders of magnitude greater than the TTri concentrations that were detected, so it is clear that these compounds are evading the air park's monitoring system during runoff treatment.

### 3.3 Observations on Water Quality Data

The following table outlines the means and standard deviations of select water quality parameters that were taken at each site across all five sampling days (the complete tables of water quality data can be found in Appendix E):

**Table 8:** Mean and standard deviation for the parameters of pH, DO (%), DO (mg/L), $NH_4^+$-[N], conductivity, and pressure measured at the three sampling sites.

| Water Quality Parameter | Lytle Creek | Indian Run | Cowan Creek |
|---|---|---|---|
| **pH** | 6.97 ± 0.13 | 7.31 ± 0.09 | 7.29 ± 0.11 |
| **DO (%)** | 98.5 ± 6.4 | 104.1 ± 4.3 | 104.1 ± 4.8 |
| **DO (mg/L)** | 12.76 ± 0.80 | 13.56 ±0.51 | 13.55 ± 0.61 |
| **$NH_4^+$ - [N] (mg/L)** | 1.00 ± 0.04 | 0.22 ± 0.04 | 0.22 ± 0.05 |
| **Conductivity (µSiemens)** | 787.6 ± 3.9 | 457.0 ± 9.9 | 638.0 ± 29.1 |
| **Pressure (mm Hg)** | 741.4 ± 3.1 | 740.9 ± 3.0 | 741.7 ± 3.3 |

The measurements for each parameter remained consistent across the three sampling sites, with the DO (both % and mg/L) and pressure values not varying too much from site to site.  However, Lytle Creek is significantly less basic, more conductive, and contains more $NH_4^+$ when compared to Indian Run and Cowan Creek.  These observations support the fact that the air park's water treatment bed that drains its runoff into Lytle Creek is actively in use throughout the winter, whereas the treatment bed that drains into Indian Run is not being used.  Whatever the mixture of chemicals (BTri compounds, glycols, detergents, etc.) entering Lytle Creek may be, it is clearly lowering the pH, adding to the amount of $NH_4^+$ present, and adding enough ions to increase the conductivity compared to what was observed to be the normal levels in Indian Run.

### 3.4 Comparison of Results to Literature and Future Work

The following table outlines the results of five studies that also investigated the presence of BTri and TTri in watershed areas that are located near airports:

**Table 9:** Locations, sample types, analysis methods, BTri concentrations, and TTri concentrations for this project and five published studies conducted in the past 13 years.

| Literature Reference | Location | Sample Type | Analysis Method | BTri Concentration | TTri Concentration |
|---|---|---|---|---|---|
| This Study | Wilmington, Ohio | Surface Water (Streams) | LC-MS | Non-detect (trace amounts) | 0.111-0.869 µg/L (Indian Run, minimum range)<br>0.167-1.248 µg/L (Indian Run, maximum range)<br>0.822-2.724 µg/L (Lytle Creek, minimum range)<br>1.201-3.435 µg/L (Lytle Creek, maximum range) |
| Giger, 2006 | Switzerland | Surface Water (Rivers) | LC-MS/MS | 0.16-2.68 µg/L (average range) | 0.04-0.47 µg/L (average range) |
| Kiss, 2009 | Germany | Surface Water (Rivers) | GC-MS | 0.038-1.474 µg/L | 0.025-0.952 µg/L (4-Me)<br>0.025-0.281 µg/L (5-Me) |
| Herrero, 2013 | Spain | Treated sewage water effluent | LC-MS/MS | 0.062-1.559 µg/L | 0.038-1.253 µg/L (4-Me)<br>0.022-1.538 (5-Me) |
| Alvey, 2016 | USA (Alaska) | Snow & meltwater discharge | LC-MS/MS | Maxima of 0.51 µg/L (snow) & 1.58 µg/L (meltwater) | Maxima of 2.55 µg/L (snow) & 5.91 µg/L (meltwater) |
| Parajulee, 2017 | Canada | Surface Water (Rivers) | LC-MS/MS | 0.00091-2.39 µg/L | 0.00144-1.99 µg/L (4-Me)<br>Non-detect-0.448 µg/L (5-Me) |

As shown in Table 8, BTri is typically present in higher concentrations than TTri in European watershed systems, while TTri tends to be present in greater concentrations than BTri in the watershed areas of North America. Since the results obtained in this project also report higher concentrations of TTri than BTri, it is reasonable to assume that the de-icing/anti-icing solutions used by Wilmington Air Park and other American airports contain a higher percentage of TTri versus BTri compared to the chemicals used by European airports. Though it would be difficult to obtain from the air park, it would be helpful to know the exact brand of de-icing/anti-icing solution that is used in order to verify that these results are an accurate reflection of the chemical composition in terms of TTri and BTri.

Currently, the amount of BTri present in Lytle Creek and Indian Run is likely at the lower end of the concentration ranges reported in surface water samples throughout the world; because BTri was not present in the analysis samples above the LC-MS detection limit of 10.0 µg/L, it is reasonable to predict that it would not have been present in the original water samples above the concentration of 0.100 µg/L. Although the TTri concentrations obtained in this study were expected to be greater than those reported in the European studies, the maximum concentration detected in Lytle Creek (2.724 µg/L or 3.435 µg/L) is still at the higher end of the concentration ranges reported in the North American studies. Furthermore, there is the potential for these values to change since the online retailer began its operations at Wilmington Air Park on June 26, 2019. This air park is a far smaller airport than the ones in the aforementioned studies (i.e., Zurich, Frankfurt, Anchorage), yet it already generates comparable TTri concentrations in its runoff water. Therefore, as the air traffic increases, the concentration of TTri in Indian

Run and Lytle Creek could increase significantly to values that are higher than those generated by these much larger airports. Additionally, if the air traffic increases to the point that the air park decides to reactivate the water treatment bed on the southeast side, the amount of BTri and TTri in Indian Run could increase and become nearly equal to the amount of these compounds in Lytle Creek. It is therefore critical that water samples from these sites continue to be analyzed for the presence of TTri and BTri to determine if there is any increase in concentration corresponding to the retailer's new air operations.

In addition to the continued monitoring of Indian Run and Lytle Creek water samples, future research should focus on further optimization of the LC-MS method. While the optimized LC-MS conditions developed herein were successful in terms of the separation and detection of BTri and TTri, it would be ideal to determine a method that could separate the two TTri isomers (4-Me-BTri and 5-Me-BTri) from each other. This is because half-maximal effective concentration ($EC_{50}$) Microtox bioassays of these two compounds (Cancilla, 1997) have shown that 5-Me-BTri is about five times as toxic to bioluminescent bacteria (*Allivibrio fischeri*) as 4-Me-BTri ($EC_{50}$ values of 4.25 mg/L and 21 mg/L, respectively). Additionally, Damalas, et al. (2018) performed acute toxicity bioassays to determine the $LC_{50}$ values (lethal concentration required to kill 50% of a population) of BTri compounds on zebrafish larvae, and found that 4-Me-BTri was twice as toxic to the larvae as 5-Me-BTri ($LC_{50}$ values of 59 mg/L and 128 mg/L, respectively). Therefore, being able to determine which isomer is present in the greater concentration would be crucial in understanding how these compounds may be impacting the health of the two streams. The separation and quantification of these isomers has been achieved (LeFevre, 2015) using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS), which is far more sensitive instrumentation than the single-quadrupole LC-MS used in this project. Achieving this level of separation on the LC-MS would require extensive adjustments and optimization of many parameters (elution solvent ratio, temperature, flow rate, etc.). However, the isomers have also been separated (Kiss, 2009) using gas chromatography-mass spectrometry (GC-MS), which is available in the instrumentation lab and would thus be a more feasible technique to utilize for further method development.

Collecting soil and sediment samples at the Indian Run and Lytle Creek sites and developing a method of extraction of BTri and TTri from those soils should also be a focus of future research. Alvey, et al. (2016) reported TTri concentrations ranging from 1.70-2.26 ng/g and 9.65-10.1 ng/g in soil samples from their two snow disposal sites in Alaska, but no BTri was found in those sites. The observed difference in soil adsorbance is likely due to the methyl group on the TTri isomers, which makes them slightly more hydrophobic and better adsorbed to soil than BTri. Both the octanol-water partition coefficient ($\log K_{ow}$, measures the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of a two-phase octanol/water system) and the soil adsorption coefficient ($K_{oc}$, measures the amount of chemical substance adsorbed onto soil per amount of water, normalized to the percent of organic carbon in the soil) values for these BTri compounds support these findings. BTri has a $\log K_{ow}$ value of 1.23 while both TTri isomers have a $\log K_{ow}$ value of 1.89 (Giger, 2006); BTri has a $K_{oc}$ range of 31.7-79.2 $mg^{-1}$, while both TTri isomers have a $K_{oc}$ range of 39.3-110 $mg^{-1}$ (Hart, 2004). Compounds with higher $\log K_{ow}$ and $K_{oc}$ values will partition better into a non-aqueous phase, and so it makes sense that TTri would adsorb to soil (especially soil high in organic matter) more than BTri would.  Another study (McNeill, 2009) reported concentration ranges of 0.05-4.10 ng/g for BTri, 2.35-167.84 ng/g for 5-Me-BTri and 0.36-424.19 ng/g for 4-Me-BTri in soil samples from three different airports in the United States.  These findings are consistent with the hypothesis that American airports use de-icing/anti-icing solutions that include a much greater percentage of TTri than BTri, and so developing a method to extract and detect these compounds from soil samples at the Indian Run and Lytle Creek sites would provide another source of verification of the relative composition of the chemicals.

# 4. Conclusions

The primary objective of this thesis was to determine if benzotriazole compounds were present in Lytle Creek and Indian Run due to runoff containing de-icing and anti-icing fluids from Wilmington Air Park. Methods for collecting water samples from the aforementioned streams and from Cowan Creek (the control site), running the samples through the solid-phase extraction process to isolate BTri and TTri, and detecting these compounds using liquid chromatography-mass spectrometry were optimized and then developed into official SOPs.

As expected, BTri and TTri were not present in the Cowan Creek samples. BTri was only detected in the Indian Run and Lytle Creek samples in trace amounts (less than 0.100 µg/L), as the BTri peak in the chromatograms was unresolvable from an unknown compound that appeared in the chromatograms of every sample from all three sampling sites. When compared with JP-8 fuel, the full scan chromatogram of the interfering substance displayed a near-identical elution time and several similar fragment ions that were 14 *m/z* units apart from each other. This indicated that the interfering substance may be hydrocarbon chain-containing chemical that is a component of car/truck fuel that is entering all three streams from the roads. Because this unknown compound is interfering with the resolution of the BTri peak, further research should focus on optimizing the SPE and LC-MS methods to achieve more distinct separation of the BTri peak from the interference peak. This would then permit the accurate quantification of the concentration of BTri present in Indian Run and Lytle Creek.

TTri was present at measurable concentrations in all of the Indian Run and Lytle Creek samples. In Indian Run, TTri was detected in the concentration range of 0.111-0.869 µg/L; in Lytle Creek, TTri was detected in the concentration range of 0.822-2.724 µg/L. The TTri levels were expected to be higher in Lytle Creek than in Indian Run because the water treatment facility that directs its runoff into Lytle Creek (located on the northwest side of the air park) is the only one actively in use at this time. Furthermore, the TTri concentrations in the two streams corresponded with the different temperature and precipitation conditions that were observed on each sampling day. The TTri concentrations were the highest on 2/01/2019 (lowest air temperature, freezing rain and snowfall), the lowest on 2/06/2019 (air temperature above 10 °C, but significant precipitation occurred), and remained at around the same value over the next three sampling days (air temperature below 10 °C with no precipitation). This indicated that the amount of TTri running off into the streams on a given day depends significantly on the temperature and precipitation conditions that necessitate the application of ADAF solutions to the aircraft at the air park.

Since the SPE process did not yield 100 percent recovery of the BTri and TTri analytes present in the water samples, a surrogate standard (5,6-dimethyl-benzotriazole) was used to determine the maximum theoretical concentrations of TTri to correct for the percent recovery. These calculations adjusted the TTri concentration ranges to 0.167-1.248 µg/L (Indian Run) and 1.201-3.435 µg/L (Lytle Creek). The corrected concentration values did not alter any trends observed with respect to the weather and precipitation data and usage of the water treatment beds, but they did provide a more accurate estimate of the potential levels of TTri in Indian Run and Lytle Creek.

Regardless of which range of values is a better representation of the true levels of TTri present in the two streams, these concentrations (as well as the trace concentrations of BTri) are too low (by five orders of magnitude) to be detected in the air park's monthly assessments of the COD levels in their effluent discharge. Therefore, the air park could stay in complete compliance with their discharge permit (OHEPA Permit 1II00031*HD) even though BTri and TTri are evading their monitoring system during runoff treatment.

While the trace concentrations of BTri detected in Lytle Creek and Indian Run do not present cause for concern in terms of the overall health of the streams, the TTri concentrations that were detected are relatively high when compared to other investigations of the presence of benzotriazoles in watershed systems surrounding airports in North America. Not only could these TTri levels rise with the retail company beginning its operations and increasing the air traffic at Wilmington Air Park, but they could rise significantly in Indian Run if the heightened activity requires the reactivation of the water treatment bed on the southeast side of the air park. It is critical, then, that water samples from these sites continue to be analyzed for the presence of TTri and BTri to determine if there is a corresponding increase in concentration with the retailer's new air operations. Additionally, it has been shown that the 4-methyl isomer of TTri is more toxic to zebrafish larvae, while the 5-methyl isomer of TTri is more toxic to the aquatic bacteria *Allivibrio fischeri*. Therefore, future research should focus on optimizing the detection method to separate the TTri isomers, either by adjusting the LC-MS conditions or by developing a new method using gas chromatography-mass spectrometry.

Although the results discussed herein have established a solid baseline for the presence of BTri and TTri in Lytle Creek and Indian Run, no soil or sediment samples were collected in this study. Both BTri and TTri have been detected in soil samples taken from watershed areas near various airports across the country, with the TTri isomers being present in higher concentrations because they are more hydrophobic and less polar than BTri. These observations are supported by the fact that TTri has higher soil adsorption coefficient and octanol-water partition coefficient values than BTri. In addition to the continued monitoring of the BTri and TTri levels present in the streams themselves, a primary objective of future research should be to collect soil samples at the Lytle Creek and Indian Run sites and develop a new method to extract and detect these compounds. This would provide the information needed to determine if most of the BTri and TTri present in the air park runoff is entering the streams or adsorbing to the soil and sediment surrounding the streams.

# 5. Appendices

## APPENDIX A

Standard Operating Procedure

### WILMINGTON AIR PARK RUNOFF WATER SAMPLING PLAN

October 8, 2019
Audrey McGowin, PhD
Jessica Wiese

## A. SCOPE AND APPLICATION

1H-benzotriazoles are complexing agents that are widely used as anti-corrosives, engine coolants, aircraft de-icers, anti-freezing liquids, and silver protection in dishwashing agents. Chemically, 1H-benzotriazoles are soluble in water, resistant to biodegradation, only partially removed in wastewater treatment, and have the potential to pass drinking water treatment. Most benzotriazole (BTri) compounds and their analogs are polar and thermally labile. In addition, BTris are toxic to certain aquatic organisms, and have the potential for impacting the health of creeks, rivers, and ground water reservoirs in which BTri and BTri analogs are deposited. The procedures outlined in this SOP were created for the collection of surface and ground water samples near Wilmington Air Park.

## B. SUMMARY OF METHOD

The purpose of this Standard Operating Procedure (SOP) is to establish a procedure for the collection of surface and ground water samples near Wilmington Air Park in order to determine the presence of 1H-benzotriazoles, tolytriazoles, and comparable analogs in runoff from the airport’s wastewater treatment plants.

## C. HEALTH AND SAFETY

The analyst must assume that all surface and ground water samples are potentially contaminated and should be treated accordingly. Personal protection equipment (PPE) should be worn at all times while out in the field; this includes long sleeves, protective gloves, safety glasses, long pants and closed-toe shoes.

## D. SAFETY AND CAUTIONS

1. Sample containers must be labeled according to the Sample Labeling Scheme outlined in Section F of this SOP.

2. During on site testing and sample collection, personnel must wear protective gloves and safety glasses.

3. Do not pour any reagents on the ground or into the water.  Collect all waste materials for proper disposal in the lab in appropriately labeled waste containers.

4. Hiking boots and a raincoat are recommended for days when precipitation is possible.

## E. EQUIPMENT AND SUPPLIES

1. Sampling protocol with Standard Sampling Form

2. Clipboard and laboratory notebook with ink pen

3. Clean amber glass bottles (500 mL) with PTFE-lined closures
4. Permanent marker for sample labeling
5. One small cooler with cool packs for sample preservation
6. Paper towels with Ziplock® bags
7. Rinsing bottle containing ASTM Type I water
8. YSI Multi-meter, pre-calibrated in the lab; DO, temperature, conductivity, pH
9. Waste containers (trash bag and waste bottle)
10. Camera
11. Clean gloves for each site
12. Proper attire for field work: eye protection, long pants, closed-toed shoes

## F. SAMPLE LABELING SCHEME

Samples will be labeled according to the following scheme:

Date (MMDDYYYY)– Sample Site – Depth – BTri – Sample Replicate Number (if needed)– Analysis Replicate Number (if needed)

For example: 10312018 – LCFR – 0 – BTri – R1

## G. SAMPLING SITES

Sampling sites are listed in the following table. The site on Indian Run was selected downstream of one of the airport's wastewater treatment facility. The site on Lytle Creek was selected downstream of the airport's second wastewater treatment facility. The site on Cowan Creek was selected upstream of Indian Run to be the control sampling site.

| Sample Site | GPS Location | Description |
|---|---|---|
| LCFR | 39.437051, -83.797386 | Lytle Creek off of Fife Road, downstream from treatment facility |
| IRJKR | 39.410702, -83.796503 | Indian Run off of Jenkin's Road, downstream from treatment facility |
| CCJKR | 39.407615, -83.798064 | Cowan Creek off of Jenkin's Road, upstream of Indian Run |

## H. SAMPLE COLLECTION PROCEDURE

1. Before going to sampling sites, clean and label sample containers and assemble sampling materials according to this protocol.

2. In the lab, calibrate the YSI Multi-meter using buffers and standards according to SOP 12.0. Remember to put an ice pack in your sample cooler.

3. When sampling the sites, stand downstream of sampling and sample into the current.

4. Upon arrival at each sampling site, put on gloves and glasses.

5. Next, collect 400 mL of site water into an amber bottle (leaving 100 mL of headroom for expansion upon freezing). Making sure the cap is on securely, place the bottle next to the ice pack in a second cooler. Repeat with second sampling bottle.

6. Use the calibrated YSI Multi-meter to measure DO, pH, specific conductance, ammonium, ammonia, and temperature of the water. Also record the ambient temperature and weather conditions. Record all readings on the Data Form.

7. Proceed to the next sampling site making sure to collect any waste. Check to be sure the GPS coordinates match. Collect all water samples and place them in the coolers. Take water quality measurements at each site. Record any additional information on the data sheet. Take photos to show conditions and anything unusual.

8. Return samples to the laboratory upon completion of sampling. Immediately place the samples into the freezer.

9. Rinse the YSI Multimeter electrodes with DI water and replace the clear plastic covers being sure that the small sponge inside has been rinsed with DI water.

## I. DATA AND RECORDS MANAGEMENT

Immediately upon returning to the laboratory, be sure Standard Sampling Forms and laboratory notebooks are secured.

## J. QUALITY ASSURANCE AND QUALITY CONTROL

Include a description of any replicate samples that are taken. Describe any events that may make samples invalid, spills, possible mislabeled samples, etc.

## K. ATTACHMENTS

Water Data Table

## Water Data Table

Date: ________________________________________

Personnel: ________________________________________________________________

| **Sample Site** | **LCFR** | **IRJKR** | **CCJKR** |
|---|---|---|---|
| Time | | | |
| Ambient T (°C) | | | |
| Water T (°C) | | | |
| pH | | | |
| DO (%) | | | |
| DO (mg/L) | | | |
| $NH_4^+$ - [N] | | | |
| $NH_3$ – [N] | | | |
| Conductivity (µSiemens) | | | |
| Pressure (mm Hg) | | | |
| Observations | | | |

# APPENDIX B

## Standard Operating Procedure

# ISOLATION OF BENZOTRIAZOLE AND ANALOG COMPOUNDS IN WILMINGTON AIR PARK RUNOFF WATER SAMPLES VIA SOLID-PHASE EXTRACTION

October 8, 2019
Audrey McGowin, PhD
Jessica Wiese

## A. SCOPE AND APPLICATION

1H-benzotriazoles are complexing agents that are widely used as anti-corrosives, engine coolants, aircraft de-icers, anti-freezing liquids, and silver protection in dishwashing agents. Chemically, 1H-benzotriazoles are soluble in water, resistant to biodegradation, only partially removed in wastewater treatment, and have the potential to pass drinking water treatment. Most benzotriazole (BTri) compounds and their analogs are polar and thermally labile. In addition, BTris are toxic to certain aquatic organisms, and have the potential for impacting the health of creeks, rivers, and ground water reservoirs in which BTri and BTri analogs are deposited. The procedures outlined in this SOP were created for the solid-phase extraction of surface and ground water samples collected near Wilmington Air Park.

## B. SUMMARY OF METHOD

The purpose of this Standard Operating Procedure (SOP) is to establish a procedure for the solid-phase extraction of surface and ground water samples collected near Wilmington Air Park in order to determine the presence of 1H-benzotriazoles, tolytriazoles, and comparable analogs in runoff from the airport's wastewater treatment plants.

## C. HEALTH AND SAFETY

The analyst must assume that all surface and ground water samples are potentially contaminated and should be treated accordingly. Personal protection equipment (PPE) should be worn at all times while in the lab; this includes lab coat, protective gloves, safety glasses, long pants and closed-toe shoes.

## D. SAFETY AND CAUTIONS

1. All personnel must abide by the safety procedures discussed in the "Wright State University Chemical Hygiene Plan". Any spills or emergency or accidents must be reported to the department of Environmental Health and Safety at Wright State University for assistance.

2. Material safety data sheets for all chemical reagents are available and should be read and understood by all personnel performing the methods described herein.

3. Do not pour any reagents down the drain. Collect all waste materials for proper disposal in the lab in appropriately labeled waste containers.

4. All personnel must wear a lab coat, gloves and appropriate eye protection when in the laboratory, including visitors.

5. Glassware and containers must be labeled with the chemical, the date, its concentration, hazard (if any), and the initials of the personnel responsible.

6. Final extracted sample containers must be labeled according to the Sample Labeling Scheme outlined in Section F of this SOP.

## E. EQUIPMENT AND SUPPLIES

1. Laboratory notebook with ink pen

2. Permanent marker for labeling glassware/containers

3. Proper attire for lab work: lab coat, eye protection, long pants, closed-toed shoes

4. Glassware & Extraction Materials
    a. Various beakers and flasks for collection/storage
    b. Several glass Pasteur pipettes
    c. 0.7-µm glass fiber filters (Whatman, GF/F, 47 mm)
    d. Whatman 47 mm glass filter funnel and 1L Erlenmeyer flask with vacuum attachment
    e. Oasis® PRIME HLB cartridges (Waters, 500 mg, 6 mL)
    f. 12-port vacuum extraction manifold
    g. 15-mL centrifuge tubes for eluate collection
    h. Tank of nitrogen gas
    i. Amber vials for storage of excess filtrates

5. Chemicals & Reagents
    a. HPLC-Grade Methanol (MeOH, CAS #67-56-1)
    b. Water (Milli-Q purified)
    c. Hydrochloric acid (12 M HCl, CAS #7647-01-0)
    d. Dichloromethane (DCM, CAS #75-09-2)
    e. 5,6-dimethyl-1H-benzotriazole (5,6-dimethyl-BTri, CAS #4184-79-6)

## F. SAMPLE LABELING SCHEME

Final extractions of samples will be labeled according to the following scheme:

Date (MMDDYYYY)– Sample Site – Depth – BTri – Sample Replicate Number (if needed)– Analysis Replicate Number (if needed)

For example: 10312018 – LCFR – 0 – BTri – R1-A

## G. SOLID PHASE EXTRACTION PROCEDURE

1. Filter each water sample through the glass fiber filters using the funnel/flask assembly.

2. Divide each filtrate into three 100-mL replicates.

3. Acidify the replicates to pH 2.5-3.0 using 3 drops of the 12 M HCl solution.

4. Spike each replicate with 54.0 ng (10 µL of a 5.0 ppm solution) of 5,6-dimethyl-BTri as the surrogate standard.

5. Connect the SPE cartridges to the ports on the vacuum extraction manifold.

6. Condition the SPE cartridges sequentially with 3 x 2 mL of MeOH and then 3 x 2 mL of Milli-Q water, applying a slight vacuum (about 5 psi).

7. Run the samples through the cartridges at a flow rate of 5 mL/min.

8. Dry the cartridges under a vacuum (15 psi) for 2 hours and 30 minutes.

9. Dissemble the vacuum extraction manifold and dispose of the water into a waste beaker; place the centrifuge tubes in the clamps beneath the ports and then reassemble the manifold.

10. Elute the analytes under a slight vacuum (5 psi) with 5 mL of DCM containing 3% MeOH, then remove the centrifuge tubes from the manifold.

11. Evaporate the eluates to dryness under a gentle stream of nitrogen gas.

12. Redissolve the dry residues in the centrifuge tubes by adding 1 mL of MeOH; store the samples in the tubes at -20 ℃ overnight.

# APPENDIX C

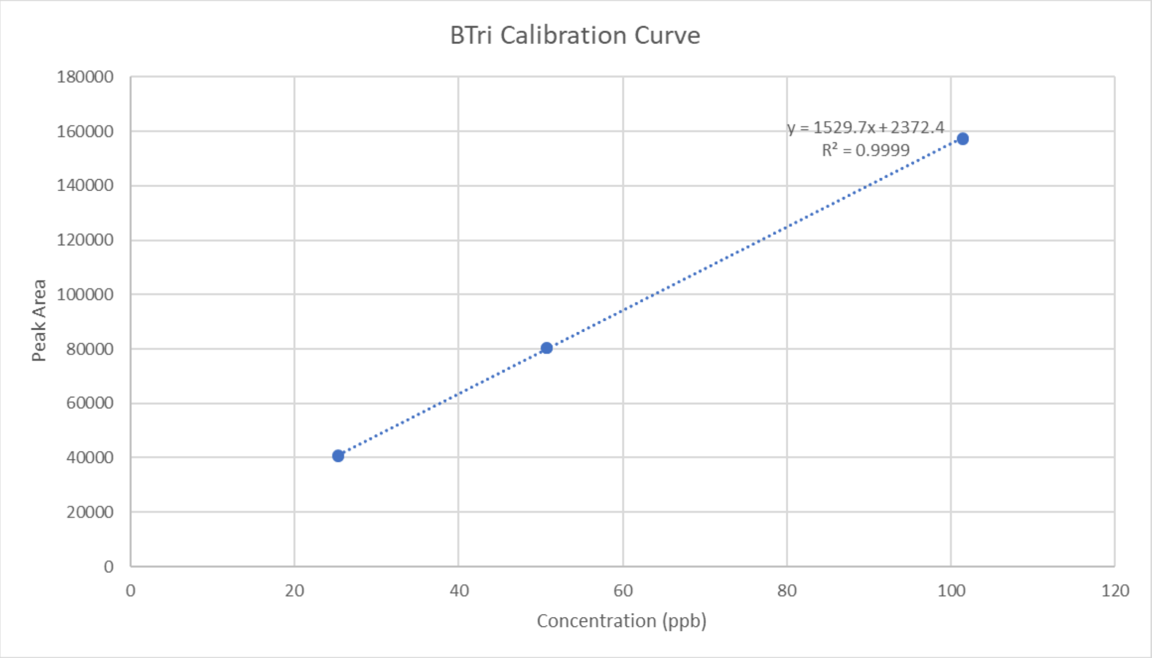


**Figure A1:** Calibration curve calculated for BTri for the 2/01/2019 samples.

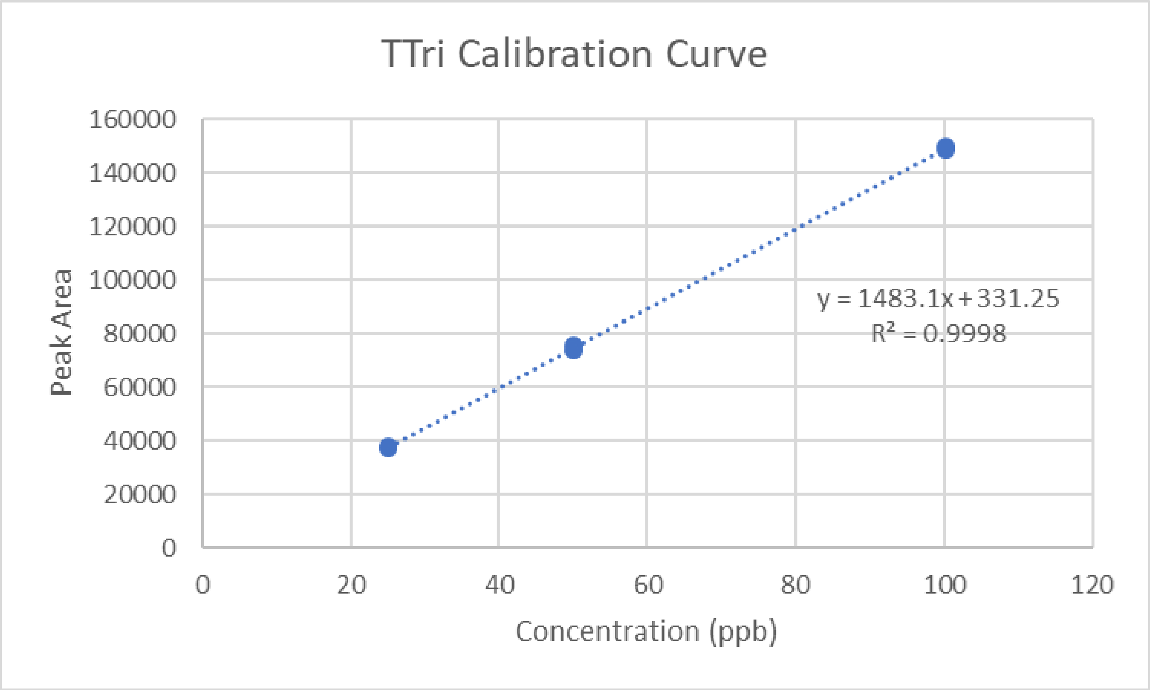


**Figure A2:** Calibration curve calculated for TTri for the 2/01/2019 samples.

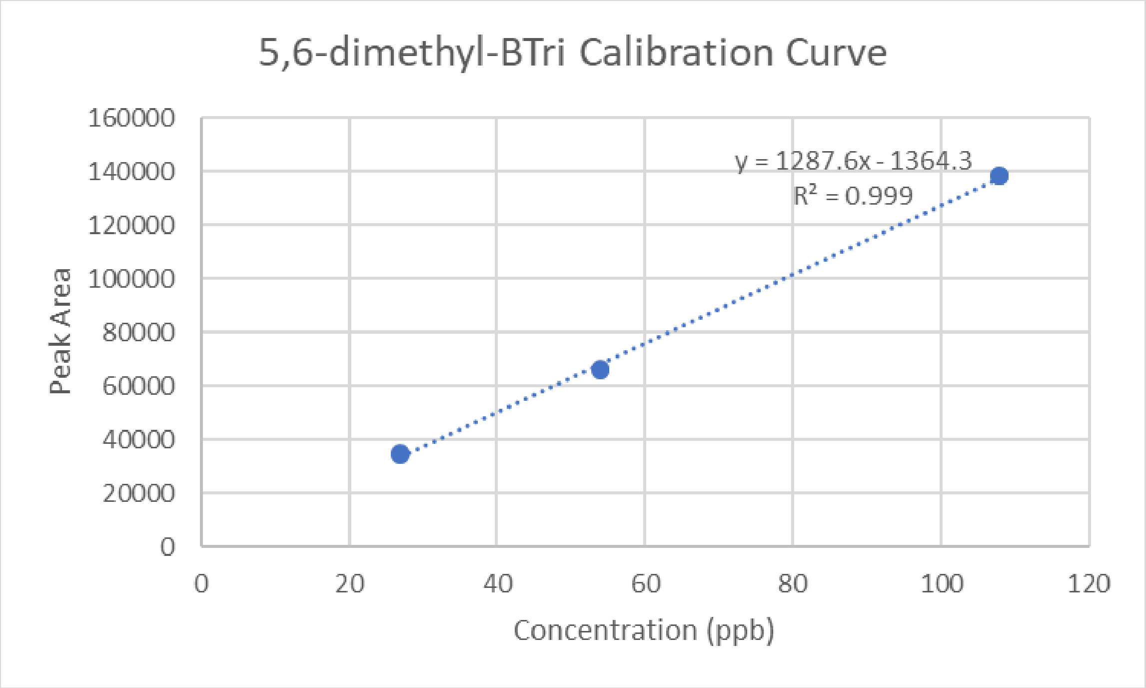


**Figure A3:** Calibration curve calculated for 5,6-dimethyl-BTri for the 2/01/2019 samples.

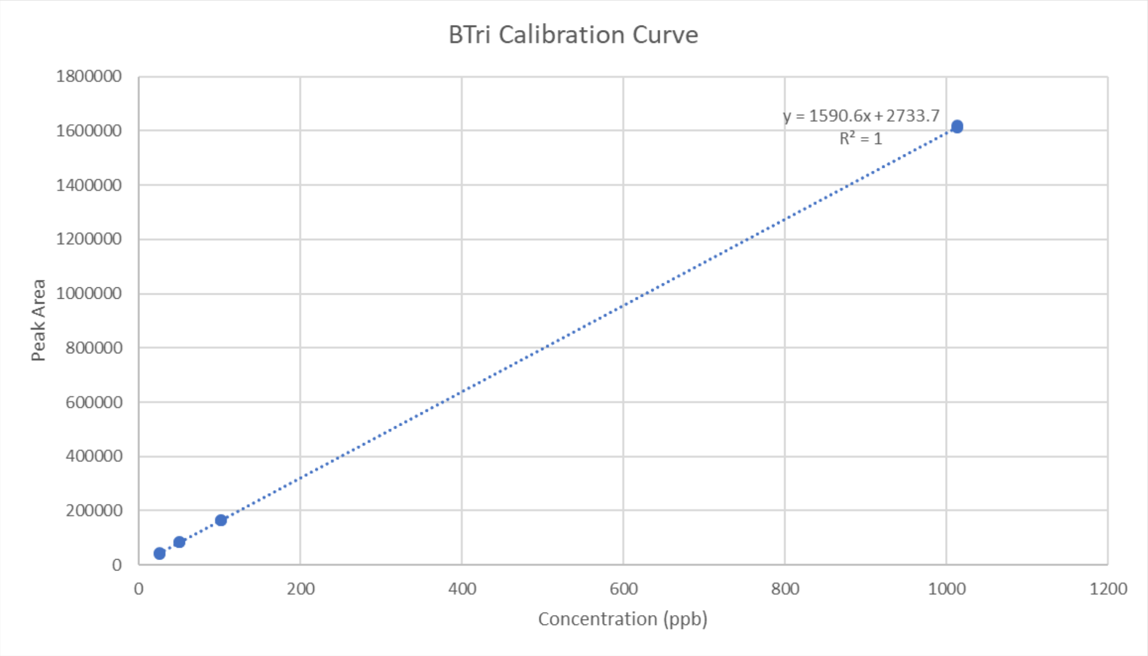


**Figure A4:** Calibration curve calculated for BTri for the 2/06/2019 samples.

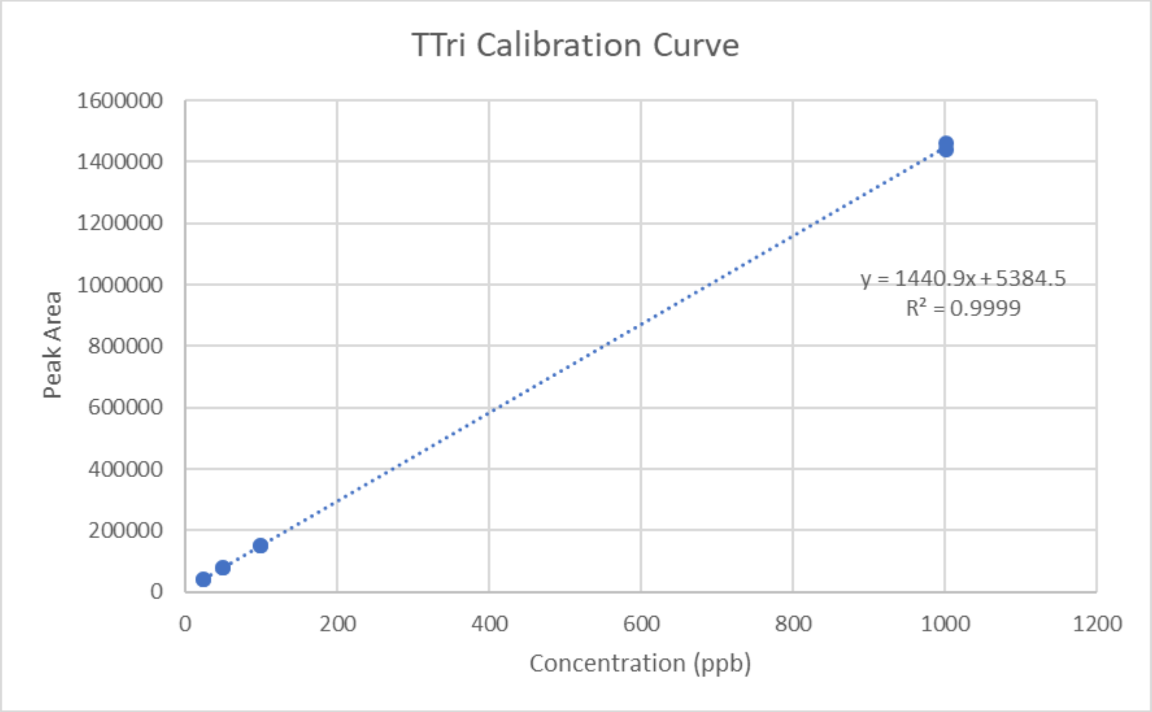


**Figure A5:** Calibration curve calculated for TTri for the 2/06/2019 samples.

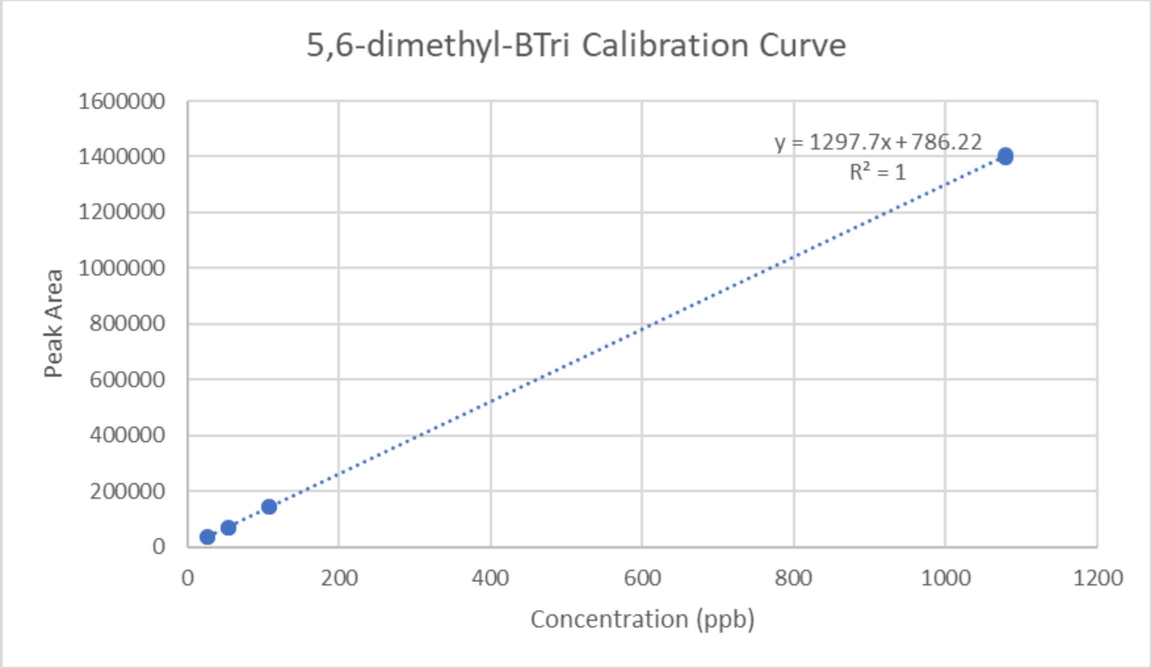


**Figure A6:** Calibration curve calculated for 5,6-dimethyl-BTri for the 2/06/2019 samples.

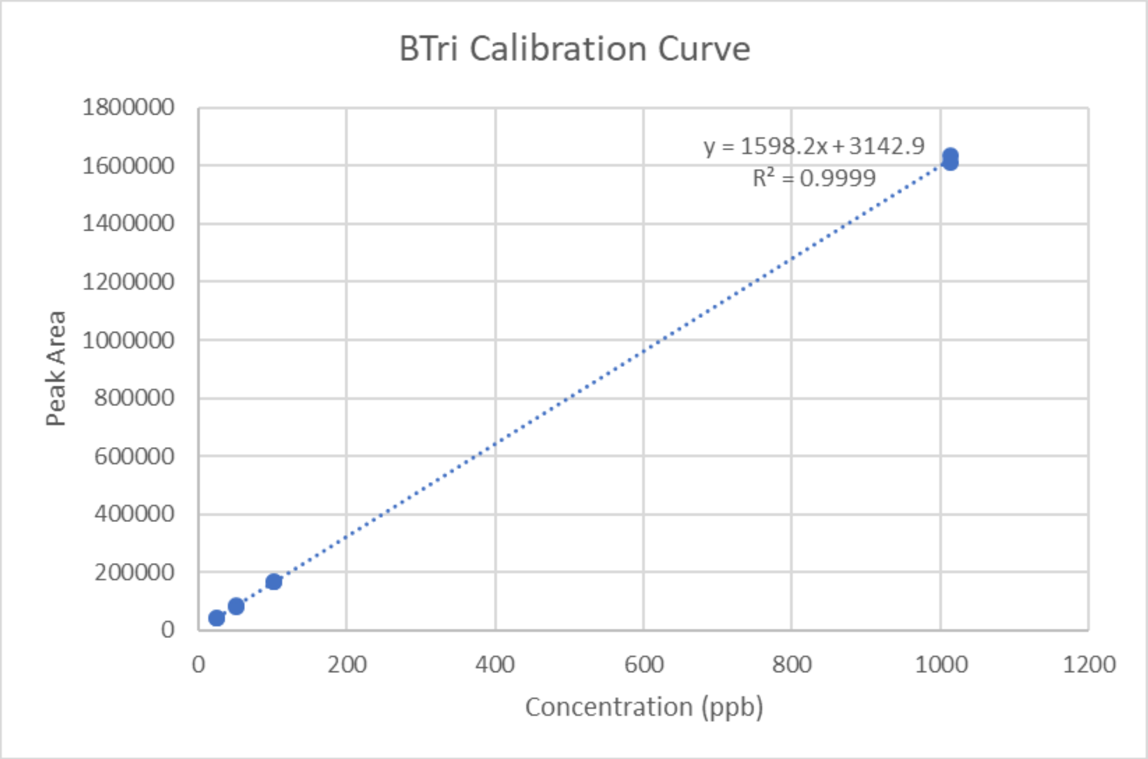


**Figure A7:** Calibration curve calculated for BTri for the 2/13/2019 samples.

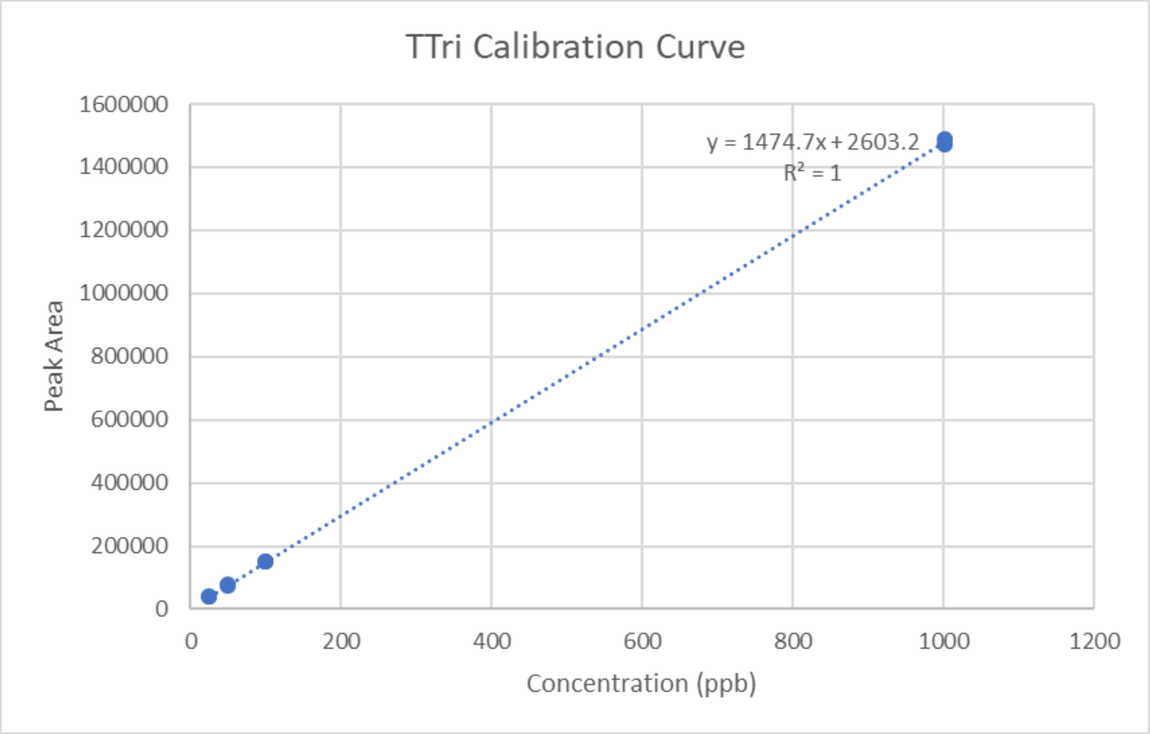


**Figure A8:** Calibration curve calculated for TTri for the 2/13/2019 samples.

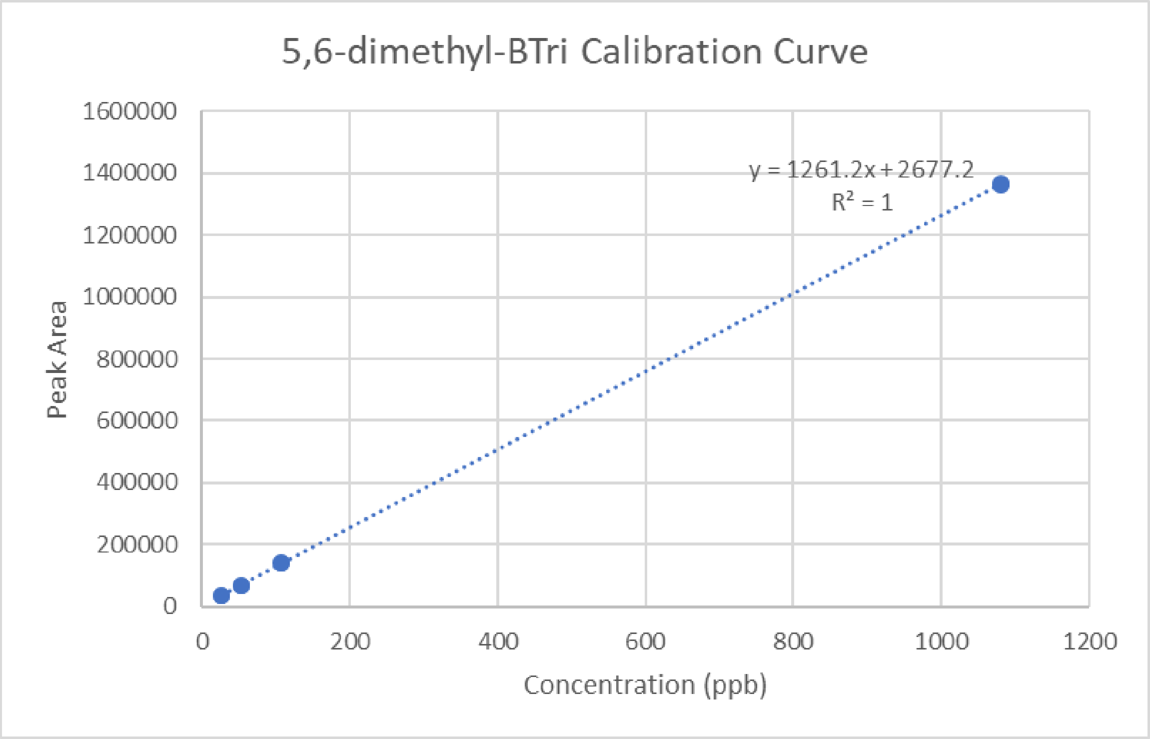


**Figure A9:** Calibration curve calculated for 5,6-dimethyl-BTri for the 2/13/2019 samples.

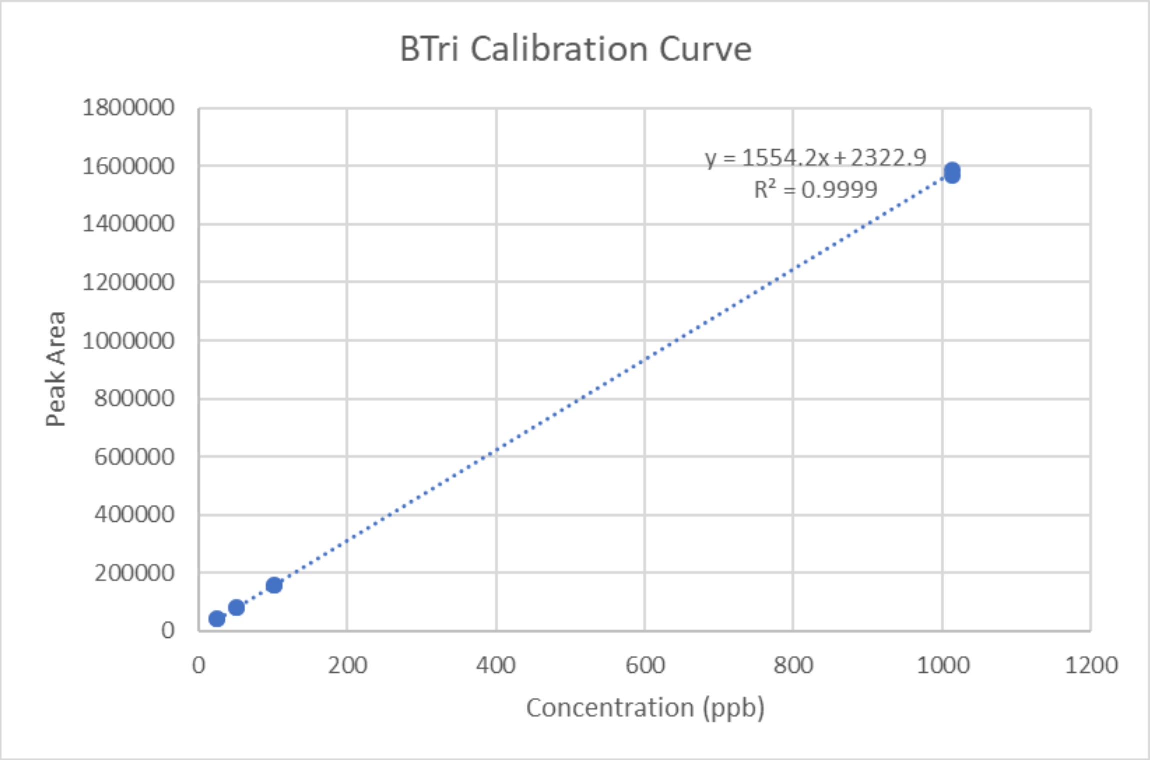


**Figure A10:** Calibration curve calculated for BTri for the 2/22/2019 samples.

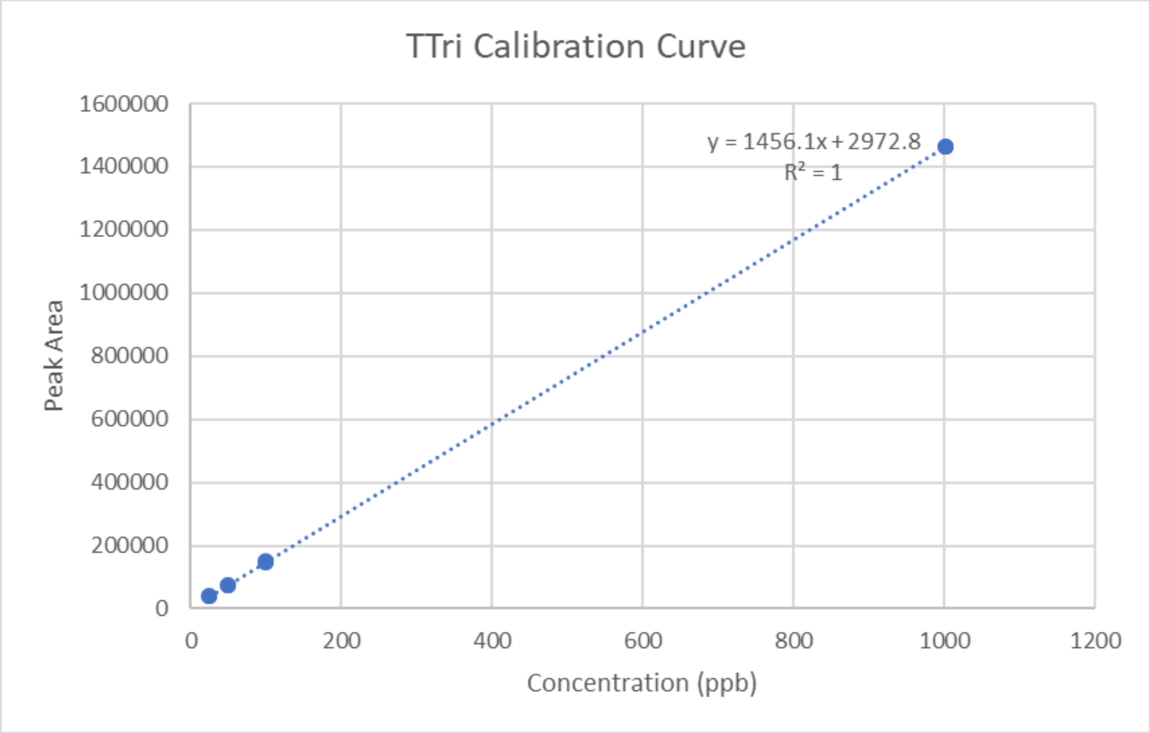


**Figure A11:** Calibration curve calculated for TTri for the 2/22/2019 samples.

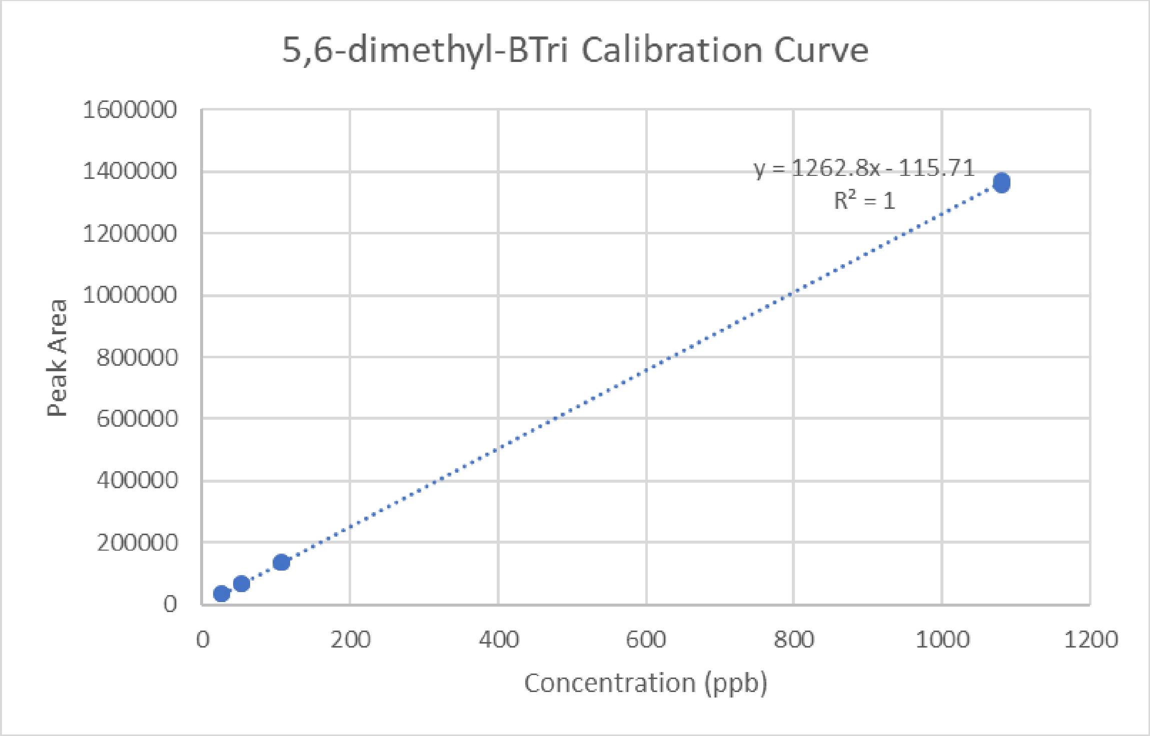


**Figure A12:** Calibration curve calculated for 5,6-dimethyl-BTri for the 2/22/2019 samples.

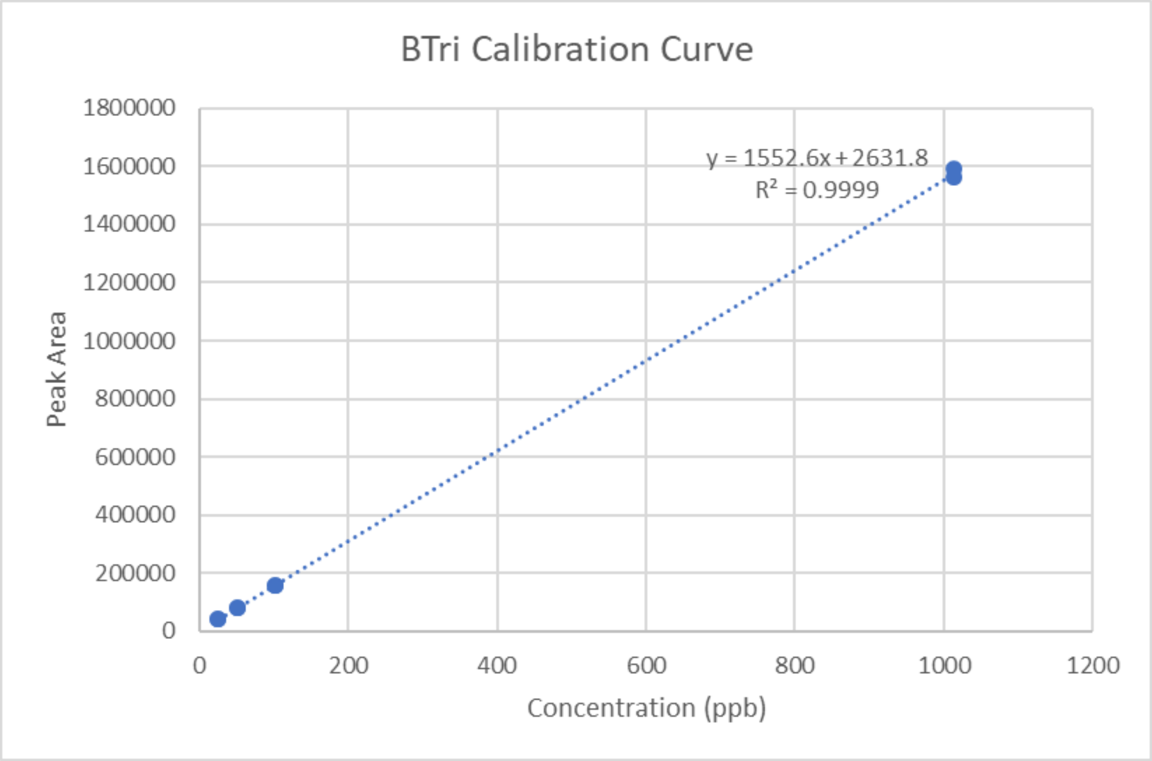


**Figure A13:** Calibration curve calculated for BTri for the 2/28/2019 samples.

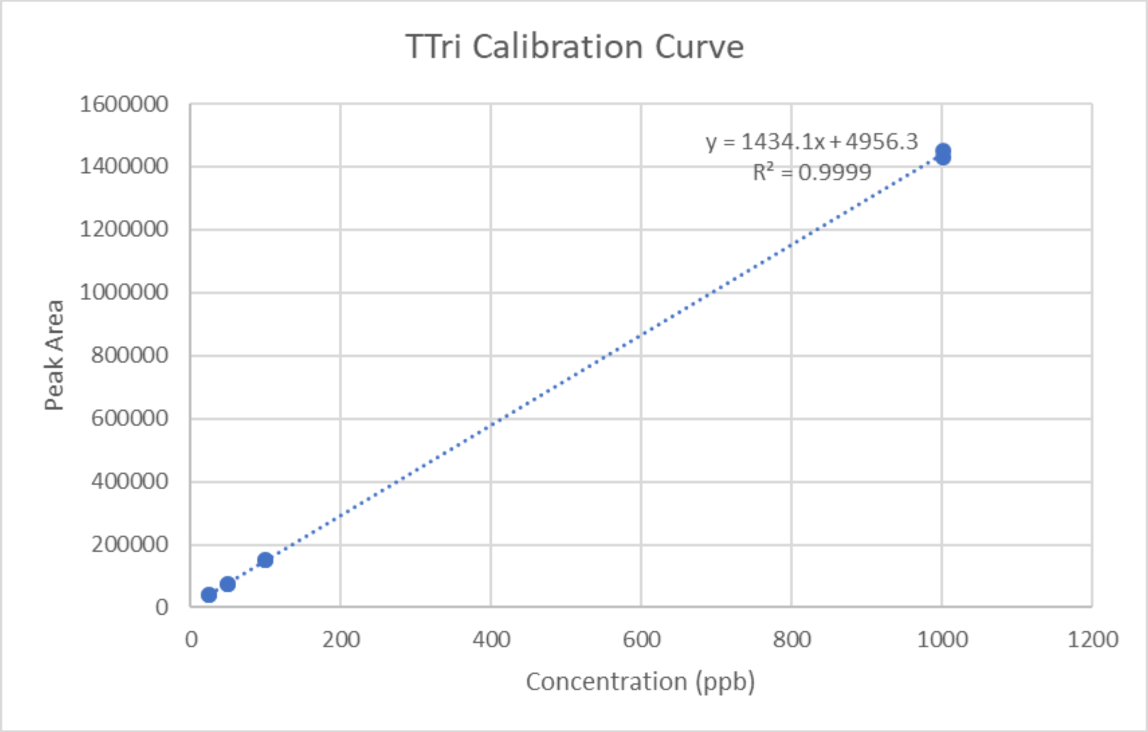


**Figure A14:** Calibration curve calculated for TTri for the 2/28/2019 samples.

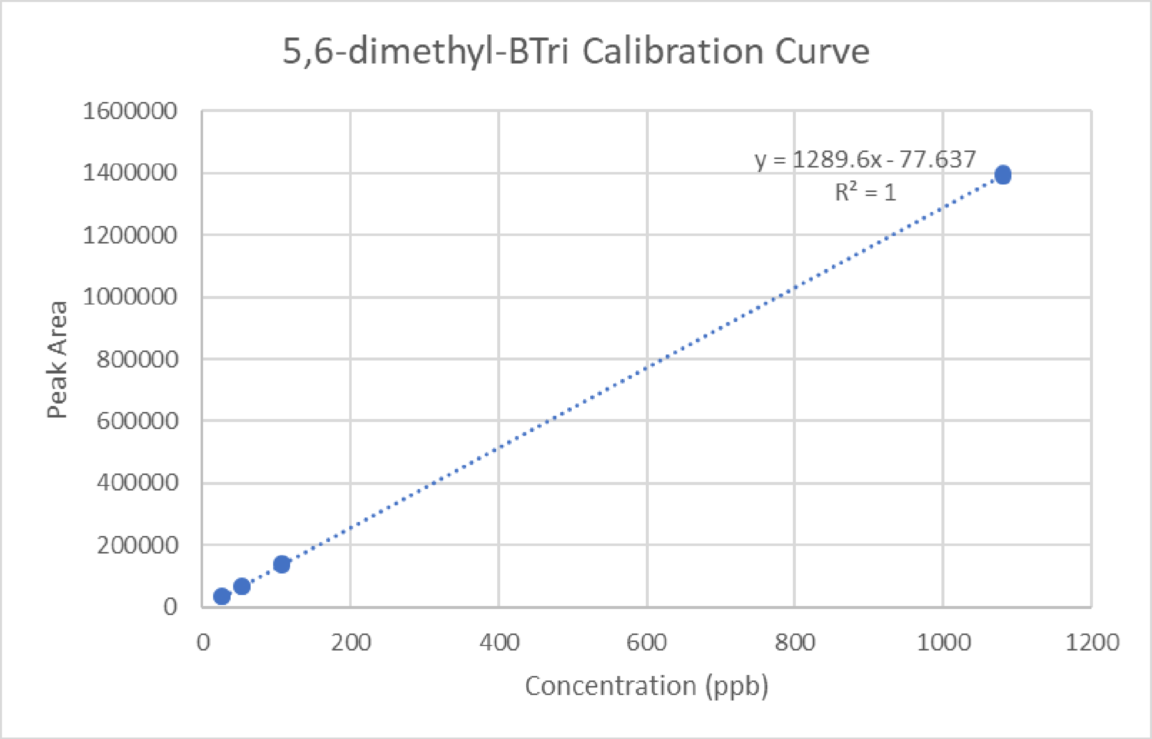


**Figure A15:** Calibration curve calculated for 5,6-dimethyl-BTri for the 2/28/2019 samples.

# APPENDIX D

Standard Operating Procedure

# DETERMINATION OF BENZOTRIAZOLE AND ANALOG COMPOUNDS BY LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY IN SURFACE AND GROUND WATER SAMPLES

October 8, 2019
Audrey McGowin, PhD
Jessica Wiese

## A. SCOPE AND APPLICATION

1H-benzotriazoles are complexing agents that are widely used as anti-corrosives, engine coolants, aircraft de-icers, anti-freezing liquids, and silver protection in dishwashing agents. Chemically, 1H-benzotriazoles are soluble in water, resistant to biodegradation, only partially removed in wastewater treatment, and have the potential to pass drinking water treatment. Most benzotriazole (BTri) compounds and their analogs are polar and thermally labile. In addition, BTris are toxic to certain aquatic organisms, and have the potential for impacting the health of creeks, rivers, and ground water reservoirs in which BTri and BTri analogs are deposited. The procedures outlined in this SOP were created for the qualitative and quantitative determination of BTri and similar compounds by Liquid Chromatography – Mass Spectrometry (LC-MS) in surface and ground water samples.

## B. SUMMARY OF METHOD

The purpose of this Standard Operating Procedure (SOP) is to establish a procedure for the qualitative and quantitative determination of 1H-benzotriazoles, tolytriazoles, and comparable analogs using LC-MS instrumentation.

## C. HEALTH AND SAFETY

The analyst must assume that all surface water samples are potentially contaminated and should be treated accordingly. Personal protection equipment (PPE) should be worn at all times while in the lab; this includes lab coat, nitrile gloves, safety glasses, long pants and closed-toe shoes. Material safety data sheets (MSDS) can be found in the back left corner of the lab. Organic solvents should be handled cautiously and used in a fume hood.

## D. SAFETY AND CAUTIONS

1. All personnel must abide by the safety procedures discussed in the "Wright State University Chemical Hygiene Plan." Any spills or emergency accidents must be reported to the department of Environmental Health and Safety at Wright State University for assistance.

2. Material safety data sheets for all chemical reagents are available and should be read and understood by all personnel performing the methods described herein.

3. All personnel must wear a lab coat, gloves, and appropriate eye protection when in the laboratory, including visitors.

4. Containers and boxes must be labeled with the chemical, the date, its concentration and hazard, the expiration date, and the name of the personnel responsible.

5. During instrument operation, personnel must wear protective gloves and safety glasses.

## E. EQUIPMENT AND SUPPLIES

1. Agilent Technologies 1220 Infinity LC quadrupole LCMS system that includes the following components:
    a. Agilent Eclipse Plus $C_{18}$ (1.8 µm I.D 2.1 x 100 mm) column
    b. Autosampler
    c. Agilent 1220 Infinity LC variable wavelength detector (VWD)
    d. OpenLAB CDS ChemStation Software
    e. Single quadrupole mass analyzer

2. 2-mL autosampler vials with Teflon caps.

3. Various glassware (Pasteur pipettes, volumetric flasks, amber jars/vials) for standard solution and eluent solution preparation.

4. Type 3 fixed needle syringes (100-µL, 250- µL, and 500- µL)

5. Chemicals & Reagents
    a. HPLC-grade Methanol (MeOH, CAS #67-56-1)
    b. Water (Milli-Q purified)
    c. Formic Acid (CAS #64-18-6)
    d. 1H-benzotriazole (BTri, CAS # 95-14-7)
    f. 4-methyl-1H-benzotriazole (4-Me-BTri, CAS #249-921-1)
    g. 5-methyl-1H-benzotriazole (5-Me-BTri, CAS #136-85-6)
    h. 5,6-dimethyl-1H-benzotriazole (5,6-dimethyl-BTri, CAS #4184-79-6)

## F. PROCEDURE – *ELUENT SOLUTION PREPARATION*

1. Add 1.0 mL of formic acid to 1 L of MeOH and mix thoroughly.

2. Add 1.0 mL of formic acid to 1 L of water and mix thoroughly.

3. Transfer each solution to a 1-L glass bottle and hook each bottle up to the LC-MS.

## G. PROCEDURE – *STANDARD SOLUTION PREPARATION*

1. Weigh out 0.00500 g of BTri and dissolve it in 50.0 mL MeOH to create the 100-ppm standard solution.

2. Take 2.5 mL of the 100 ppm solution and dilute to 25.0 mL with MeOH to create the 10-ppm standard solution.

3. Take 250 µL of the 100 ppm solution and dilute to 25.0 mL with MeOH to create the 1.0-ppm standard solution.

4. Take 250 µL of the 10 ppm solution and dilute to 25.0 mL with MeOH to create the 100-ppb standard solution.
5. Take 1.25 mL of the 1.0 ppm solution and dilute to 25.0 mL with MeOH to create the 50-ppb standard solution.
6. Take 250 µL of the 1.0 ppm solution and dilute to 10.0 mL with MeOH to create the 25-ppb standard solution.
7. Take 100 µL of the 1.0 ppm solution and dilute to 10.0 mL with MeOH to create the 10-ppb standard solution.
8. Repeat steps 1-7 for both 4-Me-BTri and 5-Me-BTri.
9. Store all standard solutions in amber glass vials/jars at -20 ℃.

## H. PROCEDURE – *SURROGATE STANDARD SOLUTION PREPARATION*

1. Weigh out 0.00025 g of 5,6-dimethyl-BTri and dissolve it in 50.0 mL of MeOH to create the 5.0-ppm standard solution.
2. Take 5.00 mL of the 5.0 ppm solution and dilute to 25.0 mL with MeOH to create the 1.0-ppm standard solution.
3. Take 1.00 mL of the 1.0 ppm solution and dilute to 10.0 mL with MeOH to create the 100-ppb standard solution.
4. Take 500 µL of the 1.0 ppm solution and dilute to 10.0 mL with MeOH to create the 50-ppb standard solution.
5. Take 250 µL of the 1.0 ppm solution and dilute to 10.0 mL with MeOH to create the 25-ppb standard solution.
6. Take 1.00 mL of the 100 ppb solution and dilute to 10.0 mL with MeOH to create the 10-ppb standard solution.
7. Store all standard solutions in amber glass vials/jars at -20 ℃.

## I. PROCEDURE – *LC-MS ANALYSIS*

1. Make sure the nitrogen tank is full. If empty, contact Dr. McGowin to replace as soon as possible. If the tank is not running already, open the two black valves on the pressure valve, and the grey valve on the tank over the "gas use" label; the pressure should read around 500 – 600 kPa.
2. If the LC-MS has not been used in a while, it is important to check that it is tuned properly.

3. Go to “MSD Tune” and click “ATUNES TUN”.

4. Select positive or negative polarity.

5. Under “Tune”, click “Check Tune”.

6. The system will run a tune check and automatically generate a report that says whether it is a “Pass” or “Fail”.

7. If it passes, proceed to Step 3; if it fails, go to “Calibrate” and run a calibration test. Make sure to save the new calibration results.

8. Run an “Autotune” check under positive, negative, or dual polarity. If it passes; proceed to Step 3; if it fails, contact Joseph Solch or Garrett Vanness for assistance.

9. If you have a method already, skip this step. If you do not, go to the "Method" tab and click "New Method".

10. In the "Sampler" section of the "Method and Run Control" window, right click and select "Method".

11. Adjust injection volume and stop time as desired; do not change the auxiliary settings.

12. Right click the "Grad. Pump" section of the "Method and Run Control" window and click "Method" to display the following parameters to be adjusted: Flow, Solvents, Stop time and Pressure Limits.
    a. The flow should not exceed more than 1-1.5 mL/min - anything greater than that will increase the pressure on the column to such an extent that it will be permanently damaged.
    b. Under the solvents tab, enter the name of the solvent as well as the percentage of each.
    c. The stop time can be adjusted to elute the last peak you desire.
    d. You must be very mindful of the pressure limits set. Do not increase the upper pressure limit to greater than 370 bar. If a long run time is planned or you are running on low volumes of eluent, the lower pressure limit can be increased to ~ 50 bar.

13. Right click the “Column” section of the “Method and Run Control” window and click “Method”. Adjust the column temperature as desired.

14. Right click the “MSD Signals” section of the “Method and Run Control” window and click “Method” to display the following parameters to be adjusted: Polarity, Full Scan and SIM.

   a. Select positive or negative polarity as desired.
   b. It is recommended that you run your method in “Full Scan” mode for your first standard solution in order to determine the times the analyte peaks of interest elute.
   c. Once you have determined your analyte’s elution time(s), you can run in “SIM” mode.

15. Right click the “UV Lamp” section of the “Method and Run Control” window and click “Method”. Adjust the wavelength detection as desired.

16. Once your method is complete, go to the "Method" tab, click "Save Method As..." and name your method to the following code: *Initials – MMDDYYYY - Primary Eluent name – MS ion mode*.

17. Now that you have a method saved, you can load it for future analyses: go to the "Method" tab and click "Load Method..."; at the top of the screen you should see your method file name.

18. Turn both the LC and MS components of the system on. To do this, click the green "ON" buttons on the screen. This will start the pumping of eluent through the column.

19. You must then purge the system in order to eliminate gas bubbles from the eluent solution.

20. Go to the "Grad. Pump" section in the "Method and Run Control" window and increase the flow rate to 5.00 mL/min. You should see that the clear tube that goes to waste be degassed. Do NOT click “OK” yet.

21. Unhinge the door to the LC component, and give the black waste knob a quarter turn counterclockwise. This switches the flow of all incoming eluent to waste.

22. Click “OK”. Turn the black knob clockwise and back a few times until no more bubbles are pumped through the eluent solution.

23. Change the flow rate back according to your sample method. Turn the black knob clockwise until it is closed and put the cover of the LC component back on. Allow the pressure to stabilize (about 10-20 minutes).

24. Set up your sequence by going to the "Sequence" tab and clicking "New Sequence Template". This creates a template to which you can save new sequences as in the future.
    a. To modify your sequence, go to the "Sequence” tab and select "Sequence Table...". This will open a spreadsheet – like window.
    b. Enter the sequence of your samples, denoting the vial position (Vial), name (Method Name) and number of injections per vial (Inj/Vial).

   c. To add lines for more samples, click "Insert". To remove sample lines, click "Cut". Exit the sequence table by clicking "OK".
   d. Go to the "Sequence" tab, click "Save Sequence Template As...", and give your file a name according to the sequence file code: *Initials_Date samples were taken (MMDDYYYY)_Samples Analysis*

25. To run all of the samples in your sequence, click “Start Sequence”. If you want to run only one or a few of the samples in your sequence, go to the “Sequence” tab and click “Partial Sequence” then “New”. This allows you to then pick and choose which vials you want to run.

26. To view the data, go to the "Data Analysis" window.

27. The “Spectrum” button displays the spectra with all of the elution times of the analytes.

28. The “Signal” button allows you to integrate the peaks and determine the areas of each peak.

29. The “Print Report” button will display a report in the "Data Analysis" window that you can view before printing. Click the "Print" button, and this will open the PDF24 Assistant. Click "Save as PDF", and save the file as your sequence name to a USB flash drive by clicking "Save".

## APPENDIX E

## Water Data Table

Date: 2/01/2019

Personnel: Jessica Wiese, Travis Luncan

| **Sample Site** | **LCFR** | **IRJKR** | **CCJKR** |
|---|---|---|---|
| Time | 9:46 AM | 10:03 AM | 10:21 AM |
| Ambient T (°C) | -6.67 | -6.67 | -6.11 |
| Water T (°C) | 2.9 | 2.2 | 2.5 |
| pH | 6.85 | 7.29 | 7.19 |
| DO (%) | 92.8 | 100.1 | 99.9 |
| DO (mg/L) | 12.05 | 13.02 | 12.97 |
| $NH_4^+$ - [N] | 0.96 | 0.17 | 0.22 |
| $NH_3$ – [N] | 0 | 0 | 0 |
| Conductivity (µSiemens) | 791.6 | 446.7 | 669.2 |
| Pressure (mm Hg) | 738.8 | 737.2 | 738.3 |
| Observations | Clear & green-ish in color, flowing slowly | Clear & green-ish in color, flowing slowly | Mostly frozen, flowing slowly |

## Water Data Table

Date: 2/06/2019

Personnel: Jessica Wiese, Travis Luncan

| **Sample Site** | **LCFR** | **IRJKR** | **CCJKR** |
|---|---|---|---|
| Time | 8:54 AM | 9:20 AM | 9:11 AM |
| Ambient T (°C) | 11.7 | 11.7 | 12.2 |
| Water T (°C) | 6.8 | 6.2 | 5.9 |
| pH | 6.98 | 7.19 | 7.22 |
| DO (%) | 103.1 | 107.3 | 107.9 |
| DO (mg/L) | 13.39 | 13.94 | 14.01 |
| $NH_4^+$ - [N] | 1.07 | 0.26 | 0.29 |
| $NH_3$ – [N] | 0 | 0 | 0 |
| Conductivity (µSiemens) | 786.9 | 468.5 | 630.1 |
| Pressure (mm Hg) | 744.6 | 743.2 | 744.3 |
| Observations | Brown & cloudy, heavy flow | Brown & cloudy, heavy flow | Brown & cloudy, heavy flow |

# Water Data Table

Date: 2/13/2019

Personnel: Jessica Wiese, Travis Luncan

| **Sample Site** | **LCFR** | **IRJKR** | **CCJKR** |
|---|---|---|---|
| Time | 7:33 AM | 8:01 AM | 8:16 AM |
| Ambient T (°C) | -1.11 | -0.56 | -0.56 |
| Water T (°C) | 4.2 | 3.5 | 3.8 |
| pH | 7.12 | 7.36 | 7.33 |
| DO (%) | 101.8 | 105.3 | 106.1 |
| DO (mg/L) | 13.22 | 13.68 | 13.78 |
| $NH_4^+$ - [N] | 0.99 | 0.23 | 0.20 |
| $NH_3$ – [N] | 0 | 0 | 0 |
| Conductivity (µSiemens) | 787.8 | 458.6 | 624.7 |
| Pressure (mm Hg) | 743.6 | 743.4 | 744.9 |
| Observations | Mostly clear, flowing slowly | Clear & brown in color, moderate flow | Clear & green in color, moderate flow |

# Water Data Table

Date: 2/22/2019

Personnel: Jessica Wiese, Travis Luncan

| **Sample Site** | **LCFR** | **IRJKR** | **CCJKR** |
|---|---|---|---|
| Time | 7:30 AM | 7:58 AM | 8:12 AM |
| Ambient T (°C) | 0.56 | 0.56 | 1.11 |
| Water T (°C) | 4.7 | 4.2 | 4.0 |
| pH | 7.08 | 7.28 | 7.25 |
| DO (%) | 104.5 | 108.6 | 108.5 |
| DO (mg/L) | 13.42 | 14.12 | 14.15 |
| $NH_4^+$ - [N] | 0.97 | 0.19 | 0.25 |
| $NH_3$ – [N] | 0 | 0 | 0 |
| Conductivity (µSiemens) | 781.5 | 464.1 | 600.3 |
| Pressure (mm Hg) | 742.4 | 742.7 | 743.1 |
| Observations | Water clear, flowing slowly | Cloudy & brown, moderate flow | Green/grey in color, moderate flow |

## Water Data Table

Date: 2/28/2019

Personnel: Jessica Wiese, Travis Luncan

| **Sample Site** | **LCFR** | **IRJKR** | **CCJKR** |
|---|---|---|---|
| Time | 7:30 AM | 8:05 AM | 7:52 AM |
| Ambient T (°C) | 1.67 | 2.22 | 1.67 |
| Water T (°C) | 4.3 | 3.7 | 4.0 |
| pH | 6.83 | 7.42 | 7.47 |
| DO (%) | 90.4 | 99.1 | 98.1 |
| DO (mg/L) | 11.74 | 13.02 | 12.82 |
| $NH_4^+$ - [N] | 1.02 | 0.24 | 0.15 |
| $NH_3$ – [N] | 0 | 0 | 0 |
| Conductivity (µSiemens) | 790.1 | 447.0 | 665.6 |
| Pressure (mm Hg) | 737.6 | 738.2 | 738.0 |
| Observations | Slow to moderate flow; rust-colored algae | Clear, slow to moderate flow | Clear & green-ish, slow to moderate flow |

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