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# Detection of benzotriazole and related analogues in surface samples collected near an Ohio airpark

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# DETECTION OF BENZOTRIAZOLE AND RELATED ANALOGUES IN SURFACE SAMPLES COLLECTED NEAR AN OHIO AIRPARK

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

by

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Clara Leedy ENTITLED Detection of benzotriazole and related analogues in surface samples collected near an Ohio airpark BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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# ABSTRACT

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Benzotriazoles are a class of contaminant of emerging concern which are commonly used as anticorrosive agents in aircraft deicer and anti-icing fluids (ADAFs). The analogues 1H-benzotriazole (BTZ), 4-methyl-1H-benzotrizole (4m-BTZ), and 5-methyl-1H-benzotriazole (5m-BTZ) are commonly found in environmental occurrence together. The two methylated isomers, collectively known as tolytriazole (TTZ), have different toxicity and stability. These contaminants are highly water soluble and resistant to biodegradation, making them persistent through water treatment. Benzotriazoles have been detected worldwide; this investigation focuses on monitoring three sites near a small airpark in Wilmington, Ohio. Two sites that receive runoff from the airpark, Lytle Creek and Indian Run, have been under investigation for decades due to documented poor water quality issues. This investigation adds to data from the two previous years documenting an increase in BTZ compounds that corresponds to an increase in activity at the airpark by an online retailer. Solid Phase Extraction (SPE) was used to isolate benzotriazoles from surface water samples. Liquid Chromatography/Mass Spectrometry (LC/MS) was used for separation and detection of analytes. Each consecutive monitoring season detected more BTZ and TTZ on average than previous seasons. The 2021 season detected

TTZ from 0.346-1.785 µg/L at Indian Run. Lytle Creek yielded BTZ from 0.051-0.158 µg/L and TTZ from 1.700-51.87 µg/L. Other occurrences have detected BTZ compounds associated with airpark runoff ranging from ng/L to mg/L. Gas Chromatography/Mass Spectrometry (GC/MS) was employed to separate the two TTZ isomers that could not be separated by LC/MS. This method revealed a ratio of 41.29% 4m-BTZ and 58.71% 5m-BTZ in selected water samples, a ratio which is similar to findings in a Wisconsin study. Based on the ratios of each isomer, hazard quotients assessed most samples analyzed as low environmental risk with a few days presenting medium to high environmental risk. Sediment samples were also examined for presence of benzotriazoles, but the results were inconclusive.

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# Introduction

## 1.1. What are benzotriazoles

### *1.1.1. Contaminants of emerging concern*

Human impact on the environment is becoming an increasingly relevant issue; chemical contamination is one aspect that is important to consider but can easily go unnoticed. Unintentional chemical contamination of the environment can come about in unexpected ways during everyday activities. Degradates from pharmaceuticals are flushed down the toilet and released into sewer systems. Chemicals found in sun block are rinsed into surface water while families enjoy a day at the lake. Cleaning products for outdoor equipment are washed into a nearby stream during a rainstorm. These examples demonstrate how everyday activities can pollute the environment. Chemical pollutants often work their way into water systems. Some chemicals are not completely removed during wastewater treatment, causing these contaminants to persist in the environment.[1] These situations have brought to light many contaminants of emerging concern.

Contaminants of emerging concern, or CECs, are pollutants that may pose a hazard to human or ecological health and are virtually unregulated under current environmental laws. The EPA developed a *White Paper Aquatic Life Criteria for Contaminants of Emerging Concern: Part I Challenges and Recommendations* detailing the technical issues and recommendations to serve as a basis for modifying preexisting guidelines in an effort to crack down on CECs.[2] The presence of CECs is not only a concern for water pollution; plants and animals can consume contaminants causing chemicals to bioaccumulate. Due to lack of regulation for CECs, information regarding

toxicity and biological impact is often unknown.[1] Monitoring and testing of suspected contaminants is a step in the direction of safer aquatic ecosystems.

One CEC that has recently become prevalent is a class of compound called benzotriazoles. Benzotriazoles have a wide range of uses and applications, but for the purposes of this study, they are investigated as additives in anticorrosive agents for use on aircraft. Their use for this application was an effort to synthesize an organic anticorrosive agent to replace inorganic nitrate- and chromate-based anticorrosives that are known to be toxic.[3] This class of compounds is becoming more and more prevalent in aquatic environments, making their presence and environmental fate important areas of interest in research for understanding the impact of these contaminants long term.

### *1.1.1. Structures, physical properties, and uses*

Benzotriazoles are a class of organic molecules comprised of a benzene ring fused with a three-membered nitrogen ring; any structure with this base compound is considered a benzotriazole. From this base compound, known as 1H-benzotriazole (BTZ), a few analogues have become apparent for study as well. Two methylated analogues, 4-methyl-(4m-BTZ) and 5-methyl-1H-benzotriazole (5m-BTZ) are commonly found in environmental occurrence with BTZ. These two methylated isomers are collectively known as tolytriazole (TTZ). **Figure 1** presents these three structures.

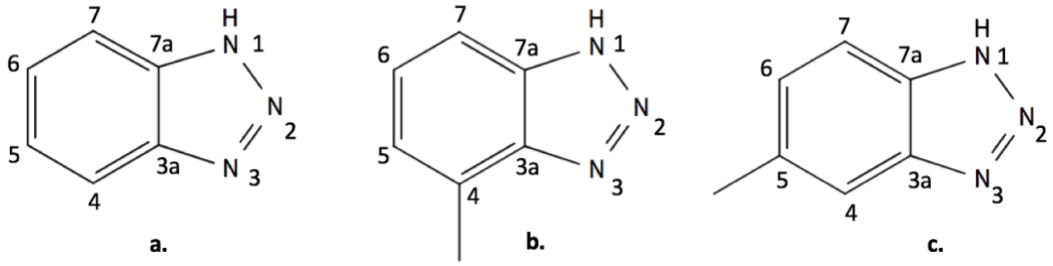


**Figure 1** depicts the labeled structures of a. 1H-benzotriazole, b. 4-methyl-1H-benzotriazole, and c. 5-methyl-1H-benzotriazole.

The three-membered nitrogen ring provides an electron rich site for binding with metals or other species. The aromatic benzene ring provides an electron rich system; electron density is increased with the addition of an electron donating group. The methyl group is electron donating; in the 5-position on the ring, the addition of this methyl group provides additional stability through resonance that the 4-position does not provide. The presence and placement of the methyl group on the benzotriazole is a small change that significantly adjusts physical and chemical properties. The difference in some properties is exemplified in **Table 1** below which compiles chemical and physical properties for these three compounds of interest.

**Table 1** Chemical and physical properties of benzotriazoles[4-8]

| Property | BTZ | 4m-BTZ | 5m-BTZ |
|---|---|---|---|
| Molecular Weight (amu) | 119.12 | 133.15 | 133.15 |
| Water Solubility (g/L) | 28 | 7 | 7 |
| Melting Point (°C) | 98.5 | 76-87* | 76-87 |
| Boiling Point (°C) | 204 | 195 (dec) | 210-212 |
| pKa | 8.20 | 9.15 | 8.85 |
| Log $K_{oc}$ | 1.02 | 1.68* | 1.68 |
| Log $K_{ow}$ | 1.23 | 1.89* | 1.89 |
| Physical Description | White or tan crystalline powder; odorless | *Tan or light brown granules; odor | Tan or light brown granules; odor |

*Values reported as tolytriazole

A few properties were recorded as collective tolytriazole or confirmed for 5m-BTZ but not 4m-BTZ. This observation supports that more research is needed to understand the differences between the two isomers. The 5m-BTZ isomer has a boiling point of 210-212 °C where at 195 °C the 4m-BTZ isomer was observed to decompose[4], reinforcing the theory that there is added stability for the placement of the methyl group in the 5-position on the ring instead of the 4-position. All three compounds exhibit high water solubility, 28 g/L and 7 g/L for BTZ and TTZ respectively, maintaining the likelihood that these chemicals will find their way to surface waterways and be transported downstream.[6] Their resistance to biodegradation coupled with these high water solubilities make it likely that benzotriazoles will reach wastewater, withstand wastewater treatment, and become prevalent in ambient water.[7] TTZ has both a higher soil adsorption coefficient ($K_{oc}$) and octanol-water partition coefficient ($K_{ow}$), suggesting that tolytriazoles may be detectable in sediments.[6]

As CECs, benzotriazoles may be accidentally leached into waterways from use in various products. BTZ and TTZ are commonly found in household detergents as well as flame retardants, anticorrosives, and deicers.[9] Aircraft Deicer and Anti-icer Fluids

(ADAFs) are primarily composed of glycols and water, with merely 1-2% of the solution consisting of additives such as alkylphenol ethoxylates (detergents) and benzotriazoles.[10] These compounds are useful as corrosion inhibitors due to the ability for the three-membered nitrogen ring to bind to metals such as copper. It has been proven that these types of structures containing heterocyclic rings with two adjacent nitrogen atoms are effective for corrosion inhibition. As an anticorrosive agent, benzotriazoles have been found to form a polymer-like film on the metal surface protecting the surface from corrosion.[11] This same ability for BTZ and TTZ to bind to metals is thought to lend itself to their toxicity; these compounds may bind with metals on membrane-bound enzymes and inhibit cellular functions and cause further complications.[12]

### *1.2. Occurrence of benzotriazoles*

#### *1.2.1. Environmental occurrence in water*

In 2004, annual benzotriazole production was 9000 tons in US.[9] The main source of benzotriazole release to the environment is through water contamination via ADAFS and detergents. BTZ and TTZ have been detected in wastewater treatment worldwide. Benzotriazoles are resistant to biodegradation making these compounds likely to persist in wastewater and later ambient water after release.[7]

Some of the highest benzotriazole concentrations in wastewater were recorded in a study by Voutsa et al in Glatt Valley, Switzerland in 2004. Analytes were detected in primary and secondary effluent in concentrations up to 100 µg/L, with median concentrations of 18 and 10 µg/L in primary and secondary effluent, respectively. It is thought these contaminants reach wastewater treatment via release from anticorrosive agents and detergents.[13]

Discharge from water treatment facilities can contaminate surface water with benzotriazoles. Many countries have reported benzotriazoles in surface water, some of the highest reported instances are from river water in Hengstbach, Germany. Grab samples were gathered from March to June of 2008 to analyze for BTZ and TTZ. Detected concentrations reached 1474 ng/L for BTZ, 281 ng/L for 5m-BTZ, and 952 ng/L for 4m-BTZ. Median concentrations for BTZ, 5m- and 4m-BTZ were 633, 95, and 476 ng/L, respectively.[10]

Benzotriazoles' resistance to biodegradation may lead them to evade other purification methods in addition to those used for typical wastewater. Tap water samples in the UK were analyzed for BTZ and TTZ. It was discovered that removal treatments may still leave 20-60 ng/L benzotriazoles in consumer tap water. Benzotriazoles were detected in all tap water samples analyzed. For BTZ, the average concentration was 30.9 ng/L, with concentrations as high as 79.4 ng/L. TTZ produced an average of 15.1 ng/L with a high concentration of 69.8 ng/L. The difference in relative concentrations is thought to be due to TTZ having a higher removal rate than BTZ in treatment. These concentrations were not deemed to pose an immediate health risk to humans, however information was not known regarding long term exposure at these levels.[14] Australia and Denmark are the only two countries with drinking water standards: the regulation for 5m-BTZ is <2400 ng/L in Australia and <20 ng/L for BTZ in Denmark.[15] It is interesting to note that the BTZ levels found in the UK are above the allowable level in Denmark. There are currently no other known benzotriazole concentration regulations.

### *1.2.2. Occurrence in sediments and air*

In addition to various water samples, benzotriazoles have been detected in sediment and air samples. Zhang et al examined sediment samples collected in China and the US for benzotriazole contamination. BTZ was observed in all samples from Detroit, US (up to 33.4 ng/g) and samples from northeastern China (up to 198 ng/g). The 5m-BTZ analogue was less frequently detected, found in 5/6 samples from the US up to 165 ng/g and in 3/5 samples in China up to 104 ng/g.[16] Few other sources recount work with sediment samples for benzotriazole detection.

Studies from China, Russia, and Spain have detected benzotriazoles in air. Yang's study recorded the presence of total compounds in China ranging from 1.53-6.28 ng/m$^3$ in air samples. Of the three cities examined, the highest concentration of analytes was found in Taiyuan, the northernmost city from this study. They theorize that more ADAFs were applied in this area due to colder temperatures, causing benzotriazoles to be more prevalent in this area. BTZ and 5m-BTZ accounted for up to 80% of contaminants detected, while 4m-BTZ was present is lower concentrations, along with other analytes.[17]

### *1.2.3. Occurrence in human samples*

Benzotriazole derivatives have been detected in human samples worldwide. Urine samples of males and females, both children and adults, were gathered in Greece, India, China, USA, Japan, Korea, and Vietnam. The study found BTZ up to 11 µg/L and TTZ up to 3.5 µg/L. Adipose tissue samples were gathered, revealing 95 ng/g wet weight BTZ and 6.6 ng/g wet weight TTZ. In amniotic fluid of pregnant females, BTZ was found up to 5.5 ng/L and TTZ was found up to 420 ng/L.[18,19] These examples prove that once consumed, benzotriazoles can be excreted, but have the ability to bioaccumulate as well.

1.3. Toxicity of benzotriazoles

*1.3.1. Toxicity for aquatic*

The above-mentioned occurrences of benzotriazoles can lead to contamination of aquatic life, animals, and humans. Benzotriazoles are known to be phytotoxic, toxic to aquatic organisms, and mutagenic to bacteria.[19] Toxicity for various aquatic organisms can be used to predict environmental risk of BTZ and TTZs. General information is provided in Table 2 below.

**Table 2** Benzotriazole toxicity to aquatic life[18]

| | BTZ | | 5m-BTZ | | 4m-BTZ | |
|---|---|---|---|---|---|---|
| Organism | Impact | Conc. | Impact | Conc. | Impact | Conc. |
| Microorganisms | Luminescence | 41.1 mg/L | Luminescence | 4.3-8.7 mg/L | Luminescence | 21 mg/L |
| Plants | Growth | 1.2-34.4 mg/L | Growth | 2.5-73 mg/L | Growth | 73 mg/L |
| Invertebrates | Immobility | 15.8-288 mg/L (48 hr) | Immobility | 4.2-109 mg/L | Immobility | 109 mg/L |
| | Mortality | 1.76-102 mg/L (2-14 days) | Mortality | 47-94 mg/L | Mortality | 95-119 mg/L |
| | Reproduction | 1 mg/L | Reproduction | 0.4-13 mg/L | - | - |
| Fish | Mortality | 65-458 mg/L | Mortality | 11-22 mg/L | Mortality | 18-95 mg/L |
| **PNEC** | **-** | **15.80 µg/L** | **-** | **5.52 µg/L** | **-** | ***21.00 µg/L*** |

Shi et al used this toxicity data to assess environmental risk using Hazard Quotients (HQs). The HQ estimates risk based on a ratio of measured environmental concentration (MEC) and predicted no effect concentration (PNEC). The PNEC listed in Table 2 is calculated for each analyte based on the toxicity data. If the HQ <0.1, the concentration is considered to be low risk. If 0.1 < HQ < 1, the concentration is medium risk. High risk is denoted by HQ > 1. The risk level refers to likelihood for harm to aquatic organisms. It was noted by researchers that the 4m-BTZ analogue data was lacking, making accurate calculation of the PNEC difficult. Other studies have estimated that the 4m-BTZ isomer may be more toxic than the 5m- isomer.[17,18] Differences in toxicity of the two methylated isomers, and the

lack of relevant data for the 4m-BTZ isomer, illuminate the importance of analytical separation of each analogue for testing and understanding occurrence and risk of benzotriazoles. Additionally, many of the studies reported in Table 2 cover the course of hours or days. The impact of benzotriazoles at lower concentrations on aquatic organisms and overall water quality over longer periods of time, such as months or years, is unknown.

### *1.3.2. Considerations for larger animals and humans*

Studies have investigated the impact of benzotriazoles on larger animals such as rats and rabbits. Exposure to benzotriazoles resulted in respiratory tract issues. Exposure to TTZ resulted in damage to lungs, liver, and kidneys. Skin and eye irritation was observed upon dermal contact with benzotriazoles in rabbits. After repeated doses, rats exhibited damage in cellular growth in the liver and the prostate or uterus. Dietary ingestion of BTZ led to tumor growth in the liver, brain, thyroid, and uterus of female rats. Similar dermal, estrogenic, and carcinogenic effects are suspected for humans if exposed over time or at high enough levels. Incidents were observed where metal workers contracted contact dermatitis on hands and forearms after interacting with lubricating oils containing BTZ. Four patch test cases revealed allergic reactions on the skin.[8,19]

### *1.3.3. A possible toxicity pathway*

The study of benzotriazoles in air by Yang considered contaminants sorbed to $PM_{2.5}$ pollution particles in three Chinese cities and revealed information about toxicity pathways. Human exposure was assessed based on body weight of those contaminated (toddlers and adults), exposure time and frequency. Calculations reported that blood and cardiovascular systems were the main areas targeted. The 4m-BTZ analogue was revealed to have the greatest cytotoxicity when tested on neonatal rat cardiomyocytes

(NRCMs) with an $LC_{50}$ concentration of 694.8 µM. The values for BTZ and 5m-BTZ were 876.5 and 806.9 µM respectively. The toxicity pathway was further investigated using 4m-BTZ. Researchers theorize that the 4-methyl isomer is more toxic due to more biochemical reactions in organisms which can induce more chemical stress. A test on mitochondrial fluorescence revealed that the presence of 4m-BTZ could induce mitochondrial dysfunction which led to apoptosis of the NRCMs. Further investigation suggested that this dysfunction was related to the biosynthesis of coenzyme A being severely impacted. This enzyme plays a vital role in many biochemical reactions including breakdown of sugars and amino acids, oxidation of fatty acids; these processes provide 90% of the body's energy. This disruption of the body's energy metabolism causes harmful effects on cardiac cells which can cause serious diseases such as myocardial infraction, heart failure, and hypertrophy. The study concluded that environmental occurrence of benzotriazoles leads to animal and human exposure, and at high enough concentrations or at frequent doses, these contaminants can lead to severe health issues.[17]

It was theorized that ADAFs were a main source of the contamination issue which eventually led to contamination through inhalation. For this reason, continued study of environmental fate and toxicity of benzotriazoles are important. Routes for contamination and risk of toxic effects are difficult to avoid due to the environmental persistence and wide occurrence of the class of contaminants.[17]

#### *1.3.4. Biological connection to tryptophan*

In addition to environmental occurrence of benzotriazoles, there is a possibility of uptake into plants. Biological processes can convert benzotriazoles into forms similar to

tryptophan, an amino acid. The possible structures and structure are shown below in Figure 2.

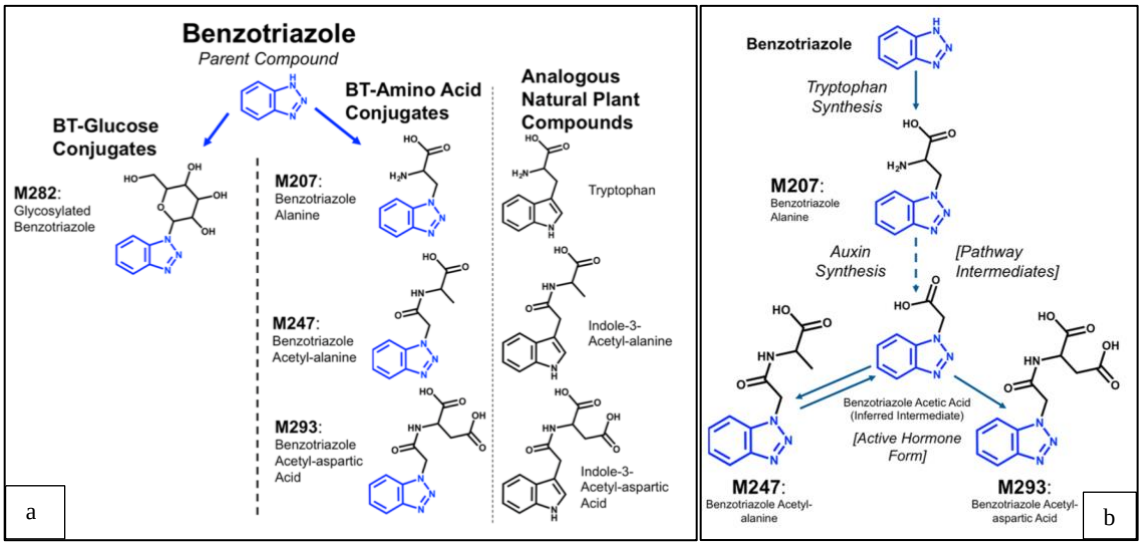


Figure 2 Depicts a. possible benzotriazole analogues produced during plant uptake and their natural plant compounds and b. the proposed synthesis route in the plants.[1]

The benzotriazole analogues synthesized during plant uptake are similar to natural plant compounds. The findings in this study suggest that plants may play a helpful role in removal of pollutants from aquatic systems. However, the effects of these synthesized compounds on animals who may consume the plants is unknown. More research can be done to determine these effects and possible use of these analogues in plants.[1] The study by Yang et al also noted that the presence of 4m-BTZ in NRCMs resulted in the observation of tryptophan metabolic pathway disruption.[17]

## 1.4. Benzotriazoles in Wilmington, Ohio

### *1.4.1. Wilmington airpark history*

The toxicity and worldwide persistence of benzotriazoles brings to light the importance of their study. Stormwater and wastewater runoff containing ADAFs are known to be a leading cause for the spread of BTZ and TTZ, making their consideration

near airparks an area of interest. One such case is that of a growing airpark in Wilmington, Ohio, USA.

Wilmington Airpark opened for operation in 1929. The site has been under ownership of many corporations since its opening including the US Air Force, Airborn Freight Corporation, DHL Express, and the current operator, an online retailer.[21] The waterways surrounding the airpark are part of the Little Miami Watershed. Starting in the 1940s, one of the nearby creeks, Lytle Creek, was used as a site for research and development of municipal wastewater treatment. As studies were conducted, the US Public Health Service and Department of Health Education and Welfare monitored the site and noted lack of fish and invertebrate diversity.[22] Two new treatment beds were constructed on either side of the airpark in 2001, one emptying into Lytle creek and one emptying into Indian Run. More recently in 2007, the site has been investigated by the EPA. Following EPA investigation, a report was released in 2009. The reported noted compromised macroinvertebrate population in Lytle Creek and Indian Run. Furthermore, the fish community of Lytle Creek was designated as "poor". Water quality continued to be designated as poor in a Clinton Country Streamkeepers report issued in 2015.[23,24] After investigating heavy metals and E-coli as other sources of contamination,[24] this study began considering presence of BTZs in runoff from the airpark to be a possible source of contamination leading to the macroinvertebrate population decline.

The aforementioned online retailer established operation in June 2019. Cargo traffic at the airpark has grown 289% since 2019 due to the growing presence of the online retailer. The airpark only services general aviation and corporate traffic with no scheduled passenger flights, making it a less congested destination for cargo shipment. In

2020, the airpark became the third largest cargo-focused airpark in the nation after 439 million pounds of cargo had been moved through the site.[25] This drastic increase in airpark activity is expected to lead to increasingly higher concentrations of BTZs in surrounding surface water.

### *1.4.2. Investigations for 2019 and 2020 seasons*

Our research group has investigated the presence of benzotriazoles near the airpark in Wilmington for two previous seasons. The project began in 2019 to set a baseline of water quality and of benzotriazole compounds found in surface water before the presence of the aforementioned online retailer. This investigation developed an initial sampling plan for three sample sites near the airpark: Cowan Creek (CCJKR), Indian Run (IRJKR), and Lytle Creek (LCFR). Samples were gathered weekly in February 2019. Initial methods were developed for processing samples using Solid Phase Extraction (SPE) and for analyzing processed samples on Liquid Chromatography/Mass Spectrometry (LC/MS) for presence of BTZ and TTZ. BTZ was detected in trace levels (below 0.100 µg/L) at Indian Run and Lytle Creek. TTZ was detected up to 0.869 µg/L at the Indian Run site and up to 2.724 µg/L at Lytle Creek. These concentrations support the airpark's compliance with their Ohio EPA discharge permit.[23]

The investigation continued the following year by monitoring water quality and gathering samples every two to three weeks from November 2019 to March 2020 (denoted 2020 season). This investigation presented a broader picture of the presence of benzotriazoles over the course of the winter season. The work optimized the previously developed SPE process and LC/MS methods to achieve better analyte recoveries and better chromatographic analyte separation. BTZ was found up to 3.467 µg/L at the Lytle Creek site. TTZ was found up to 5.649 µg/L and 11.943 µg/L at Indian Run and Lytle

Creek sites respectively. The increase in analytes detected is presumably due to the increased activity of the online retailer at the airpark. These increased BTZ concentrations are thought to pose a low to medium environmental risk according to hazard quotients determined by *Shi et al*.[18,26]

### *1.4.3. Approach for the 2021 season*

The 2021 season continued monitoring water quality and collecting samples for detection of benzotriazoles at the same three sites. The same optimized methods for SPE and LC/MS were used. In addition to water data, sediment samples were collected on one sample day to see if tolytriazoles sorb to surface level sediments. Furthermore, a Gas Chromatography/Mass Spectrometry (GC/MS) method was developed in an attempt to separate the 4m- and 5m-BTZ isomers that cannot be separated using LC/MS. The differences in boiling points between the two analogues makes GC/MS a possible option that our group has not previously investigated. Investigation of analytes in sediments and analytical separation of the two isomers are important steps for understanding environmental fate and for assessment of the contamination risk to the environment and to humans.

# Sampling and Experimental Methods

## 2.1. Sampling near Wilmington Air Park

### *2.1.1. Sampling Materials*

Listed below are materials required for collecting surface water samples according to the water sampling plan SOP in Appendix A.

- YSI Multimeter Pro Plus
- ASTM Type 1 Water, 18 MΩ-cm resistance (HQ water)
- Gloves
- 500-mL glass amber bottles with Teflon caps (7)
- Cooler and ice packs
- Water Data Collection Form (SOP)
- Clipboard

The YSI Multimeter Pro Plus was calibrated one day before each sampling excursion.

Solutions required for sampling include the following:

- YSI Confidence Solution (Cat No. 15-176-216)
- YSI Conductivity Solution (Cat. No. 09-390-16)
- YSI pH Buffer solutions of pH 4, 7, and 10 (Cat No. 15-176-208 for set)
- YSI Ammonium solutions 1 and 10 ppm (Cat. No. 15-178-103)

For sediment sampling, a few materials were required in addition to the water sampling materials:

- Clear 60-mL glass sample jars with Teflon caps (10)
- Clean plastic spoons (10)
- Small scoop or shovel
- Sediment Data Sheets (10)

### *2.1.2. Sampling Process*

Sampling for this investigation was conducted in Wilmington, Ohio according to the SOP in Appendix A at three sample sites Cowan Creek (CCJKR), Indian Run (IRJKR) and Lytle Creek (LCFR). The sample sites and procedure are comparable to the process conducted for the 2019 and 2020 sampling seasons of this project. Water samples were collected on five sampling days for this 2021 season: December 9, 2020; January 13, February 24, March 4, and June 24, 2021. The YSI Multimeter Prop Plus was calibrated one day before each sample day. Seven 500 mL glass amber bottles were cleaned thoroughly with HQ water allowing two sample bottles for each site and one trip blank. Sample bottles were pre-labeled according to the SOP in Appendix A as follows:

MMDDYYYY-Site-R#-BTZ

Ex: 12092020-CCJKR-R1-BTZ

Only the respective sample bottles were carried to each site, while the other bottles remained in the cooler to avoid cross contamination or mislabeling of samples.

Upon arrival at each site, the YSI Multimeter Pro Plus was allowed to equilibrate in the surface water for a few minutes while the water sample was collected. Each pre-labeled sample bottle was rinsed in the respective site water before being filled roughly three-fourths full with sample water. Water quality data from the YSI meter was used to fill out the Water Data Sheet for each site (found in SOP). If the probe could not carefully be placed in an area near where the sample was collected that had a steady water flow, the probe was moved slowly back and forth in the water to allow water to flow continuously over the probe electrodes. Once samples were collected, they were placed in the cooler with ice packs until return to the lab.

On March 4, 2021, sediment samples were also collected in addition to water samples according to the protocol in Appendix B. Sediments were collected at the same sites as water samples. At each site one sample was taken from the center (C) of the surface water source and one from either a left descending- or right descending bank (L or R Bank, direction with respect to downstream water flow). Labels were similar to those for water samples but included specific location at each site:

Ex: 03042021-CCJKR-C-BTZ

Samples were collected near the sediment surface using either a clean plastic spoon or the shovel, cleaned thoroughly between uses. One sediment data sheet was filled out for each sample detailing conditions of the sample. Samples were kept cool with ice packs until return to the lab.

Samples were immediately placed in deep freeze (-40 °C) upon return to the lab. Special care was taken to ensure bottles were not too full, disposing of excess water sample down the sink. Water samples were frozen laying down sideways allowing more surface area in an effort to prevent breaking sample bottles. Sediment samples were placed in a box with a lid to protect from excess light exposure.

The chemistry of each sample was better understood after sample analysis, however understanding the conditions of each sample location helps paint the bigger picture. Figure 3 below shows the layout of the sample sites with respect to the airpark.


**Figure 3** shows the location of each sample site relative to the airpark.[27]
GPS locations for each site are provided below in Table 3.

**Table 3** GPS locations for benzotriazole sample sites in Wilmington

| Sample Site Code | GPS Coordinates |
|---|---|
| **CCJKR** | 39.407615, -83.798064 |
| **IRJKR** | 39.408914, -83.799194 |
| **LCFR** | 39.437051, -83.797386 |

Cowan Creek (CCJKR) was used as the control site where surface water flowed upstream from the airpark. The site is located in a wooded area off of Jenkins Road, shown below in **Figure 4.**

**Figure 4** depicts the Cowan Creek sample site from ground level on 12/9/2020 (left) and from the bridge over the site on 2/24/2021(right).

On days of lower flow, samples were taken near the bottom of the bridge on Jenkins Road. When water levels were high, samples were gathered by tossing a bucket down from the bridge. The difference between water levels can be seen based on the two images from **Figure 4.** Higher animal activity was observed at this site, such as the presence of deer and small fish.

Water flows from Cowan Creek and joins Indian Run (IRJKR). The Indian Run site is accessed by crossing through the wooded area next to Jenkins Road, then crossing through a field. This site, shown in **Figure 5,** is directly downstream from the airpark located off of airpark property.

**Figure 5** presents Indian Run sample site on 2/24/2021.

On low flow days, the site was accessed by climbing down the bank. On high flow days such as the sample day shown above, a bucket was used to gather water for sampling.

The third sample site, Lytle Creek, was located downstream from the treatment facility  off of Fife road. The site was accessed by walking along the road toward a drain pipe that ran under Fife road.

**Figure 6** shows the Lytle Creek sample site on 12/9/2020 (left) and on 2/24/2021 (right). Sampling for this site never required the use of a bucket, as the bank was easier to access than at the other two sites, even under higher water conditions. This site usually had a smaller water volume and lower flow than the other sites, and litter was more common with proximity to the busier road.

## 2.2. Sample Processing

### *2.2.1. Processing materials*

The following materials were required for processing samples using Solid Phase Extraction (SPE):

- Beakers and flasks for sample storage and collection
- 0.7-µm glass fiber filters (Whatman, GF/F, 47mm)
- Whatman 47-mm glass filter funnel
- 1.00-L Erlenmeyer flask with vacuum attachment
- Whatman Oasis HLB 6 cc Vac Cartridges 500-mg, 60-µm
- 12-port vacuum extraction manifold
- Glass Pasteur pipettes

- 25-µL laboratory grade syringe
- 15-mL graduated centrifuge tubes
- Fisher Concentrated Hydrochloric Acid (12M HCl, CAS #7647-01-0)
- Fisher HPLC Grade Methanol (CAS #67-56-1)
- ASTM Type 1 Water, 18 MΩ resistance (HQ water)
- Fisher Dichloromethane (DCM, CAS #75-09-2)
- Sigma Aldrich 5,6-dimethyl-1H-benzotriazole (5,6-DMBTZ, CAS #4184-79-6, 100%)
- Tank of nitrogen gas (Cas # 7727-37-9)
- Centrifuge

### *2.2.2. Water Sample Processing Via Solid-Phase Extraction (SPE)*

Water samples were processed according to the SOP in Appendix C. Frozen sample bottles were removed from the deep freeze (-40 °C) and left to thaw on a bench top overnight before processing. Sample jars were placed in 600-mL beakers to catch the sample in case the jars cracked during the thawing process. Teflon caps were opened and left loosely on top of jars to release pressure during thawing.

After samples were completely thawed, each sample was filtered through a 0.7-µm Whatman glass fiber filter and then divided into three 100-mL aliquots. Each aliquot was acidified to pH 3 with a few drops of concentrated HCl and spiked with 10 µL (60 ng) of 6.00-mg/L 5,6-DMBTZ surrogate standard. The addition of acid helps condition the cartridge and ensures that the desired analytes are protonated before analysis. The known surrogate standard is injected for the purpose of tracking analyte recovery during the extraction process. Each set of samples was processed with an HQ water method blank to check for sources of contamination. Before the samples were passed through the cartridges, each cartridge was conditioned with sequential rinses of methanol and water. The samples were then passed through the cartridges at a rate of roughly 2 mL/min at 5

psi. The cartridges were then allowed to drip dry for 2.5 hours under 15 psi. Once the drying phase was complete, the analytes were eluted from the cartridges. To elute the analytes, the cartridges were loaded with 5 mL of 95/5 DCM/methanol. This elution mixture was allowed to equilibrate in the cartridge for 10 minutes before the sample analytes were eluted from the cartridge into glass centrifuge tubes at a rate of 2 mL/min under 5 psi. The cartridges were allowed to drip to dryness for about 30 minutes before the centrifuge tubes containing eluted analytes were removed.

The elution solvent was either immediately blown off under gentle stream of nitrogen, or the samples were placed in the freezer (-20 °C) overnight to have solvent removed the next day. After the samples were blown down to dryness, the dried analytes were diluted with 1.0 mL of methanol. These diluted samples were transferred to prelabeled autosampler vials for analysis on either LC/MS or GC/MS. Samples were labeled according to their sample code, replicate number, and aliquot number (T1-3) as shown below.

Ex:01132021-IRJKR-R1-T2

Samples were stored at -20 °C after analysis.

### *2.2.3. Sediment Sample Processing*

Sediments were processed according to the protocol in Appendix D, adapted from Zhang.[16] A portion of sediment samples taken on March 4, 2021 were freeze-dried using a VirTis freeze-drying apparatus with condenser temperature set to -55.9°C and vacuum set to 380 torr. An image of the freeze-drying apparatus set up is available as Figure D1 in the Appendix. The freeze-dried samples were kept in the deep freeze (-40°C) until further processing. To extract the desired analytes from the sediment matrix, solvent

extraction was performed. Approximately 1 g sediment was weighed on an analytical balance and stored in a glass centrifuge tube. Images of each sediment sample are available in Appendix D. The weighed sediment was spiked with 10 µL (60 ng) of 6.00-mg/L 5,6-DMBTZ surrogate standard and allowed to equilibrate for one hour before continuing the extraction process. Two sequences of extractions were conducted where 5 mL of methanol were added to the centrifuge tube. The sample was hand-shaken before being sonicated at 35°C for 30 minutes. The sample was hand-shaken again, then placed in a centrifuge for 10 minutes to allow sediment to settle out of solvent. The solvent layer was decanted off the top before the second sequence of extraction was performed. Both solvent layers were combined in a new prelabeled glass centrifuge tube for further processing. The methanol solvent was blown to near dryness under steady stream of nitrogen gas. The sample was then diluted to roughly 10 mL with HQ water to prepare for SPE.

A modified version of the SPE process outlined in SOP C was used to clean up analytes during sediment processing. To begin the SPE process, each 10-mL sample was acidified to pH 3 with 1 drop of concentrated HCl. This step mirrors the water sample SPE process, using less acid to account for the difference in sample volume. The cartridges were then conditioned with sequential rinses of methanol and water. The sample was passed through the cartridge at a rate of 1 mL/min. Once this step was complete, the cartridge was washed with 5% methanol in HQ water according to the procedure by Zhang. The cartridges were allowed to dry for 30 minutes before desired analytes were eluted from the cartridges with 5 mL of 95/5 DCM/methanol at a rate of 1 mL/min. The DCM solvent layer was blow to near dryness under steady stream of

nitrogen gas. The samples were then diluted to 1.0 mL with methanol. Sample labels were tagged for freeze dried sediment by adding “FDS” to the end to differentiate from water samples, as shown below:

Ex: IRJKR-C-R1-FDS

Samples were stored in the freezer (-20°C) until analysis on LC/MS.

## 2.3. Sample Analysis

### *2.3.1. Analysis Materials*

Preparation of analytical standards and analysis of samples on instrumentation required the following materials:

- Various volumetric flasks
- Various laboratory grade syringes
- 2-mL autosampler vials with Teflon caps
- Glass amber vials with Teflon caps
- Fisher HPLC Grade methanol (CAS #67-56-1)
- Sigma Aldrich 1H-benzotriazole (BTZ, CAS #95-14-7, 98%)
- Sigma Aldrich 4-methyl-1H-benzotriazole (4m-BTZ, CAS #249-921-1, 90%)
- Sigma Aldrich 5-methyl-1H-benzotriazole (5m-BTZ, CAS #136-85-6, 98%)
- Sigma Aldrich 5,6-dimethyl-1H-benzotriazole (5,6-DMBTZ, CAS #4184-79-6, 100%)
- ASTM Type 1 Water, 18 MΩ resistance (HQ water)
- Fisher Formic Acid (CAS #64-18-6)

### *2.3.2. Analytical Standard Preparation*

Before sample analysis began, standards for the desired analytes and surrogate standard were made according to the SOP in Appendix E. Stock solutions of each analyte were made using analytical grade solid weighed on an analytical grade balance and dissolved in methanol. The initial desired concentration of stock for BTZ, 4m-BTZ, and 5m-BTZ was 100-mg/L in a 50-mL volumetric flask, requiring 0.00500 g of solid analyte. The desired concentration for DMBTZ was 5.0-mg/L which required 0.00025 g of solid analyte in a 50-mL volumetric flask. The mass of each analyte weighed is recorded in Table 4 below. The concentration of each standard was calculated, considering correction based on analyte purity.

**Table 4:** Mass of analyte used to make stock standards

| Analyte | Mass Weighed (g) | Analyte Purity (%) | Corrected Concentration (mg/L) |
|---|---|---|---|
| 1H-benzotriazole | 0.00421 | 98 | 82.51 |
| 4-Methyl-1H-benzoriazole | 0.00501 | 90 | 90.18 |
| 5-Methyl-1H-benzoriazole | 0.00505 | 98 | 98.98 |
| 5,6-Dimethyl-1H-benzoriazole | 0.00030 | 100 | 6.00 |

The mass of BTZ and DMBTZ deviated significantly from the desired mass recorded in the SOP. There was insufficient BTZ left in the analyte bottle to weigh the desired amount. Therefore, dilution steps outlined in the SOP were slightly adjusted to make the desired standard concentrations. Additionally, mixed 4m- and 5m-BTZ, or TTZ, standards were made for LC/MS analysis due to the previous discovery that these two analytes have the same retention time on LC/MS. The desired concentrations for BTZ and TTZ standard solutions were 10- and 1.0-mg/L and 100-, 50-, 25-, and 10-µg/L. Additional higher-level standards of TTZ were made when it was discovered that some

sample concentrations were above the calibration curve. These concentrations were 5.0-, and 2.5-mg/L and 500-µg/L. Desired DMBTZ concentrations were 1.0-mg/L, 100-, 50-, 25-, and 10-µg/L. An additional 75-µg/L standard was made because the 10 µL of surrogate spike in each sample should have an instrument response of 60-µg/L, adjusted for recovery. A few additional deviations from the original SOP were used to make adjusted volumes of standards. After standards were made, it was determined that concentrations needed to be corrected based on analyte purity. All deviations from the SOP are recorded below in Table 5.

**Table5:** LC/MS standard preparation steps adjusted from SOP

| Analyte | Standard Concentration | Higher Standard Used | Final Volume (mL) | Purity Corrected Concentration |
|---|---|---|---|---|
| BTZ | 10.00-mg/L | 3.00 mL of 82.51-mg/L | 25 | 9.90-mg/L |
| BTZ | 1.00-mg/L | 1.00 mL of 10-mg/L | 10 | 0.990-mg/L |
| BTZ | 100.0-µg/L | 100.0 µL of 10-mg/L | 10 | 99.00-µg/L |
| BTZ | 50.00-µg/L | 500.0 µL of 1.0-mg/L | 10 | 49.50-µg/L |
| BTZ | 25.00-µg/L | - | - | 24.75-µg/L |
| BTZ | 10.00-µg/L | - | - | 9.90-µg/L |
| TTZ | 10.00-mg/L | 1.25 mL each of 4m- and 5m-BTZ stock | 25 | 9.40-mg/L |
| TTZ | 5.00-mg/L | 500 µL of 10-mg/L | 1.0 | 4.70-mg/L |
| TTZ | 2.50-mg/L | 250 µL of 10-mg/L | 1.0 | 2.35-mg/L |
| TTZ | 1.00-mg/L | - | - | 0.940-mg/L |
| TTZ | 500.0-µg/L | 5 mL of 1.0-mg/L | 10 | 470.0-µg/L |
| TTZ | 100.0-µg/L | 100 µL of 10-mg/L | 10 | 94.00-µg/L |
| TTZ | 50.00-µg/L | 500 µL of 1.0-mg/L | 10 | 47.00-µg/L |
| TTZ | 25.00-µg/L | - | - | 23.50-µg/L |
| TTZ | 10.00-µg/L | - | - | 9.40-µg/L |
| DMBTZ | 1.00-mg/L | 4.16 mL of 6.0-mg/L | 25 | - |
| DMBTZ | 75.00-µg/L | 750 µL of 1.0-mg/L | 10 | - |

All prepared standards were run on LC/MS. The resulting instrument responses created linear calibration curves shown in Appendix E, with responses comparable to previously made standards.

Mixed standards for GC/MS analysis were made using the 10-mg/L TTZ standard (5-mg/L of 4m- and 5m-BTZ). Dilutions for these standards and calibration curves are

included in Appendix F. All standards for benzotriazole analysis were stored in glass amber vials at -20°C until use.

### *2.3.3. Analysis on LC/MS*

All processed surface water samples and respective blanks were analyzed on an Agilent Technologies 1220 Infintiy LC equipped with a 6120 Quadrupole LC/MS. The column was an Agilent Eclispe Plus $C_{18}$ (1.8 I.D. 2.1 x 100 mm) column. A previously developed and validated LC/MS method[23,26] according to the SOP in Appendix E determined the optimum parameters for this LC/MS method analysis, shown in Table 6.

**Table 6** LC/MS parameters for benzotriazole analysis

| LC/MS Parameter | Setting | LC/MS Parameter | Setting |
|---|---|---|---|
| **Column Polarity** | Positive | **Eluent** | 45 water: 55 methanol (0.1% formic acid) |
| **Pressure** | 310 bar | **Flow Rate** | 1.2 mL/min |
| **Injection Volume** | 2 µL | **Stop Time** | 8.00 min |

For best sensitivity, samples were run on Selective Ion Monitoring (SIM) mode. The desired ions and their respective retention times are shown in Table 7.

**Table 7** LC/MS retention times for benzotriazoles

| | BTZ | TTZ | DMBTZ |
|---|---|---|---|
| **SIM Ion (*m/z*)** | 120 | 134 | 148 |
| **Retention Time (min)** | 4.01±0.23 | 5.18±0.09 | 6.69±0.14 |

LC/MSD ChemStation software was used for data processing. Example chromatograms showing each standard at 50-µg/L are shown below.

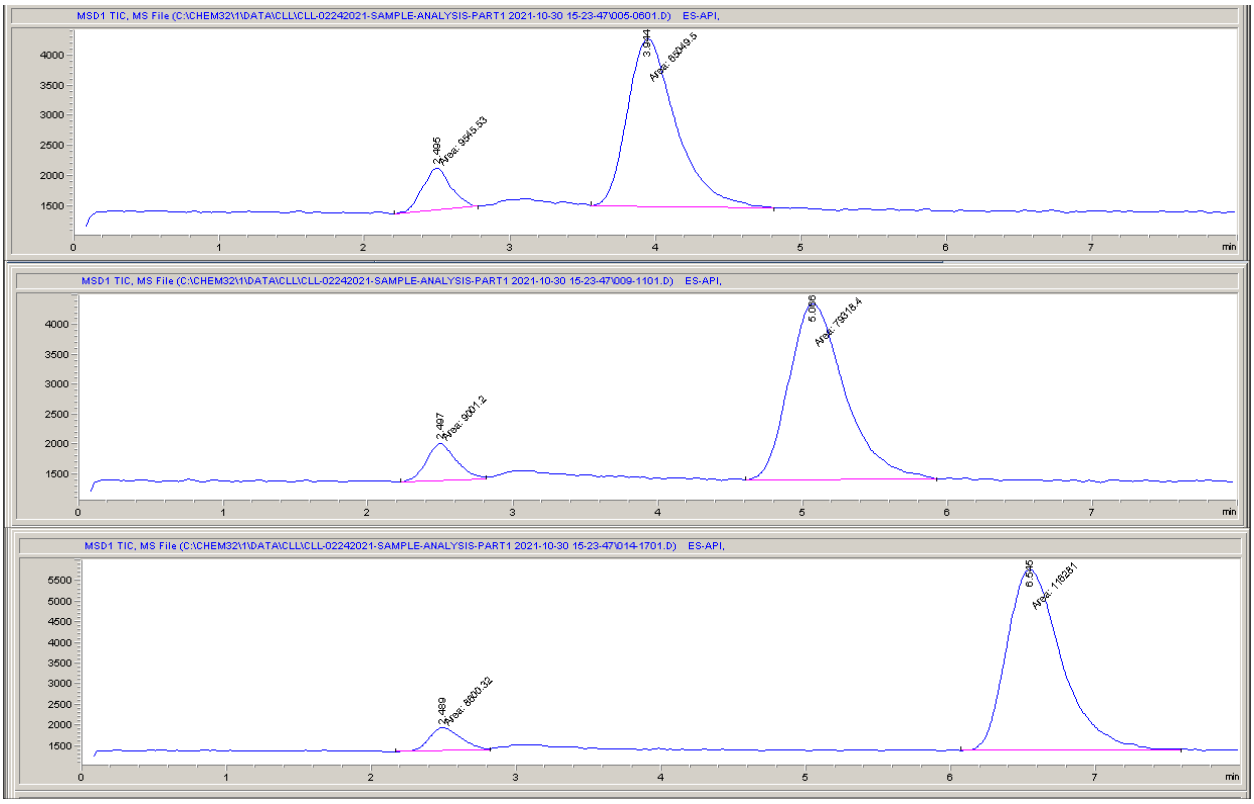


**Figure 7** shows BTZ (top), TTZ (middle), and DMBTZ (bottom) each as a 50 µg/L standard.

The LC/MS method was able to achieve appropriate separation of BTZ, TTZ, and DMBTZ. The limit of detection was determined to be 5 µg/L instrument response or 0.05 µg/L in a concentration corrected sample.[26] It was not possible to separate the 4m- and 5m-BTZ isomers on LC/MS, so GC/MS was employed to attempt the separation.

### *2.3.4. Analysis on GC/MS*

A GC/MS method was developed based on the method from Corsi et al.[28] The method is outlined in the SOP in Appendix F. An Agilent 7890B GC System was used for chromatographic separation. The column was a 30m x 250 µm x 0.25 µm PDMS capillary column. Parameters used for this analysis are included in Table 8.

**Table 8** GC/MS Parameters for benzotriazole analysis

| Parameter | Setting | Parameter | Setting |
|---|---|---|---|
| Injection Volume | 2 uL | Inlet Temperature | 260 C |
| Flow | 1 mL/min | Inlet Mode | Splitless |
| Temperature Program | | | |
| Initial Oven Temp | 70 C | Hold Time | 2 min |
| Temperature Ramp | 14 C/min | - | - |
| Final Oven Temp | 275 C | Hold Time | 2 min |

An Agilent 240 Ion Trap was used for MS detection. On full scan mode, the MS was set to scan from 50-150 m/z, because the highest mass analyte was DMBTZ with m/z of 147. MS Workstation 7.0.1 software was used for data processing. Full scan mode was used first to identify retention times for each analyte and to utilize the library match function. The library match for each of the four analytes is provided in Appendix F as Figures F1-4. An example chromatogram is shown below for a TTZ mixed standard containing equal concentrations of 4m- and 5m-BTZ.

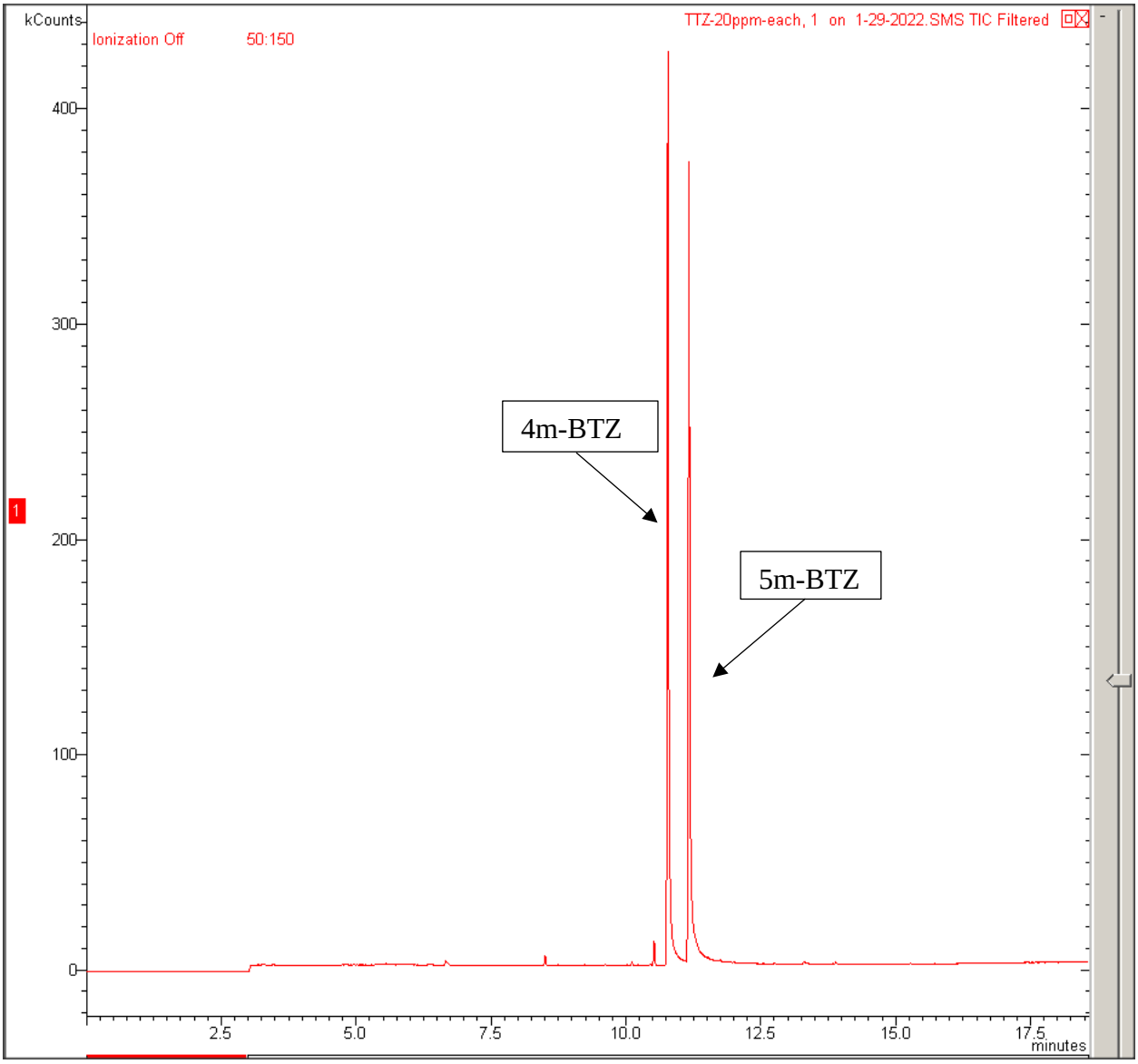


**Figure 8** depicts a GC/MS chromatogram with 4m- and 5m-BTZ at 20-mg/L each.

The above chromatogram confirms that separation of the two isomers is possible based on the developed GC/MS method. Once the retention time of each analyte was confirmed, SIM mode was used to analyze select samples. Table 9 below includes the retention time and *m/z* ion for each.

**Table 9** GC/MS retention times for benzotriazoles

| | BTZ | 4m-BTZ | 5m-BTZ | DMBTZ |
|---|---|---|---|---|
| SIM Ion *(m/z)* | 119 | 133 | 133 | 147 |
| Retention Time (min) | 10.18 | 10.75 | 11.14 | 12.36 |

It was later determined that the best response would be obtained if only TTZ (133 *m/z*) was monitored. Based on concentrations found from LC/MS analysis of water samples, it was known that BTZ never had an instrument response above 50-µg/L. DMBTZ was around a 50-µg/L instrument response for each surrogate injection. A 50-µg/L standard of each of those two analytes was run to determine that neither could be observed at the levels present in real samples. Chromatograms testing BTZ and DMBTZ response at these concentrations are provided in Appendix F as Figures F5 and 6. Because these low levels could not be detected, but TTZ consistently had a higher response, select real samples were considered on GC/MS running only SIM 133 *m/z*.

Before developing the final method that was used, a few tests were run to determine the best detection method. The inlet temperature was adjusted, trying 120°C, 170°C, and 260°C. The results for the 120 and 170 test are provided in Appendix F as Figures F7 and 8, while the chosen temperature of 260 is shown below.

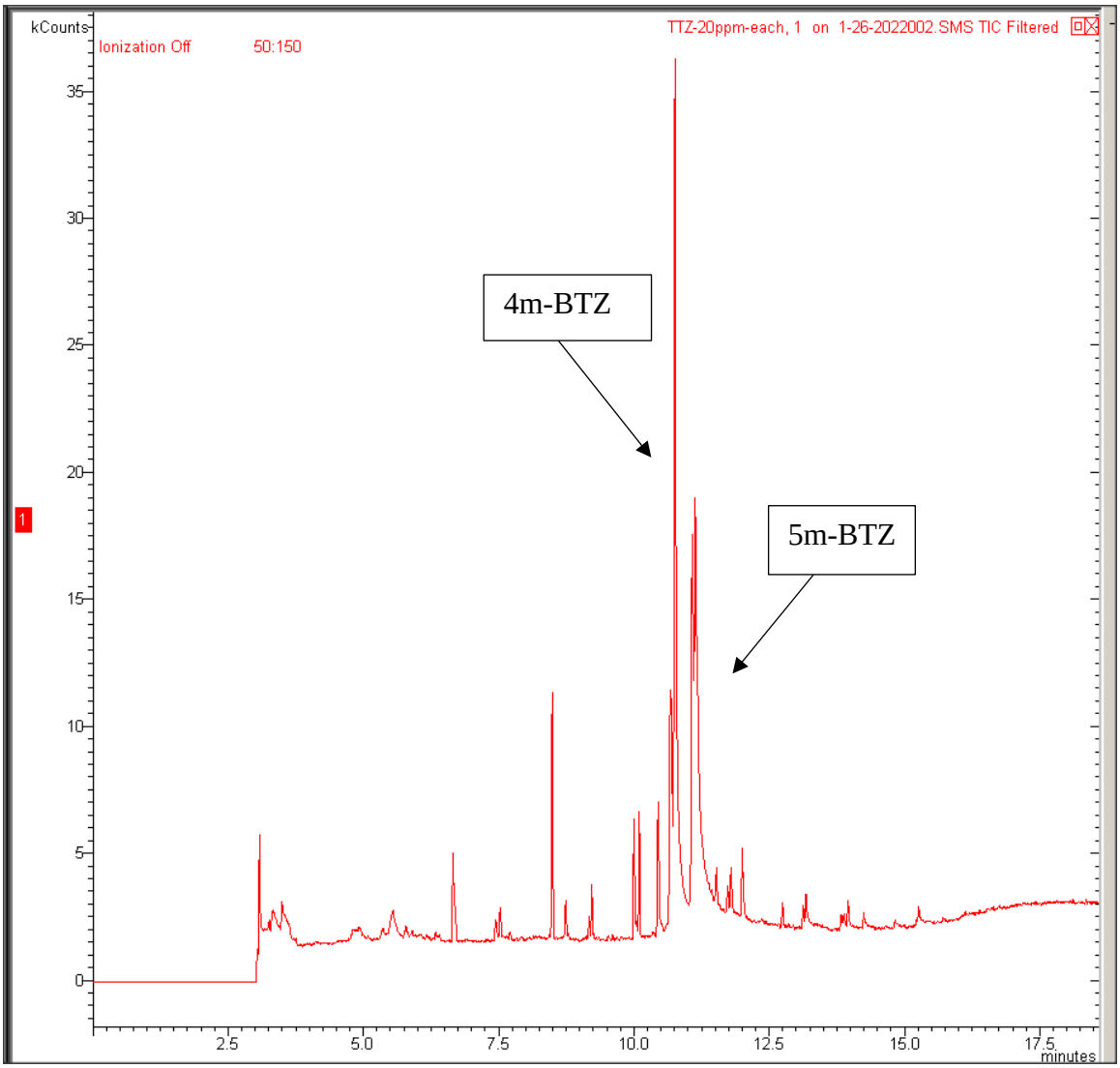


**Figure 9** depicts a TTZ standard with 20-mg/L of each isomer, original inlet and inlet temperature set to 260°C.

Based on these three figures, it can be seen that the highest temperature tested has the best response. The run for 120°C has no detectable response, and 260°C produced higher count peaks than 170°C. Still, the counts for the two peaks were not very high. Additionally, the peaks appear to be split and the response of 5m-BTZ compared to 4m-BTZ is much lower. In an effort to remedy this issue, the original inlet liner which contained silanized glass wool was replaced with an inlet liner without glass wool. Comparing Figure 8 with Figure 9, the difference from removing the glass wool is

observed. The use of an inlet liner without glass wool produced significantly higher peak counts and removed the issue of the split peaks. This observation of higher peak counts suggests that the analytes were reacting with the glass wool creating a lower response. Therefore, the final method used for this analysis employed an inlet without glass wool, set at a temperature of 260°C.

The limit of detection (LOD) for both 4m- and 5m-BTZ isomers was determined to be 1-mg/L instrument response, or 10-µg/L method limit of detection. This determination was done by shooting standards to make a calibration curve and observing the concentration where peak area leveled out. Standard concentrations used were 500-µg/L, 800-µg/L, 1-mg/L, 2-mg/L, 3-mg/L, 4-mg/L, and 5-mg/L. Two injections of each standard were run to confirm consistency of resulting peaks. Average injections of the lowest three standards produced roughly the same peak area; examples are provided in Figures F9 and 10. The differences in response can be seen between these two figures compared to the 2-mg/L standard in Figure F11. The standards were plotted to visually examine the relationship between peak area and standard concentration.

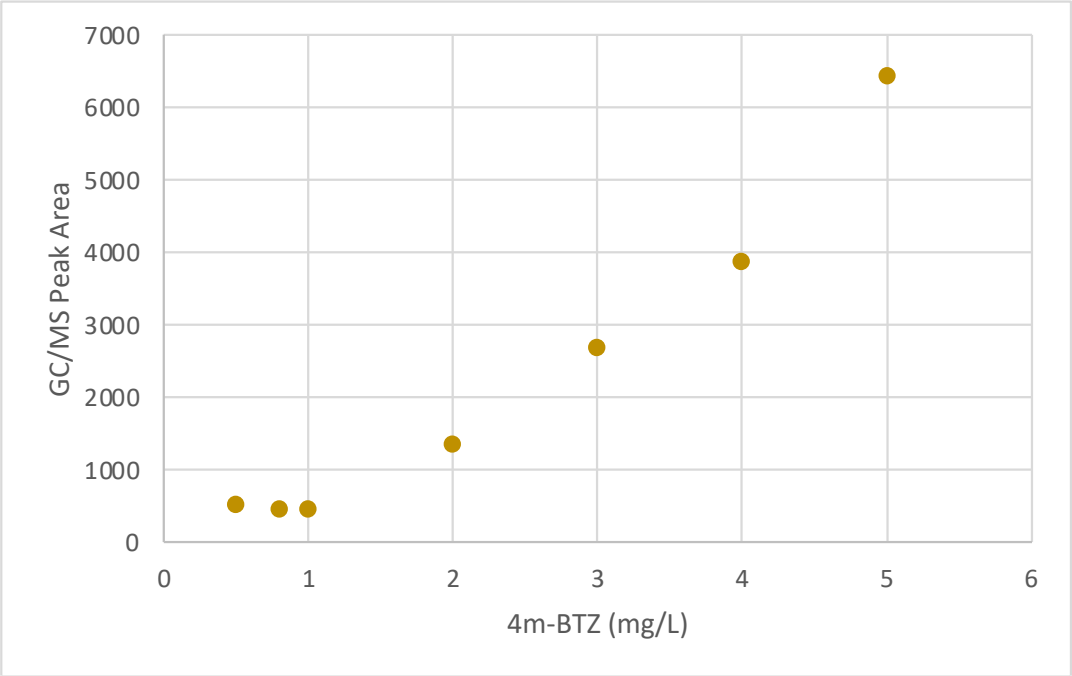


**Figure 10** Depicts the 4m-BTZ standards plotting GC/MS peak area vs standard concentration.

The plot for 5m-BTZ is provided in the Appendix F. For analysis purposes, the lowest points were removed from the plot to create linear calibration curves. These are provided as Figures F13 and 14. Select water samples were examined with duplicate injections on GC/MS to determine the ratio of 4m- and 5m-BTZ isomers in tested samples.

# Results and Discussion

*3. Samples analyzed on LC/MS*

*3.1. Blanks and controls*

Each set of samples was processed with an ASTM Type 1 water method blank. This method blank was filtered, acidified, and spiked at the same time as the real samples to check the process for contamination. An example of a typical method blank chromatogram is shown below in **Figure 11.**

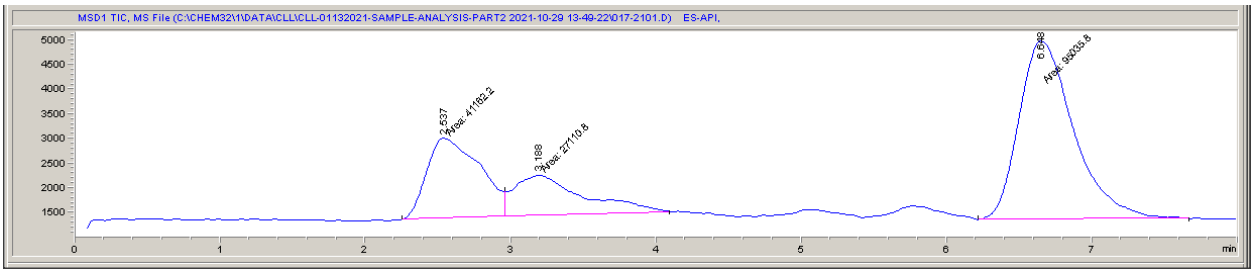


**Figure 11** depicts a typical LC/MS chromatogram for a method blank, sample 10272021-DI-BLK, which was processed with the sample set 01132021-R2.

The peak observed at retention time 6.6 minutes is the surrogate standard injected in each sample. The two peaks at the front of the chromatogram around retention times 2.5 and 3.2 minutes are unidentified**.** The peak around retention time 2.5 minutes persisted in all samples run on LC/MS other than a few methanol blank samples. The peak around retention time 3.2 minutes was seen only in samples that underwent the SPE process. Both were observed in previous studies.[23,26] Previous investigation considered contamination from multiple sources such as methanol, DCM, and HCl. None of the tests confirmed the identity of the contamination peaks.[26] An additional test in this

investigation involved pre-treating a cartridge with DCM and collecting the subsequent wash, then running a mock sample and collecting the eluted sample as described in the SOP. Both peaks persisted in this test, leaving the identity of the peaks unknown.

The Cowan Creek (CCJKR) sample site was used as a control site due to the surface water flow from this site originating upstream from the airpark. A typical chromatogram for a sample from this site is shown below in **Figure 12.**

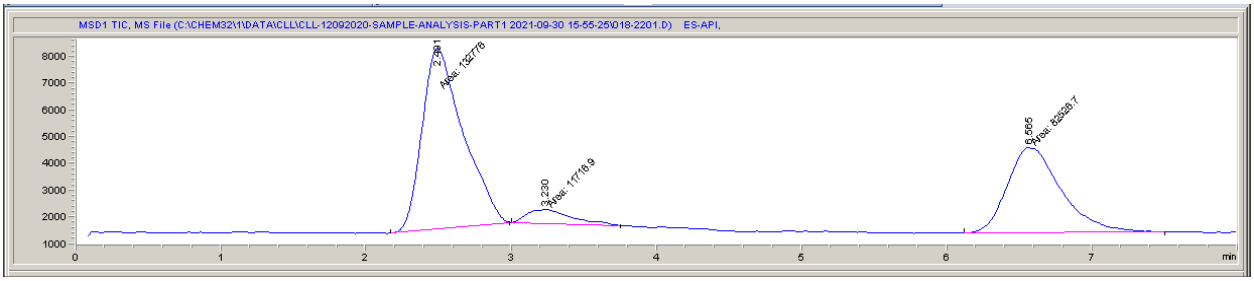


**Figure 12** depicts a typical LC/MS chromatogram for a Cowan Creek sample site, sample 12092020-CCJKR-R1-T1.

This chromatogram presents the two mystery peaks as well as a peak for the surrogate standard. No real analytes were detected in the Cowan Creek site samples, though a few chromatograms had small peaks suggesting negligible random contamination. The source could be from cross contamination in the lab processing steps. Because no real analytes were detected in the Cowan Creek site samples, it is determined that the benzotriazoles found in the two other sample sites were released as runoff from the airpark.

*3.1.2. Indian Run and Lytle Creek sites*

The two sites monitored for contamination downstream from the airpark treatment facilities were Indian Run (IRJKR) and Lytle Creek (LCFR). Both sites had detectable analytes on each sample day. Average concentrations, corrected for recovery, are provided below in **Table 10**.

**Table 10** Average concentrations for benzotriazoles at each sample site from LC/MS

| Sample Date | Sample Site | BTZ (µg/L) | TTZ (µg/L) |
|---|---|---|---|
| 12092020 | IRJKR | ND | 0.629±0.04 |
| | LCFR | 0.051±0.01 | 2.205±0.1 |
| 01132021 | IRJKR | ND | 0.346±0.03 |
| | LCFR | Trace, Below LOD | 1.700±0.04 |
| 02242021 | IRJKR | ND | 1.785±0.1 |
| | LCFR | 0.146±0.05 | 51.87±2 |
| 03042021 | IRJKR | ND | 0.924±0.08 |
| | LCFR | 0.097±0.004 | 16.26±0.7 |
| 06242021 | IRJKR | Trace, Below LOD | 0.982±0.03 |
| | LCFR | 0.158±0.03 | 8.476±0.3 |

*ND signifies analyte not detected, below LOD

** LOD for all analytes on LC/MS was 5 µg/L (Method LOD 0.05 µg/L)

The concentrations reported in **Table 10** were found by converting the instrument response as peak area to concentration in µg/L. Next the recovery of each sample based on DMBTZ surrogate standard instrument response was considered. For TTZ on the 12092021-R2 sample analysis, LCFR-R2-T3 can be converted as shown based on the TTZ calibration curve, Figure E2 in the Appendix, where y is peak area in µS*min and x is analyte concentration in µg/L:

$$y = 1718.3x - 3883.5;\ y = 302229.0\ \mu S*min$$
$$x = (302229.0 + 3883.5)/1718.3 = 178.1\ \mu g/L$$

Each instrument response had a concentration factor of 100:1 due to the SPE process of concentrating analytes from the original 100-mL aliquot down to dryness and rediluting the sample to 1.00 mL in methanol. Therefore, once the calibration curves were used to

convert peak areas to analyte concentrations in µg/L, the instrument concentration was divided by 100 to find the analyte concentration in the100-mL aliquot.

178.1 µg/L /100 = 1.781 µg/L

The concentrations were then corrected for recovery. Each 100-mL sample had 10 µL or 60 ng of 6.00 mg/L DMBTZ injected, which would warrant an instrument response of 60 µg/L or 0.60 µg/L after the concentration factor. The example for the sample in question is shown below:

DMBTZ µg/L: 52.77 µg/L /100= 0.5277 µg/L
0.5277 µg/L /0.60 µg/L = 0.8795 or 87.95% recovery

To find the final analyte concentration, samples were corrected by adjusting for the surrogate deviation from 0.60 µg/L.

Concentration = 1.781 + [1.781 x (1-0.8795)] = 2.000 µg/L

For the example shown, the replicate in question would be reported to contain 2.000 µg/L TTZ. The average of all six replicates was reported in Table 10. The IRJKR site did not produce any detectable BTZ. The LCFR sample site consistently yielded higher concentrations of TTZ than the IRJKR site.

Full tables including results for all analytes and surrogates along with recoveries can be found in Tables E1-5 in the Appendix. The range for recovery was 24.426-129.966%. Each end of the range proved to be outliers with justification noted in laboratory notes. Low recoveries occurred for one set of processed samples where analytes were blown to dryness with nitrogen according to the SPE SOP, but not re-

dissolved in methanol until the day of analysis the next day. This mistake likely caused loss of analytes resulting in low recoveries. The sample with a recovery of over 100% was accidentally spiked twice with surrogate standard at the beginning of processing. Most recovery percentages fell between high fifties and low seventies, with an average value of 63.730%.

The range of TTZ concentrations for the Indian Run site was 0.346-1.785 µg/L. Example chromatograms for the site are shown below in Figure 13.

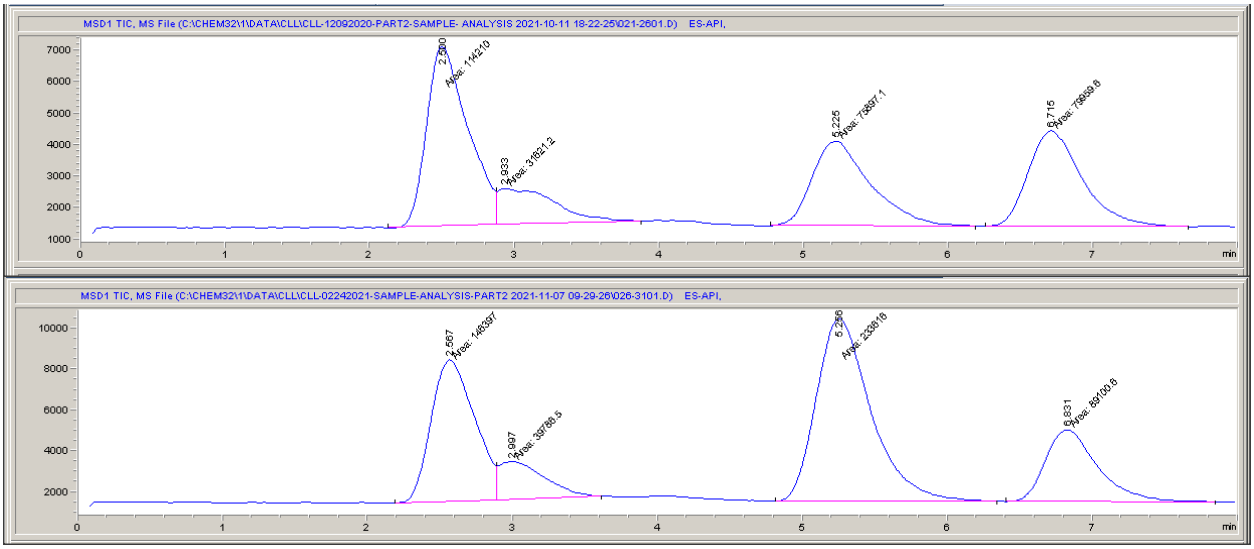


**Figure 13** Shows example LC/MS chromatograms from sample 12092020-IRJKR-R2-T1 (top) and 02242021-IRJKR-R2-T3 (bottom).

Both chromatograms exhibit peaks at similar retention times; the two mystery peaks followed by TTZ at 5.2 minutes and then DMBTZ at 6.7minutes. The noticeable differences in TTZ peak area indicate that more TTZ was observed on the February sample day than on the December one.

The Lytle Creek site consistently exhibited both BTZ and TTZ in detectable concentrations. The range for BTZ was 0.051-0.158 µg/L. The range for TTZ was 1.700-51.87 µg/L. The large range of sample concentrations throughout the season can be visualized by the chromatograms of LCFR sample sites below in **Figure 14.**

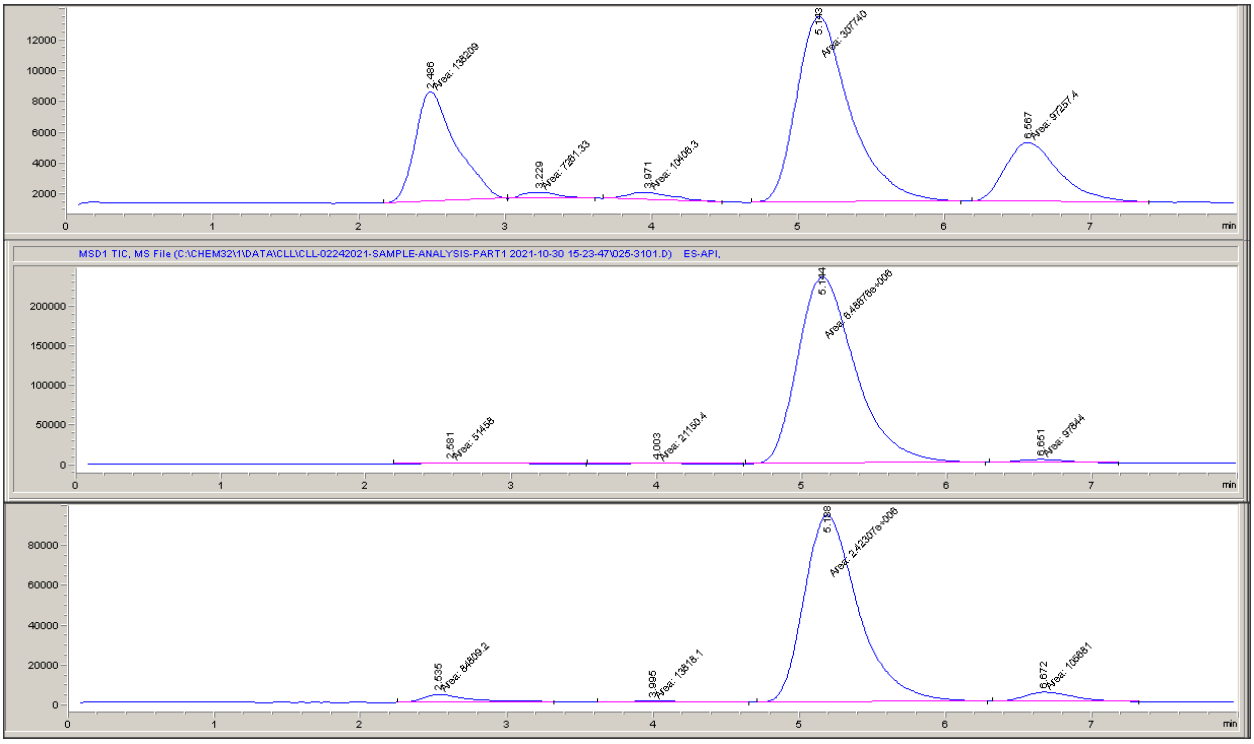


**Figure 14** Shows LC/MS chromatograms from LCFR on sample days 12092020-LCFR-R1-T2 (top), 02242021-LCFR-R1-T2 (middle), and 03042021-LCFR-R1-T3 (bottom).

All the anticipated peaks are present in each chromatogram. BTZ can be observed at retention time 3.9 minutes as a small peak in front of TTZ. While BTZ did not produce a large range of sample concentrations, TTZ is seen to dramatically increase from the December sample day to the two days later in the sample season. The DMBTZ surrogate standard peak is roughly the same peak area in each pictured chromatogram, but the significant increase in TTZ concentration diminishes the appearance of the surrogate peak. The increase in total analytes detected in LCFR samples compared to IRJKR samples suggests that more runoff from the airpark is directed to the LCFR site, or there is more airpark activity on the side near Lytle Creek.

An interesting observation for the 2021 season was a summer sample day on June 24th. It was expected that because the airpark is not likely to apply deicers to aircraft in

the summer, summer sample sites would be void of contamination or the observed concentrations would be comparably low. However, this sample day produced the highest BTZ average of 0.158 µg/L at LCFR. Additionally, both sites had notable TTZ concentrations of 0.982 and 8.476 µg/L for IRJKR and LCFR respectively. The presence of such high analyte concentrations over the summer reveals further questions about benzotriazole presence near the airpark. One possibility is that additional agents may be applied to aircraft over the summer which also contain benzotriazoles. Another possibility is that water used for processing runoff may already contain benzotriazoles. If groundwater is contaminated and being used for this purpose, BTZ and TTZ may be continuing to cycle through surface- and groundwater, becoming ubiquitous in the nearby water systems.

### *3.2. Weather and Water Quality*

Water quality data related to site conditions was gathered at each sample site using the YSI probe. Additional information was recorded about weather on sampling days. Data tables for each sample day can be found as Water Data Tables in Appendix A. Combining this data with data gathered from the National Weather Service provides insight into sample results. Temperature and precipitation data is recorded below in Table 11.

**Table 11** Temperature and precipitation data leading up to sample days[29]

| Sample Date | Day of Sampling | 1 Day Before | 2 Days Before | 3 Days Before | Month Average Temp | Month Total Precipitation (cm) |
|---|---|---|---|---|---|---|
| 12/9/2020 | Avg. Temp: 5.3 °C | Avg. Temp: 0.3 °C | Avg. Temp: -0.3 °C | Avg. Temp: 0.5 °C | 1.5°C | New Snow: 9.9 Other Precip: 5.1 |
| 1/13/2021 | Avg. Temp: 1.9 °C | Avg. Temp: -3.6 °C | Avg. Temp: -3.8°C | Avg. Temp: -1.3°C | 0.0°C | New Snow: 9.9 Other Precip: 7.1 |
| 2/24/2021 | Avg. Temp: 7.2 °C Snow Accumulated: 2.5 cm | Avg. Temp: 3.3°C Snow Accumulated: 10.2 cm | Avg. Temp: 2.5 °C Snow Accumulated: 15.2 cm | Avg. Temp: -5.6 °C Snow Accumulated: 20.3 cm | -3.8°C | New Snow: 47.8 Other Precip 7.1 |
| 3/4/2021 | Avg. Temp: 2.2°C | Avg. Temp: 5.6°C | Avg. Temp: 0.3°C | Avg. Temp: 4.4°C | 7.6°C | New Snow: 0.0 Other Precip 8.0 |
| 6/24/2021 | Avg. Temp: 21.6 °C | Avg. Temp: 17.5°C | Avg. Temp: 15.6°C | Avg. Temp: 22.8°C | 22.5°C | New Snow: 0.0 Other Precip 15.0 |

*Precipitation denoted as *precip*

The above data were gathered for the Wilmington area from the National Weather Service website. Previous work has revealed that temperature and precipitation are two of the most important environmental factors leading to an increase in benzotriazole release. Deicers are required to be applied when the ambient temperature reaches 10 °C, where there is potential for ground icing due to precipitation or there is potential for planes to accumulate ice upon accent. Heavy precipitation can wash contaminants out of treatment beds containing airpark runoff or off of surrounding land and into surface water. If there is too much precipitation, airpark runoff treatment beds can overflow, causing accidental release of untreated contaminants. Additionally, in the case of heavy snowfall, contaminants can be trapped in snow and ice and released as the snow melts.

No notable precipitation was observed on days leading up to any sampling days. However, as can be seen from the site images in Figures 4-6, snow was accumulated

during the February sample day. According to the National Weather Service, heavy snow began falling February 15th, up to 8.9 cm new snowfall a day and 20.3 cm accumulating. The temperature on February 15th was -8.3°C, with temperatures remaining below freezing until February 22nd. As can be seen from Table 11, the accumulated snow began melting as temperatures warmed up each day leading up to the sampling event on February 24, 2021.[29] This sample date revealed the highest TTZ concentration at LCFR of 51.87 µg/L. It is possible that more ADAFs were applied to aircraft over this time due to heavy precipitation and cold weather. Additionally, this heavy precipitation likely caused treatment beds to overflow releasing untreated runoff that the airpark cannot be held liable for.

The IRJKR site also exposed the highest TTZ concentration of February 24th of 1.785 µg/L. However, it is interesting that the LCFR site had a much more significant spike in analyte concentrations than the IRJKR site considering both sites were subject to the same weather conditions. The drastic range of TTZ of 1.700-51.87 µg/L detected at LCFR compared to the range of 0.346-1.700 µg/L suggests that other factors besides weather are responsible for the higher analyte concentrations at LCFR.

While temperature and precipitation data provide insight about likely ADAF application prior to sampling, water quality data provides information about samples and the sample site on the day of sampling. Water quality parameters monitored using the YSI Probe are summarized below in Table 12, with full data from each sample day in Tables A1-5 in the Appendix.

**Table 12** Range and average for water quality parameters at each site

| | CCJKR | | IRJKR | | LCFR | |
|---|---|---|---|---|---|---|
| Parameter | Range | Average | Range | Average | Range | Average |
| Ambient Temp °C | 1.1-18.6 | 3.0±1.8 | 0.9-18.4 | 4.4±2.6 | 1.7-19.6 | 3.7±2.5 |
| Water Temp °C | 0.2-17.6 | 3.4±3.1 | 1.4-16.5 | 4.4±3.0 | 1.6-17.7 | 4.5±3.4 |
| pH | 7.75-8.39 | 8.07±0.23 | 7.83-8.36 | 8.07±0.19 | 7.69-8.16 | 7.95±0.19 |
| DO (mg/L) | 8,12-14.88 | 13.76 ±0.82 | 7.63-14.10 | 12.35 ±1.48 | 5.65-12.45 | 10.97 ±1.27 |
| NH4+ | 0.08-0.24 | 0.12±0.07 | 0.16-0.60 | 0.27±0.19 | 0.53-6.41 | 2.16±2.44 |
| NH3 | 0 | 0 | 0-0.01 | 0.002±0.004 | 0.01-0.07 | 0.03±0.03 |
| SPC | 421.5-603.6 | 536.9±77.3 | 673.3-738.2 | 709.7±24.3 | 1153-2670 | 1826±604 |

The temperature and Dissolved Oxygen (DO) data for the June sample day was omitted from the average in the above table due to the values being drastically different than those from the winter sample months.

It is interesting to notice that across all three sample sites over the sampling season, LCFR appears to be the outlier. The average pH is slightly lower at LCFR than the other two sites. The DO is slightly lower at LCFR on each sample date, with the June reading of 5.65 mg/L being significantly low**.** The DO data recorded from the YSI probe on each sample day is presented below in **Figure 15.**

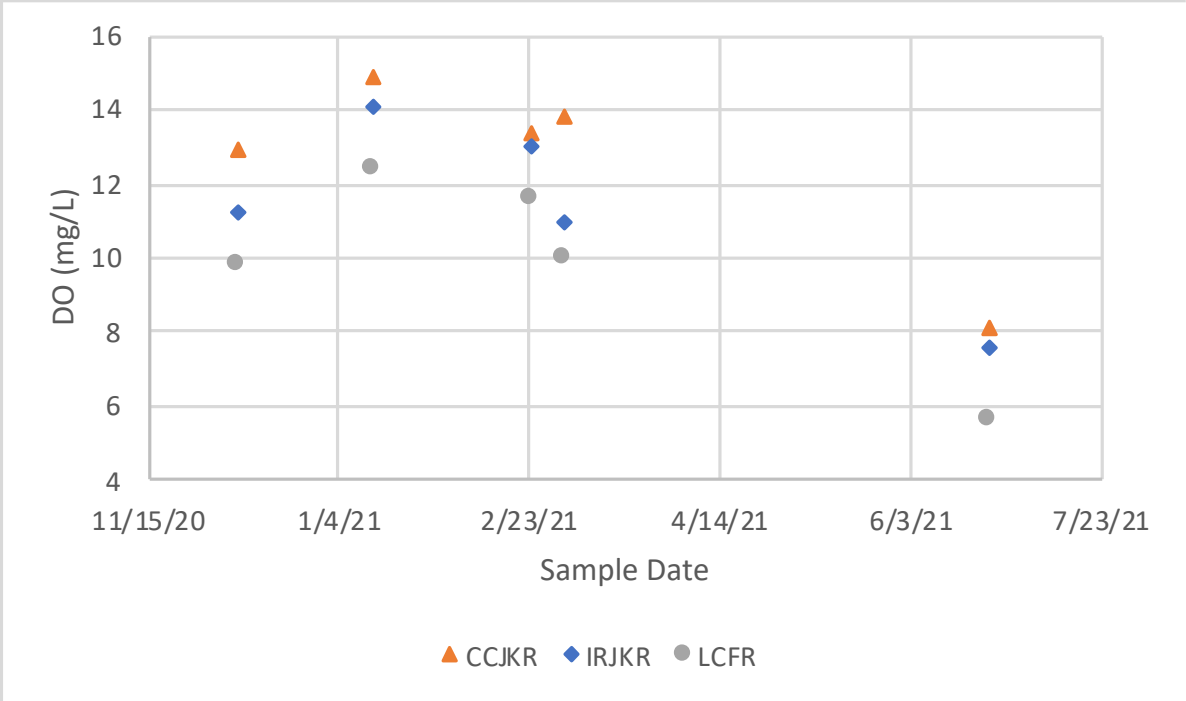


**Figure 15** Dissolved oxygen (DO, mg/L) data gathered from the YSI probe for each sample day in the 2021 sampling season.

DO is an important water quality parameter, as aquatic life requires oxygen to survive; a DO concentration below 4 mg/L is dangerous for aquatic life. Dissolved oxygen is closely correlated with temperature; colder water can contain more DO than warmer water. The above figure reveals that DO followed temperature patterns. The June sample date was significantly warmer than the winter sample days and the DO on this day is significantly lower. Additionally, the LCFR site consistently followed the trend of the other two sites but has a notably lower DO concentration. The presence of microbes in treatment biodegradation processes commonly require oxygen reducing the dissolved oxygen in water.

The average and range of ammonium recorded is highest at LCFR, and the highest value from each range was recorded on the February sample date. Ammonium acetate is a common deicer spread on surfaces, leaving ammonium to be detected in

treated water. Another significant difference is the specific conductance (SPC) readings for each site. Not only does LCFR have a higher average reading than the other two sites, but the range is significantly larger. The change in SPC over time for each site can be observed below in Figure 16.

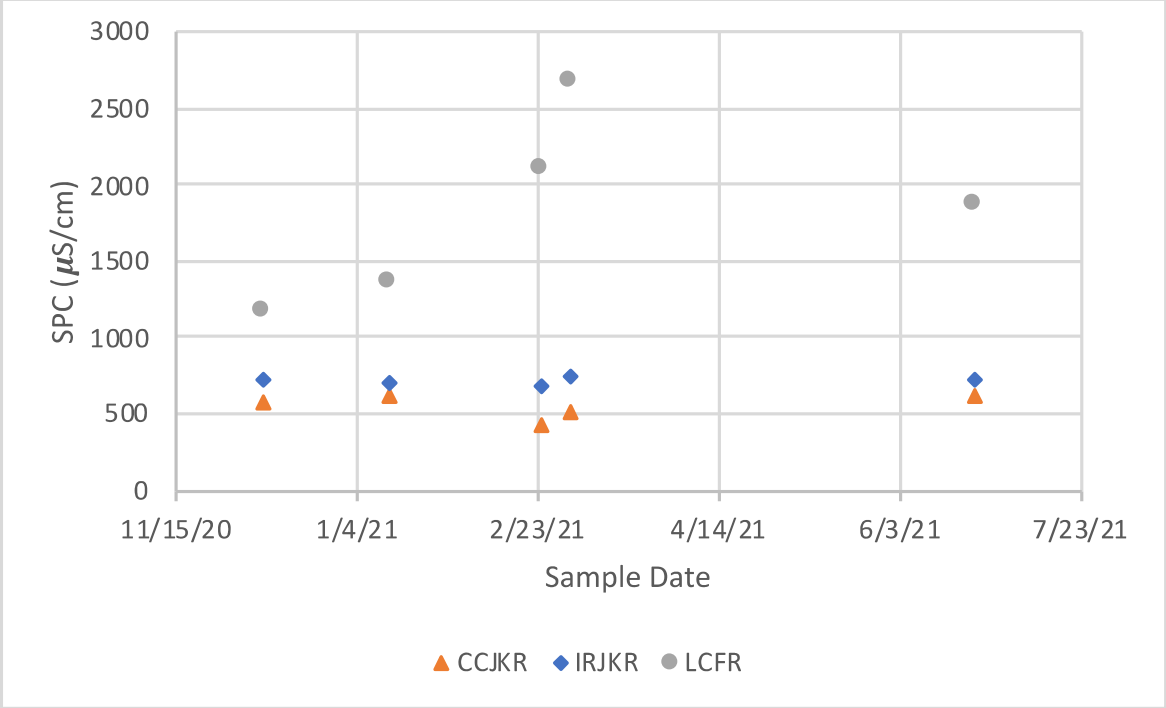


**Figure 16** Depicts SPC reading for all three Wilmington sample sites for the duration of the sampling season.

The SPC readings at LCFR change drastically overtime, while the other two sites remain comparatively consistent. High conductivity is indictive of the water treatment process. The presence of such high conductivity only at the Lytle Creek site reinforces the claim that a higher volume of treated water is directed to this site as opposed to Indian Run. If equal volumes of treated water were dispersed from beds near each site, it would be expected that Indian Run would also have a drastic fluctuation of SPC values, and both sites would differ significantly from the control Cowan Creek site, which does not receive treated water from the airpark.

3.3. *Comparison with past occurrences*

The 2021 sampling season was the third year of monitoring water quality and benzotriazole presence in Wilmington. The 2019 sampling season gathered data for February 2019, which set a baseline before the increase in airpark traffic due to the online retailer in June of that year. The 2020 season gathered data from November 2019-March 2020. Data from the 2021 season can be compared to these past two seasons to gain a clearer picture of how weather patterns and airpark use play a role in detection of benzotriazoles. Full data tables for the past two seasons are provide in Appendix G. The range of analytes detected during the consecutive seasons at each site are provided below in Table 13.

**Table 13**: Range of benzotriazoles for each sampling season

| Season | 2019 | | 2020 | | 2021 | |
|---|---|---|---|---|---|---|
| Site | BTZ (µg/L) | TTZ (µg/L) | BTZ (µg/L) | TTZ (µg/L) | BTZ (µg/L) | TTZ (µg/L) |
| IRJKR | ND | 0.111-0.869 | ND | 0.214-5.649 | ND | 0.346-1.785 |
| LCFR | Below LOD | 0.822-2.724 | 0.625-3.470 | 0.725-11.943 | 0.051-0.158 | 1.700-51.87 |

Based on the range of data from each season, it can be seen that LCFR consistently produced higher analyte concentrations than IRJKR. TTZ increased during each season, with the LCFR results for TTZ in the 2021 sampling season producing significantly higher concentrations than observed in past years. Plots were made for IRJKR and LCFR to visualize the TTZ concentration throughout each season, shown in **Figures 17 and 18**.

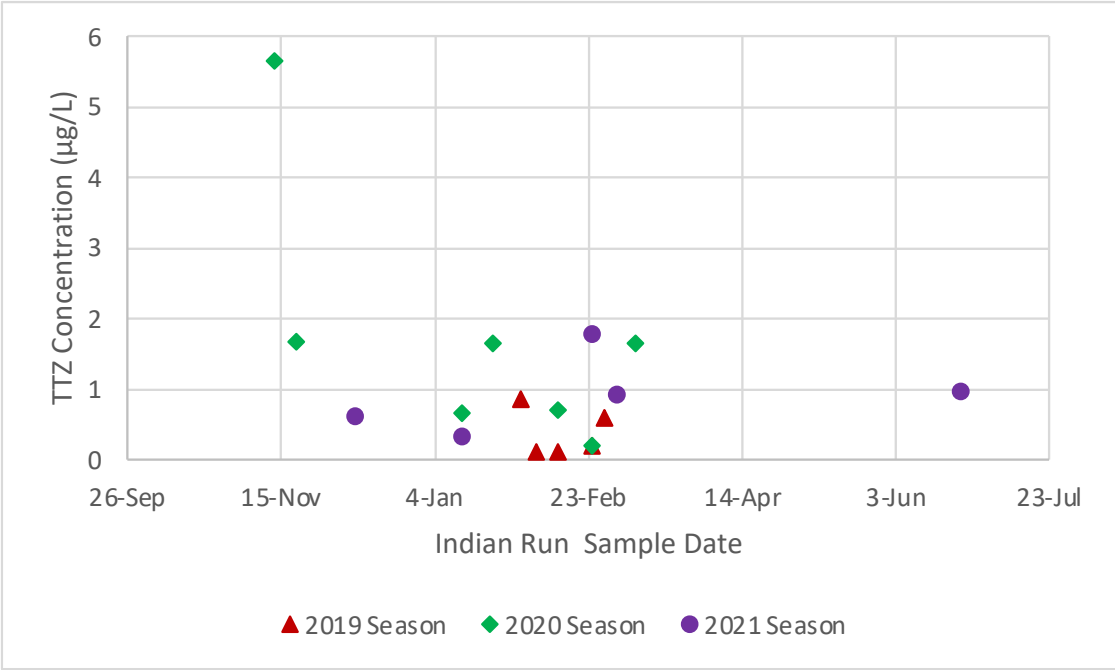


**Figure 17** Plots of TTZ concentration in µg/L for each sample day overlaid with all three seasons at Indian Run.

It can be seen from the plot of Indian Run TTZ results that aside from the sample on November 13, 2019, the 2020 and 2021 seasons have a similar range of values. Covering the same time frame of December-March, the 2020 Indian Run range was 0.214-1.679 µg/L (average 0.988 µg/L) and the 2021 range was 0.346-1.785 µg/L (average 0.921 µg/L). This observation shows that throughout the winter months, the Indian Run data was not severely impacted by the increase in airpark traffic from 2020-2021 seasons. These two seasons do have higher concentrations than the 2019 season, which had a range of 0.111-0.869 µg/L (average 0.378 µg/L). The 2019 sampling is a short snapshot of the entire winter season, but the given data indicates that there was a slight increase in TTZ detection at Indian Run after the online retailer established operation in June 2019.

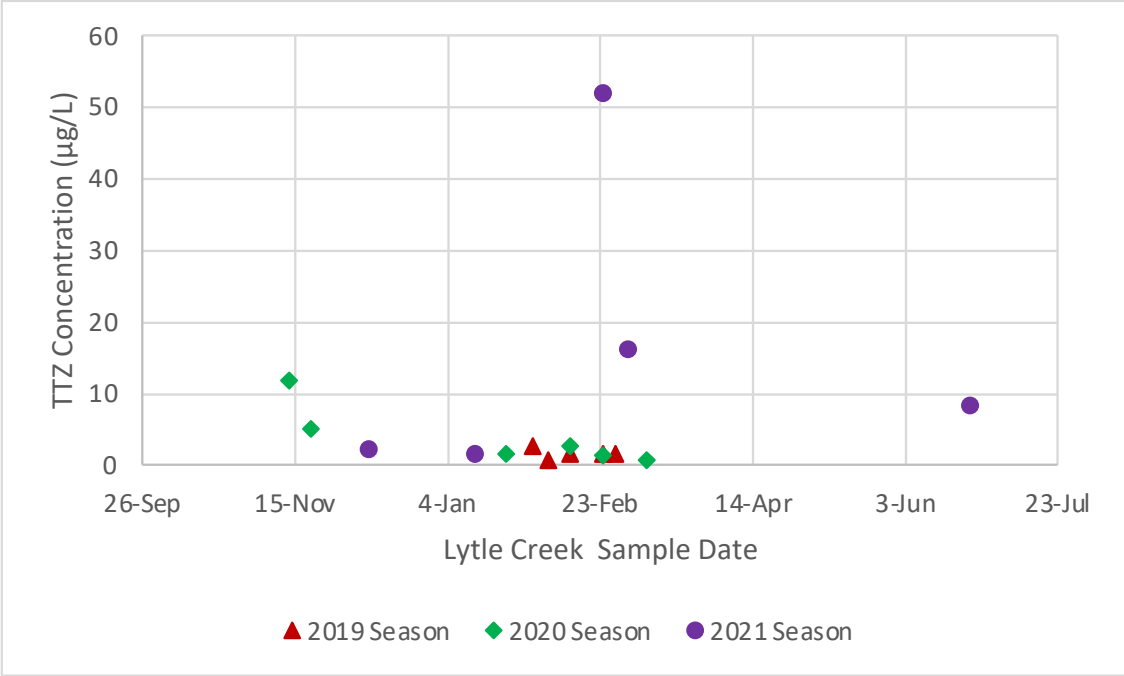


**Figure 18** Plot of TTZ concentration in µg/L for each sample day overlaid with all three seasons at Lytle Creek.

The plot of Lytle Creek data for each season revealed a similar trend that November 13, 2019 was significantly higher than the rest of the 2020 seasons data. The 2019 season found a baseline range of 0.822-2.724 µg/L TTZ (average 1.730). The 2020 season detected TTZ at 11.943 µg/L on November 13, with a range of 0.725-5.058 µg/L (average 2.206 µg/L) for the rest of the winter months sampled. The 2021 season had two days in a similar range with previous sample years (Dec 9 and Jan 13), with the other days sampled having significant variability in values. The highest TTZ detection of 51.87 µg/L was a 462% increase from the highest level detected in the 2020 season. These observations clearly indicate that TTZ detection significantly increased each year at Lytle Creek. However, while there was nearly a 300% increase in airpark traffic from the 2020 to the 2021 season, weather patterns of each year provide additional insight into the increase in analyte detection. A plot of Figures 17 and 18 overlayed for comparison is

available as Figure G1 in the appendix. Figure 19 presents BTZ data, which was only quantifiable at LCFR in 2020 and 2021 seasons.

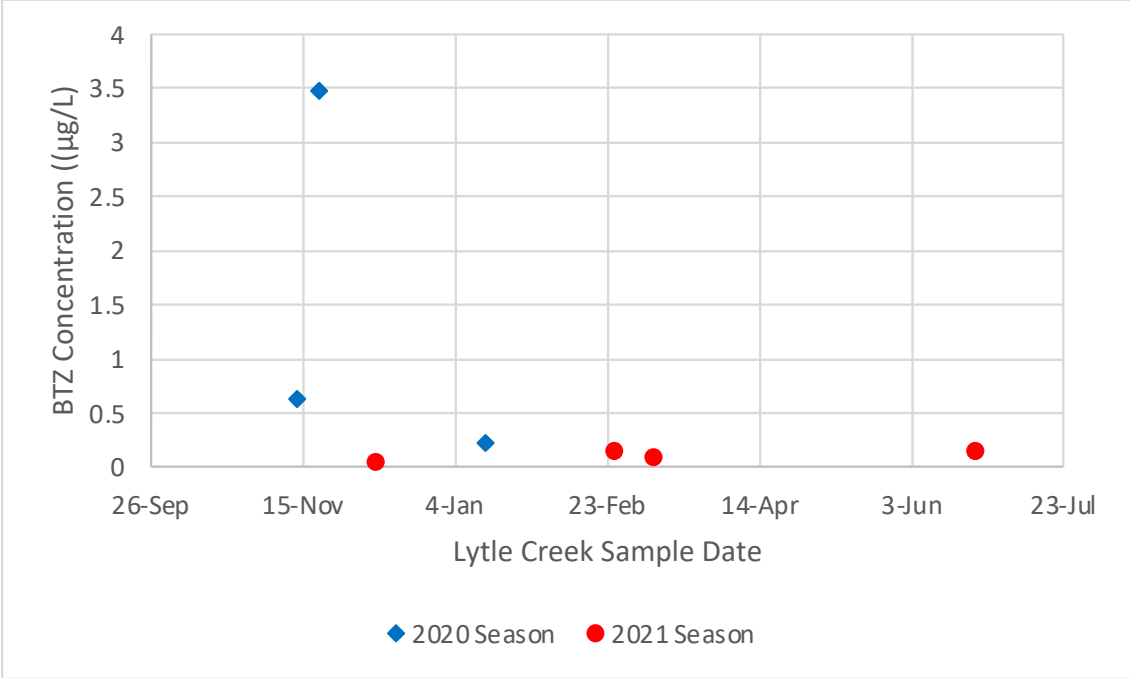


**Figure 19** Plot of quantifiable BTZ concentration in µg/L for each sample day overlaid with 2020 and 2021 seasons at Lytle Creek.

Each season only detected BTZ at the Lytle Creek site. BTZ was detected but not quantifiable in the 2019 season. The 2020 season did yield higher BTZ concentrations than the 2021 season, again observed in November 2019. BTZ was detected in all 2020 season LCFR samples, but only quantifiable in the two November days and the first January day. BTZ was quantifiable in all 2021 season samples except the January date. Therefore, even though detected concentrations are lower for the 2021 season, BTZ was more frequently detected in the 2021 season, with 4/5 sample days yielding BTZ levels above the LOD verses 3/7 sample days from the 2020 season.

The observation that the November 2019 samples produced significantly higher analyte concentrations than the rest of that season suggests that it is possible the time of year impacts the treatment process. It is possible that the microbes in the treatment

process need additional time to actively begin processing contaminants.[26] This theory cannot be verified with 2021 season data because sampling did not begin until December. Additional weather patterns should be considered to compare data that was gathered over the same time frame from year to year.

The 2019 and 2020 winter seasons were mild compared to the 2021 season. Both seasons followed the same trend, that the highest detected analyte concentrations fell on the coldest sample day periods. For the 2019 season, the highest TTZ concentrations of 2.724 µg/L (LCFR) and 0.869 µg/L (IRJKR) were found on February 1, 2019. The range of temperatures from three days prior up to the sample day was -16.7 – (-6.9) °C. The entire month of February 2019 experienced scattered precipitation, no more than 3.8 cm in a day (one instance). The 2020 season yielded the highest analyte concentrations in November 2019. The November 13, 2019 sample day had the highest TTZ detection with 11.943 µg/L (LCFR) and 5.649 µg/L (IRJKR). The range of temperatures from three days prior up to the sample day was -8.6 – 6.4 °C. There was a light snow leading up to the sampling event of roughly 0.46 cm of snow on November 10, which may have prompted additional use of ADAFs. Information regarding temperature and precipitation for the previous sample seasons is available in Appendix G.

While temperature was shown to correlate to highest analyte concentrations for the previous two seasons, the 2021 season yielded different results. The coldest sample day segment was that of January 13$^{th}$, ranging from -3.8 - 1.9 °C. However, the greatest TTZ concentration discovery occurred in February, when there was a dramatic snow melt event. This information reveals that significant precipitation is a leading factor in anticipating benzotriazole contamination. There were no significant snow events during

the 2019 and 2020 monitoring seasons compared to that in February 2021. Therefore, the most likely cause for the drastic increase in analyte detection for the 2021 season was the significant snow event that occurred in February.

The data gathered in this study can be compared to similar environmental occurrences. The table below provides environmental occurrences of benzotriazoles associated with ADAF application in runoff from various airports.

**Table 14** Wilmington work compared to other airpark runoff occurrences

| Study | Instrumentation | Location | Analytes |
|---|---|---|---|
| Kiss et al 2009[10] | GC/MS | Germany Frankfurt Intl Airport | BTZ: 72-472 ng/L<br>4m-BTZ: 25-148 ng/L<br>5m-BTZ: 25-80 ng/L |
| Griger et al 2006[7] | LC-MS/MS | Glatt Valley, Switzerland | BTZ: 0.16-2.68 µg/L<br>TTZ: 0.04-0.32 µg/L |
| **Weise 2019[23]** | **LC/MS** | **Wilmington, OH (USA) Wilmington Airpark** | **TTZ: 0.111-2.724µg/L** |
| **Raska 2020[26]** | **LC/MS** | **Wilmington, OH (USA) Wilmington Airpark** | **BTZ: 0.625-3.470 µg/L<br>TTZ: 0.214-11.943 µg/L** |
| **This Study 2021** | **LC/MS** | **Wilmington, OH (USA) Wilmington Airpark** | **BTZ: 0.051-0.158 µg/L<br>TTZ: 0.346-51.87 µg/L** |
| Sulej et al 2013[30] | GC/MS | Poland Intl Airport | BTZ: 29.1 µg/L<br>5m-BTZ: 89.3 µg/L |
| Corsi et al 2003[28] | GC/MS | Milwaukee, WI (USA) Gen Mitchell Intl Airport | 4m-BTZ: 1.67 mg/L<br>5m-BTZ: 2.16 mg/L |
| Olds et al 2021[31] | LC-MS/MS | Milwaukee, WI (USA) Gen Mitchell Intl Airport | 4m-BTZ: 1.80 mg/L<br>5m-BTZ: 3.00 mg/L |

While benzotriazoles have been detected worldwide in various sample mediums, these airpark runoff samples were focused in the US and Europe. Kiss et al monitored samples from two rivers near Frankfurt International Airport in Germany in March-June 2008. Their study calculated trace levels of all three benzotriazole analogues considering river mass flow. Due to large river water mass flow, roughly 50% of the study's samples were below their LOD of 8-12 ng/L. Based on samples quantified, their study recognized seasonal variation between frequency of BTZ and methylated isomers.[10] Griger et al

monitored treated airport effluent in Glatt Valley, Switzerland from November 2003-April 2004, considering BTZ and TTZ detection compared to known ADAF use. Their study concluded that 55% of benzotriazoles applied at the airpark were released and detected in water samples shortly after deicing occurred. The study theorized that the other 45% was retained longer in treatment processes, was degraded, or otherwise evaded detection.[7] Sulej et al considered runoff water samples from water near various Polish airports. The study did a heavy investigation f sample preparation for PAHs, glycols, and benzotriazoles. The study found the first two classes of analytes in all samples, and BTZ/TTZs in most samples. They conclude that more extensive study of the fate of CECs in airpark runoff is necessary for understanding environmental impact.[30] The studies by Corsi and Olds covered the same sample area in airports in Wisconsin, USA. Their studies noted a decrease in DO for treated water, similar to this study. The noted a change in benzotriazole detection from 2010-2013, suggesting that ADAFs were manufactured with reduced benzotriazoles.[28,31] The maximum data from this study was found to fall in the middle of the studies. This study did not take in to consideration mass of water flow as did some of the studies listed. It is interesting to note that the studies reporting separation of TTZ isomers commonly used GC/MS, with one study using an LC-MS/MS tandem system. These results provide support for utilization of GC/MS in the current investigation.

*3.4. Sediment sample results*

Select sediment samples were analyzed to check for presence of analytes. After sampling, sediment samples were frozen. A portion of frozen samples were freeze dried to remove water from samples before further processing. Table 15 below records masses of sediment samples before and after freeze drying.

**Table 15** Masses of sediment before and after freeze drying

| Sample ID | Wet Sediment Mass (g) | Dry Sediment Mass (g) | Percent Moisture (%) |
|---|---|---|---|
| CCJKR-C-BTZ | 45.888 | 35.567 | 22.492 |
| CCJKR-LBANK-BTZ | 40.867 | 32.037 | 21.607 |
| IRJKR-C-BTZ | 42.728 | 26.506 | 37.966 |
| LCFR-RBANK-BTZ | 36.142 | 25.463 | 29.547 |

*All samples from date 03042021

After the samples were freeze dried, portions were weighed for further processing. Freeze dried samples were mixed and sieved before being weighed. The CCJKR-C sample was notably rocky and sandy making it difficult to remove an adequate sediment sample. The CCJKR-LBANK sample was a heterogeneous mixture of sand, sediment, and small pebbles. The IRJKR sample was full of larger particulates, which were sieved out to reveal usable sediment sample. The LCFR-RBANK sample was comparably homogeneous fine clay-like sediment. Images of each sample are available in Appendix D. Dry, weighed sediment samples were processed using methanol extraction followed by modified SPE, then analyzed on LC/MS. A reagent grade sand blank (RGS-BLK) was used to test for contamination. The chromatogram for this sample is shown below in Figure 20.

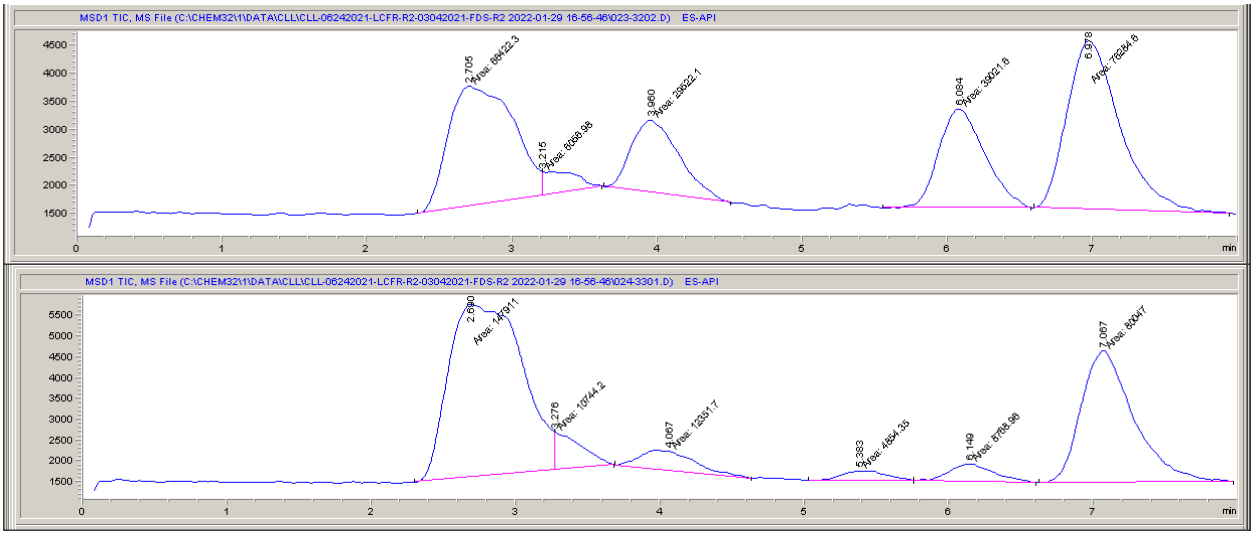


**Figure 20** LC/MS chromatograms of samples 12142021-RGS-BLK (top) and 03042021-CCJKR-C-FDS (bottom).

The two peaks from the water sample chromatograms can be observed in these samples. Additionally, BTZ and TTZ are both present in the sample. Similar peak areas of BTZ and TTZ were observed in the two samples from the control site, CCJKR as well. It is likely that a source of contamination impacted each sample, such as contamination in the methanol used to process samples. Similar peak areas of BTZ were observed in the IRJKR and LCFR samples as well, so it was determined that the observed BTZ was due to contamination and not a real analyte detection. However, both IRJKR and LCFR samples produced TTZ peak areas significantly higher than those of the blank/control sites. These chromatograms are provided below as Figure 21.

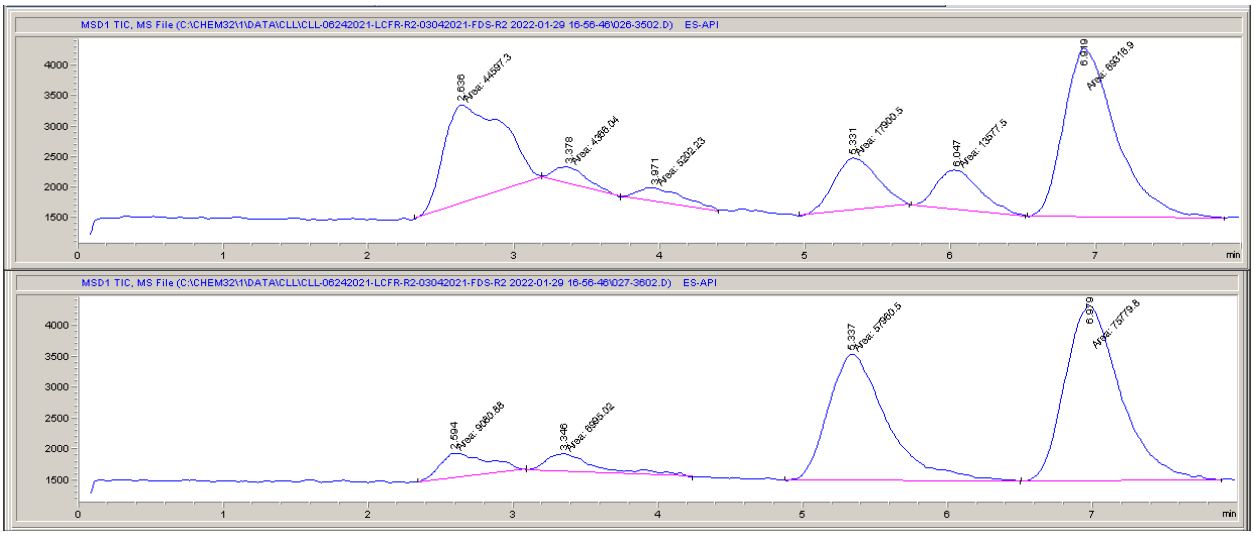


**Figure 21** LC/MS chromatograms of IRJKR-C-FDS (top) and LCFR-RBANK-FDS (bottom).

The previously observed peaks persist in the sediment sample chromatograms. Additionally, a new unidentified peak was observed around retention time 6.0 minutes. Small BTZ and TTZ peaks were observed as well. Due to the presence of these peaks in the method blank RGS-BLK sample, it was determined that the levels of BTZ and TTZ in the CCJKR samples are negligible. From the sediment analysis TTZ calibration curve, Figure D4 in the Appendix, the average concentration for these samples was -1.281 ±0.259 µg/L. To correct for this contamination, the average peak area of TTZ from these samples was subtracted from the TTZ peak area for IRJKR and LCFR samples. The resulting concentrations are shown below in Table 16.

**Table 16** Tolytriazole concentrations in sediment samples from LC/MS

| Sample ID | Dry Sediment Weighed (g) | TTZ from LC/MS (µg/L) | TTZ ng/ g (Dry Wt.) | µg TTZ/ L water in sediment |
|---|---|---|---|---|
| RSG-BLK-FDS | 1.0360 | 0 | - | - |
| CCJKR-C-FDS | 1.0376 | 0 | - | - |
| CCJKR-LBANK-FDS | 1.0917 | 0 | - | - |
| IRJKR-C-FDS | 1.0312 | 0.065 | 0.063 | 0.166 |
| LCFR-RBANK-FDS | 1.0069 | 0.461 | 0.458 | 1.550 |

*All samples from date 03042021

Concentrations for TTZ from the LC/MS were found according to the process outlined for water samples. From that calculation, the work to find ng/g dry wt. and µg TTZ/ L water in sediment is provided in Appendix D. These calculations were done to provide further clarity to the concentrations found from the LC/MS. Converting the LC/MS value found to ng/g allows the TTZ dry wt. concentration detected to be compared with other occurrences. The levels of TTZ discovered by Zhang et al were three orders of magnitude higher at 165 ng/g dry wt. 5m-BTZ in the sample from the US.[16] The calculation for µg TTZ/ L water was done to determine if the TTZ detected was definitely sorbed to the sediment sample. The TTZ concentrations in water samples for IRJKR and LCFR on March 4$^{th}$ were 0.986 and 17.417 µg/L respectively. Because the µg TTZ/ L water in sediment was significantly lower than the concentrations found in water, the presence of TTZ in the sediment samples is inconclusive.

*3.5. Select water samples on GC/MS*

The water sample analysis on LC/MS was a previously developed and optimized method for trace detection of benzotriazoles.[23,26] The separation of the 4m- and 5m-BTZ isomers was not possible using this previously developed method. However, the differences in boiling point of the two isomers makes GC/MS a possible option for

separation of the isomers. The 4m-BTZ isomer was observed to decompose around 195 °C and the 5m-BTZ isomer boils from 210-212 °C.[4] The GC/MS method developed was able to adequately separate the two isomers. An example water sample is shown below as Figure 22.

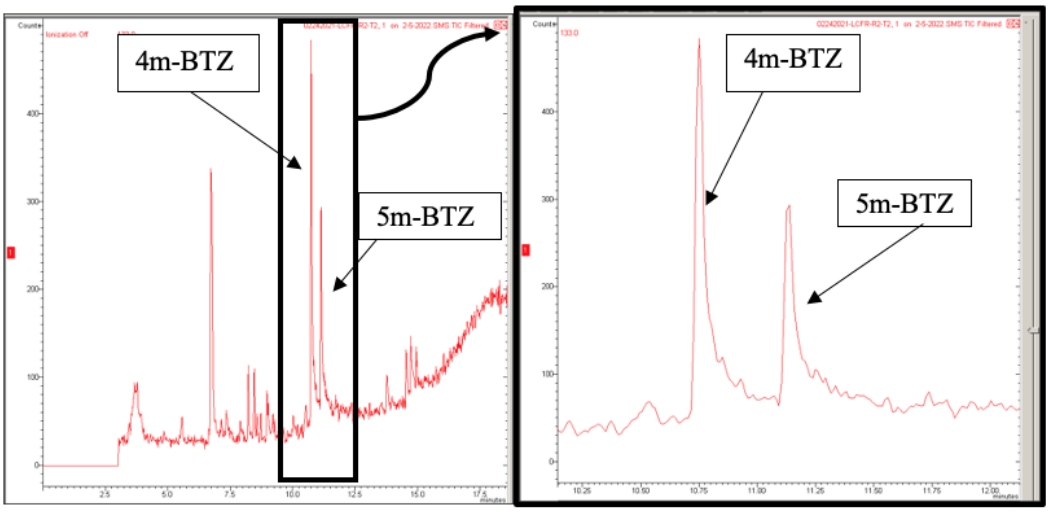


**Figure 22** GC/MS chromatogram of sample 02242021-LCFR-R2-T2 full spectrum (left) and zoomed (right).

The left side of Figure 22 depicts the full chromatogram of the sample, while the right side is zoomed to cover only the analytes of interest. The two peaks are clearly defined where 4m-BTZ comes out first and 5m-BTZ comes out second with average retention times of 10.75 and 11.13 respectively. The limit of detection for both isomers was determined to be 1-mg/L based on instrument response, with a method limit of detection of 10-µg/L. Select replicates from previous LC/MS water sample analyses were chosen that were expected to have a concentration above the determined detection limit of the GC/MS. The sample in Figure 22 is a replicate from the February sample day which yielded the highest average TTZ concentration. Other example chromatograms are provided in Appendix F. Concentrations recorded in Table 17 were found for these replicate samples based on the calibration curves in the Appendix Figures F13 and 14.

**Table 17** Concentrations of select water samples from GC/MS

| Sample ID | 4m-BTZ (µg/L, corrected) | 5m-BTZ (µg/L, corrected) | Recovery (from LC/MS, %) |
|---|---|---|---|
| 02242021-LCFR-R1-T1 | 24.77 | 33.20 | 68.89 |
| 02242021-LCFR-R2-T3 | 21.71 | 30.76 | 80.80 |
| 02242021-LCFR-R2-T2 | 28.19 | 37.73 | 78.27 |
| 03042021-LCFR-R1-T1 | 20.03 | 30.46 | 63.21 |
| 03042021-LCFR-R2-T3 | 17.40 | 25.45 | 75.80 |
| 03042021-LCFR-R2-T2 | 15.89 | 23.23 | 85.42 |
| 06242021-LCFR-R1-T3 | 29.48 | ND | 78.61 |
| 06242021-LCFR-R2-T2 | 31.70 | ND | 59.95 |
| 11132019-LCFR-R1-A | 16.42 | ND | 71.00 |
| 11132019-LCFR-R1-C | 31.76 | ND | 72.80 |

Both isomers were observed for the selected February and March samples. After separation of the two isomers was achieved, the next goal was to determine the ratio between the isomers. The percent of each isomer was found based on the total TTZ detected on the GC/MS, recorded in Table 18.

**Table 18** Percent ratios of each TTZ isomer in water samples from GC/MS

| Sample ID | Total TTZ (µg/L) | 4m-BTZ Percent Total | 5m-BTZ Percent Total TTZ |
|---|---|---|---|
| 02242021-LCFR-R1-T1 | 57.97 | 42.73 | 57.27 |
| 02242021-LCFR-R2-T3 | 52.47 | 41.38 | 58.62 |
| 02242021-LCFR-R2-T2 | 65.93 | 42.76 | 57.24 |
| 03042021-LCFR-R1-T1 | 50.48 | 39.67 | 60.33 |
| 03042021-LCFR-R2-T3 | 42.85 | 40.60 | 59.40 |
| 03042021-LCFR-R2-T2 | 39.11 | 40.62 | 59.38 |
| **Average** | **-** | **41.29** | **58.71** |

Both sets of replicates where two peaks where observed produced a comparable percent ratio, averaging 41.29 % 4m- and 58.71 % 5m-BTZ in the samples. Understanding the ratio of 4m- to 5m-BTZ can provide insight into toxicity and risk assessment of the concentrations detected on the LC/MS. Even though sample concentrations between the

two instruments are not exactly comparable, the discovery of a ratio of the isomers relative to each other provided insight to the LC/MS TTZ total concentrations discovered.

The June 2021 and November 2019 samples both yielded only the 4m-BTZ peak. The example chromatograms are provided below in Figure 23.

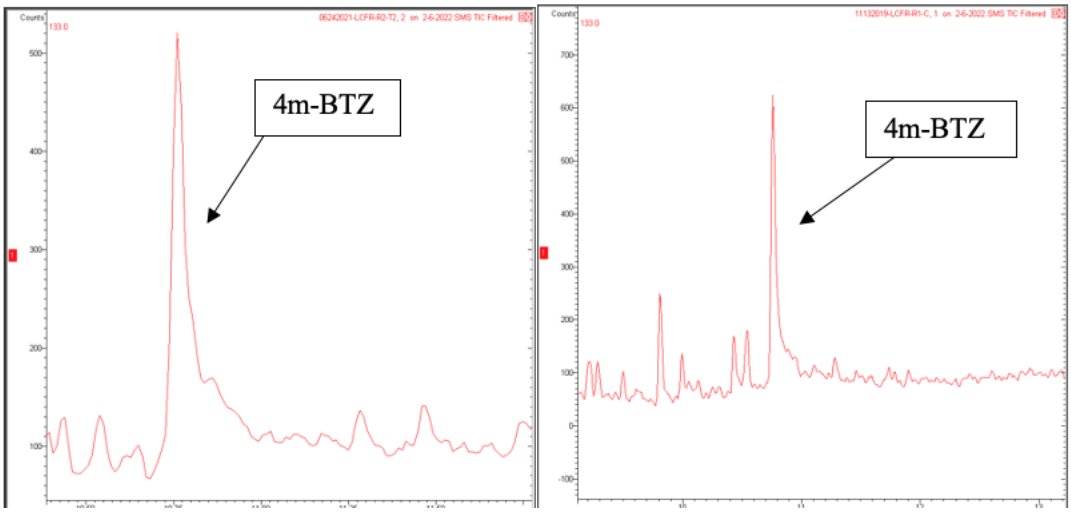


**Figure 23** GC/MS chromatograms of 06242021-R2-T2 (left) and 11132019-R1-C (right), both zoomed to consider the analytes of interest.

The reason for the absence of the 5m-BTZ peak in these two replicate sets is unknown. It may be possible that the 5m-BTZ peak is below the limit of detection and cannot be discerned from the background. However, even lower-level standards below the determined limit of detection produced discernable peaks. Another possibility is that the time of year the samples were gathered plays a role. The sample from 2021 was gathered in June while the other 2021 samples were gathered during the winter months. The agents applied to aircraft may have a different composition. Kiss et al reports that the 5m-BTZ isomer is more likely to biodegrade into other analogues, which were not considered in this study, while the 4m-BTZ isomer was said to be "recalcitrant".[10] Other sample day replicates from the 2021 season were tested with no observable TTZ peaks, but this response was expected due to the detection limitations for this analysis.

*3.6. Implications and future work*

The ability of the GC/MS to determine isomer ratios between 4m- and 5m-BTZ isomers allows environmental risk assessment based on hazard quotients (HQs) determine d by *Shi et al.*[18] The predicted no effect concentration (PNEC) for BTZ, 4m-BTZ, and 5m-BTZ were 15.80 µg/L, 21.00 µg/L, and 5.52 µg/L, respectively. The HQs for each sample day can be calculated as a ratio of these PNECs and the measured environmental concentration (MEC). The MEC was determined using the isomer percent ratios determined by the GC/MS data, which were 41.29% for 4m-BTZ and 58.71% for 5m-BTZ. These ratios were compared to the TTZ concentration from the LC/MS for the corresponding samples. The MEC for each isomer on the sample days run on GC/MS is provided in Table 19.

**Table 19** Ratio of TTZ isomers and HQs

| | Total TTZ (LC/MS, µg/L) | 4m-BTZ (µg/L) | HQ | 5m-BTZ (µg/L) | HQ |
|---|---|---|---|---|---|
| February 24 | 51.87 | 21.41 | 1.020 | 30.46 | 5.518 |
| March 4 | 16.26 | 6.714 | 0.320 | 9.546 | 1.729 |

Table 19 records total TTZ from the LC/MS and the corresponding concentration for each isomer based on the ratio percent from the GC/MS. From this data, it was determined that the February 2021 sample day was a high environmental risk event (HQ>1) and the March 2021 sample day was a medium risk (0.1<HQ<1) to high risk event. The June 2021 sample day is likely a medium to low risk event due to observation of 8.476 µg/L total TTZ from the LC/MS, and only a 4m-BTZ peak on the GC/MS. The other 2021 sample days were not high enough concentration to be analyzed on GC/MS.

Based on total TTZ for the LC/MS, other sample days of lower concentration are likely low environmental risk.

A continuation of this investigation could consider a few other avenues. Based on the discovery of benzotriazoles in November and June samples, further study could involve one monthly sample for a whole calendar year to monitor yearly occurrence. If access to a nearby well is possible, samples could be gathered from well water to determine if benzotriazoles are present in groundwater. Furthermore, data could be gathered to assess water mass flow of each site.

# Conclusion

The main goals of this investigation were to continue monitoring water quality and benzotriazole presence in the Little Miami Watershed, and to separate the two TTZ isomers using GC/MS for the purpose of assessing environmental risk. For the purposes of BTZ and TTZ analysis in samples from the Little Miami Watershed in Wilmington, use of SPE followed by LC/MS continues to be a practical technique. All water samples were able to be analyzed using LC/MS, due to the low limit of detection of 5-µg/L instrument response (corresponding to 0.05-µg/L method limit of detection).

No notable analytes were detected from the Cowan Creek control samples. Indian Run water samples consistently produced detectable TTZ ranging from 0.346-1.785-µg/L. Aside from one sample gathered in November 2019, the levels of TTZ discovered at Indian Run for the 2021 season fell in same range as the 2020 season. Both succeeding seasons yielded higher concentrations than the initial 2019 season. BTZ was never notably detected in Indian Run samples.

BTZ was detected in all Lytle Creek water samples, with a quantifiable range of 0.051-0.158-µg/L. These levels are lower than those detected for BTZ at LCFR in the 2020 season. TTZ was detected with a range of 1.700-51.87-µg/L at Lytle Creek. The range for TTZ was much larger at Lytle Creek than Indian Run, indicating that it is likely more treated water is diverted to the lagoons and treatment beds near this site. Two LCFR sample days produced concentrations that were significantly higher than those found in the 2020 season, where the highest concentration was 11.943-µg/L. The highest analyte

concentrations detected in the 2020 season were from November sample days. A possible explanation for this observation is that the water treatment process was not able to effectively treat benzotriazoles so early in the colder months. It is unknown if the 2021 season could have yielded similar or higher results for a November sample day because sampling began in December 2020 for the 2021 season. The highest concentration for the 2021 season was detected in February.

Weather conditions leading up to sample days and water quality conditions the day of sampling provided insight into why certain days and sites revealed more analytes than others. Temperature and precipitation data from the National Weather Service revealed that snow continued to fall and accumulate a week and a half leading up to the February 2021 sampling event. It is likely that the increase in precipitation caused treatment lagoons to overflow releasing untreated water for which the airpark cannot be held liable. Additionally, water soluble analytes can be retained in snow and released to waterways as the snow melts. A notable snow melt was occurring during the February sampling day. However, such high TTZ concentrations at LCFR compared to significantly lower IRJKR results suggest more airpark activity on the Lytle Creek side leading to more runoff in treatment beds near Lytle Creek, releasing more treated runoff to this stream. When considering water quality from the YSI meter, low DO, high ammonium and high SPC readings at LCFR confirm this theory.

Sediment samples from March were processed using alcohol extraction and modified SPE before analysis on LC/MS. TTZ was detected in the samples from Indian Run and Lytle Creek, 0.063 and 0.461 ng/g at each respective site. However, because the µg TTZ/ L water in sediment was low compared to TTZ found in water samples on that

day, the results are inconclusive for whether or not TTZ is sorbed to the sediment. More samples could be taken and tested again for follow up work. However, due to the extensive time required for sediment processing compared to the low concentrations found, it may not be worth time and resources to focus on TTZ in sediment at these Wilmington sites.

A GC/MS method was able to effectively separate the 4m- and 5m-BTZ isomers, however the limit of detection was determined to be 1-mg/L instrument response for each analogue (10-µg/L method limit of detection). Therefore, only select water samples from LCFR with higher TTZ concentrations were able to be run on GC/MS. Based on GC/MS information from February and March 2021 water samples, the ratio between the two isomers was determined to be 41.29% 4m-BTZ and 58.71% 5m-BTZ. Based on this ratio, Hazard Quotients (HQ) derived from data by Shi et al were used to assess environmental risk. While most Wilmington water samples present low risk benzotriazole concentrations, the March sample day was medium to high risk and the February day was classified as high environmental risk. These medium- and high-risk events are likely to contribute to significant impact on aquatic life at LCFR over time.

Overall, it was observed that each sampling season discovered more TTZ than the last, and BTZ was able to be reliably detected as well. While airpark traffic plays a role in increased analyte presence, heavy precipitation events are a leading factor for anticipating benzotriazole contamination risk. While GC/MS was a useful tool for identifying isomer ratios, LC/MS remains the more practical method for the majority of water samples from the sites examined. Future year-around monitoring could be helpful to assess environmental risk moving forward. Well water samples could provide information into

whether or not analytes have reached groundwater. While a few medium- and high risk events were recorded, the majority of data from the past three years of monitoring represents low environmental risk for the sample day. According to these observations, the airpark is in compliance with their Ohio EPA Permit 1II00031 based on allowed effluent COD requirements. However, little research is available for the impact of benzotriazole exposure at low levels over the course of months to years. This long-term exposure could be a leading cause for the decline in water quality in Lytle Creek and Indian Run. Intentional use of vegetation for contaminant uptake may be a possible route to lower contamination risk.

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# Appendices

## Appendix A Water Sampling

Standard Operating Procedure
WILMINGTON AIR PARK RUNOFF WATER SAMPLING PLAN
January 23, 2021
Audrey McGowin, PhD
Jessica Wiese
Lee A. Raska

## 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 (BTZ) compounds and their analogs are polar and thermally labile. In addition, BTZ are toxic to certain aquatic organisms, and have the potential for impacting the health of creeks, rivers, and ground water reservoirs in which BTZ and BTZ 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. Cell phone
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 – BTri – Sample Replicate Number (if needed)– Analysis Replicate Number (if needed)

For example:
012320 – LCFR – BTri – R1

**G. SAMPLING SITES**

Sampling sites are listed in the following table. Indian Run Site 1 and Site 2 are both 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 both Indian Run Sites to be the control sampling site.

| Sample Site Name | Coordinates | Site Description |
|---|---|---|
| Cowan Creek (CCJKR) | 39.407615, -83.798064 | Sample next to bridge on Jenkins Road crossing Cowan Creek |
| Indian Run Site 1 (IRJKR1) | 39.411386, -83.795392 | Sample after crossing field, downstream from treatment facility on Jenkins Road |
| Indian Run Site 2 (IRJKR2) | 39.408914, -83.799194 | Sample after going through wooded area next to Cowan Creek and crossing field, downstream from treatment facility on Jenkins Road |
| Lytle Creek (LCFR) | 39.437051, -83.797386 | Downstream and across the road from treatment facility, Lytle Creek right off Fife Road. Sample next to large pipe |

**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 Temp. (°C) | | | |
| Water Temp. (°C) | | | |
| pH | | | |
| DO (%) | | | |
| DO (mg/L) | | | |
| $NH_4^+$ (mg/L) | | | |
| $NH_3$ (mg/L) | | | |
| Conductivity (µS/cm) | | | |
| Pressure (mmHg) | | | |
| Observations | | | |

**Water Data Table**

Date: 12/9/2020

Personnel: Clara Leedy, Lee Raska, Travis Luncan

| **Sample Site** | **LCFR** | **IRJKR** | **CCJKR** |
|---|---|---|---|
| Time | 9:43 | 10:15 | 10:30 |
| Ambient T (°C) | 2.8 | 4.2 | 4.1 |
| Water T (°C) | 4.6 | 4.8 | 2.8 |
| pH | 7.84 | 8.10 | 8.13 |
| DO (%) | 79.9 | 91.5 | 99.6 |
| DO (mg/L) | 9.86 | 11.24 | 12.95 |
| $NH_4^+$-[N] | 0.80 | 0.19 | 0.08 |
| $NH_3$-[N] | 0.01 | 0.00 | 0.00 |
| Conductivity (µS) | NA | NA | NA |
| Specific Conductance (µS) | 1153 | 719.9 | 558.7 |
| Pressure (mmHg) | 730.9 | 731.5 | 731.5 |
| Observations | Lots of moss<br>Clear water<br>Some flow | Clear<br>Calm | Water clear, calm<br>Light flow |

**Water Data Table**

Date: 1/13/2021

Personnel: Clara Leedy, Lee Raska, Travis Luncan

| **Sample Site** | **LCFR** | **IRJKR** | **CCJKR** |
|---|---|---|---|
| Time | 10:20 | 10:45 | 11:00 |
| Ambient T (°C) | 1.7 | 0.9 | 1.14 |
| Water T (°C) | 1.6 | 1.4 | 0.2 |
| pH | 8.11 | 8.36 | 8.39 |
| DO (%) | 92.9 | 103.6 | 105.8 |
| DO (mg/L) | 12.45 | 14.10 | 14.88 |
| $NH_4^+$-[N] | 0.53 | 0.16 | 0.10 |
| $NH_3$-[N] | 0.01 | 0.00 | 0.00 |
| Conductivity (µS) | 750 | 384.4 | 317.2 |
| Specific Conductance (µS) | 1355 | 701.1 | 603.6 |
| Pressure (mmHg) | 734.2 | 735.2 | 735.1 |
| Observations | Sunny<br>Clear | Sunny<br>Clear<br>Slight haze in water | Small fish<br>Good flow<br>Clear |

**Water Data Table**

Date: 2/24/2021

Personnel: Clara Leedy, Lee Raska, Travis Luncan

| **Sample Site** | **LCFR** | **IRJKR** | **CCJKR** |
|---|---|---|---|
| Time | 8:55 | 9:30 | 9:20 |
| Ambient T (°C) | 2.8 | 5.3 | 5.7 |
| Water T (°C) | 2.4 | 3.0 | 2.8 |
| pH | 7.96 | 7.83 | 7.75 |
| DO (%) | 89.2 | 101.0 | 104.6 |
| DO (mg/L) | 11.61 | 13.05 | 13.40 |
| $NH_4^+$-[N] | 6.41 | 0.60 | 0.24 |
| $NH_3$-[N] | 0.07 | 0.00 | 0.00 |
| Conductivity (µS) | 1195 | 390.4 | 243.6 |
| Specific Conductance (µS) | 2099 | 673.3 | 421.5 |
| Pressure (mmHg) | 730.8 | 731.2 | 731.3 |
| Observations | Water higher than usual<br>Snow melting<br>Steady flow<br>Clear water | High, murky water<br>Slow, steady flow | High, murky water<br>Heavy flow |

**Water Data Table**

Date: 3/4/2021

Personnel: Clara Leedy, Travis Luncan, Audrey McGowin

| **Sample Site** | **LCFR** | **IRJKR** | **CCJKR** |
|---|---|---|---|
| Time | 3:34 | 2:55 | 2:33 |
| Ambient T (°C) | 7.3 | 7.2 | 5.2 |
| Water T (°C) | 9.2 | 8.4 | 7.6 |
| pH | 8.16 | 8.06 | 8.10 |
| DO (%) | 89.9 | 97.3 | 118 |
| DO (mg/L) | 9.96 | 11.0 | 13.8 |
| $NH_4^+$-[N] | 2.04 | 0.22 | 0.10 |
| $NH_3$-[N] | 0.05 | 0.00 | 0.00 |
| Conductivity (µS) | 1878 | 522.0 | 332.4 |
| Specific Conductance (µS) | 2670 | **738.2** | 498.7 |
| Pressure (mmHg) | 737.7 | 738.2 | 738.3 |
| Observations | Clear<br>Moderate flow | Murky<br>Moderate flow | Murky<br>Moderate flow |

**Water Data Table**

Date: 6/24/2021
Personnel: Clara Leedy, Travis Luncan

| **Sample Site** | **LCFR** | **IRJKR** | **CCJKR** |
|---|---|---|---|
| Time | 9:50 | 10:31 | 10:59 |
| Ambient T (°C) | 19.6 | 18.4 | 18.6 |
| Water T (°C) | 17.7 | 16.5 | 17.6 |
| pH | 7.69 | 7.99 | 8.00 |
| DO (%) | 61.4 | 79.7 | 87.7 |
| DO (mg/L) | 5.65 | 7.63 | 8.12 |
| $NH_4^+$-[N] | 1.03 | 0.18 | 0.09 |
| $NH_3$-[N] | 0.02 | 0.01 | 0 |
| Conductivity (µS) | 1423 | 599 | 518 |
| Specific Conductance (µS) | 1853 | 716 | 602 |
| Pressure (mmHg) | 738.5 | 739.2 | 738.9 |
| Observations | Clear Colorless | Clear Colorless No aquatic life observed | |

# Appendix B Sediment Sampling

## Sediment Sampling Protocol

March 4, 2022

Audrey McGowin, PhD
Clara Leedy
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 (BTZ) compounds and their analogs are polar and thermally labile. In addition, BTZ are toxic to certain aquatic organisms, and have the potential for impacting the health of creeks, rivers, and ground water reservoirs in which BTZ and BTZ analogs are deposited. The procedures outlined in this protocol were created for the collection of surface sediment samples near Wilmington Air Park.

## B. SUMMARY OF METHOD

The purpose of this protocol is to establish a procedure for
the collection of surface sediment 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 protocol.
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 60-mL glass jars with lids
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
9. Plastic spoons or shovel for sample collection
10. YSI Multi-meter, pre-calibrated in the lab; DO, temperature, conductivity, pH
11. Waste containers (trash bag and waste bottle)
12. Cell phone
13. Clean gloves for each site
14. 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 -Site Location– BTZ – Sample Replicate Number (if needed)– Analysis Replicate Number (if needed). Site location is determined as the side of bank descending compared to downstream water flow.

For example:
012320 – LCFR-LBank– BTZ – R1

**G. SAMPLING SITES**

Sampling sites are listed in the following table. Indian Run Site 1 and Site 2 are both 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 both Indian Run Sites to be the control sampling site.

| Sample Site Name | Coordinates | Site Description |
|---|---|---|
| Cowan Creek (CCJKR) | 39.407615, -83.798064 | Sample next to bridge on Jenkins Road crossing Cowan Creek |
| Indian Run Site 2 (IRJKR2) | 39.408914, -83.799194 | Sample after going through wooded area next to Cowan Creek and crossing field, downstream from treatment facility on Jenkins Road |
| Lytle Creek (LCFR) | 39.437051, -83.797386 | Downstream and across the road from treatment facility, Lytle Creek right off Fife Road. Sample next to large pipe |

**H. SAMPLE COLLECTION PROCEDURE**

10. Before going to sampling sites, clean and label sample containers and assemble sampling materials according to this protocol. Jars and spoons were soaked in ASTM Type 1 water for 24 hours and dried prior to sampling.

11. 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.

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

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

14. Use a fresh, clean plastic spoon or cleaned shovel to collect surface sediment at the center and bank of each stream site.

15. 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.
16. Proceed to the next sampling site making sure to collect any waste. Check to be sure the GPS coordinates match. Collect all sediment 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.
17. Return samples to the laboratory upon completion of sampling. Immediately place the samples into the freezer.
18. 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**
Sediment Sampling Form

**APPENDIX D - Standard Sampling Form**



## Ohio EPA Sediment Data Collection Sheet

Project:_____

Collection Date:_____ Collection Time:_____

Collector(s):_____

Weather Conditions: _____

**Sample Location Description (Provide Diagram of Sampling Location(s) on opposite Side) :**

Waterbody Name: _____ River Mile Location: _____

Latitude: _____ Longitude: _____

Sample Site Description: _____

**Ambient Site Information (water):**

Conductivity _____ Dissolved Oxygen _____ pH _____

Temperature _____ Current Velocity _____

**Sediment Collection Information:**

Water Depth Above Sample: _____ Sediment Sample Depth: _____

Collection Device: Scoop _____ Eckman Dredge _____ Corer _____ Other _____

Sample Type: Grab _____ Composite: _____

Sample Replicate Collected? YES or NO Sample Duplicate Collected? YES or NO

Replicate ID/Name: _____ Duplicate ID/Name: _____

**Sample Information:**

Sediment pH (undisturbed) _____ Sediment pH (post-homogenization) _____

Color (Munsell Soil Color Chart Number): _____

Texture (particle size description): _____

Odor: _____

Additional Comments: _____

**Sand -** Particles 0.06-2.0 mm in diameter, possessing a gritty texture when rubbed between fingers. Loose materials (not cohesive) that often cannot be molded into shapes (non-plastic).

**Silt -** Particles 0.004-0.06 mm in diameter, generally fine material possessing a greasy or smooth, talc-like feel when rubbed between fingers. Non-plastic and not cohesive.

**Clay -** Particles less than 0.004 mm in diameter, which forms a dense, gummy surface that is difficult to penetrate with tools (hardpan). Clay is both plastic and cohesive.

**Marl -** Calcium carbonate, usually greyish-white, often containing fragments of mollusc shells.

**Detritus -** Dead, unconsolidated organic material including sticks, wood, leaves, and other partially decayed coarse plant material.

**Peat -** Partially decomposed plant materials characterized by an acidic pH; parts of plants such as Sphagnum moss sometimes visible.

**Muck -** Black, extremely fine, flocculant material composed of completely decomposed organic material (excluding sewage).

**Sludge -** Organic matter that is decidedly of human or animal origin.

**Figure B1 Travis Luncan gathering sediment samples at Cowan Creek on March 4, 2021.**

**Figure B2 Dr. Audrey McGowin gathering sediment samples at Cowan Creek on March 4, 2021.**

Appendix C Water Sampling Process (SPE)

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.53
4. Spike each replicate with 54.0 ng (10 μL of a 5.0 ppm solution) of 5,6-dimethylBTri 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 D Sediment Sample Processing
Sediment Processing Protocol

# ISOLATION OF BENZOTRIAZOLE AND ANALOG COMPOUNDS IN SEDIMENT SAMPLES VIA SOLID-PHASE EXTRACTION

December 13, 2021

Audrey McGowin, PhD
Clara Leedy
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 (BTZ) compounds and their analogs are polar and thermally labile. In addition, BTZs are toxic to certain aquatic organisms, and have the potential for impacting the health of creeks, rivers, and ground water reservoirs in which BTZ and BTZ analogues are deposited. The procedures outlined in this protocol were created for the solid-phase extraction of surface sediment samples collected near Wilmington Air Park.

**B. SUMMARY OF METHOD**

The purpose of this Standard protocol is to establish a procedure for the solid-phase extraction of surface sediment samples collected near Wilmington Air Park in order to determine the presence of 1H-benzotriazoles, tolytriazoles, and comparable analogs in sediment as a result of the airport's wastewater treatment plants.

**C. HEALTH AND SAFETY**

The analyst must assume that all sediment 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 protocol.

**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. 12-port vacuum extraction manifold
    d. 15-mL centrifuge tubes for eluate collection
    e. Tank of nitrogen gas
    f. Centrifuge
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 – Site Location– BTZ – Sample Replicate Number (if needed)– Analysis Replicate Number (if needed)-FSD (Freeze Dried Sediment)
For example: 03042021 – LCFR –C – BTZ – R1-FSD

**G. ALCHOHOL EXTRACTION PROCEDURE**

1. Weigh approximately 1 g of freeze-dried sediment sample into a clean, labeled centrifuge tube.
2. Spike each sample tube with 10 µL 6.00 DMBTZ surrogate standard (60 ng) and allow sample to equilibrate for 1 hour.
3. Add 5.0 mL methanol to the sample tube. Hand-shake the tube.
4. Sonicate the same tube at 35 °C for 30 minutes.
5. Hand-shake the sample aging. Centrifuge sample for 10 minutes.
6. Use a clean glass Pasteur pipette to remove the solvent layer.
7. Repeat steps 3-6. Combine both solvent layers in a new centrifuge tube.
8. Blow down the solvent layer to near dryness under steady stream of $N_2$ gas.
9. Dilute the sample residue to a volume of 10 mL with ASTM Type 1 water. Shake well.

**H. SOLID PHASE EXTRACTION PROCEDURE**

1. Acidify the 10-mL samples to pH 2.5-3.0 using 1 drop of the 12 M HCl solution.
2. Connect the SPE cartridges to the ports on the vacuum extraction manifold.
3. 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).
4. Run the samples through the cartridges at a flow rate of 1 mL/min.
5. Wash each cartridge with 5 mL 5% methanol in water.

6. 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 °C overnight.


**Figure D1 Freeze drying sediments; apparatus and set-up**

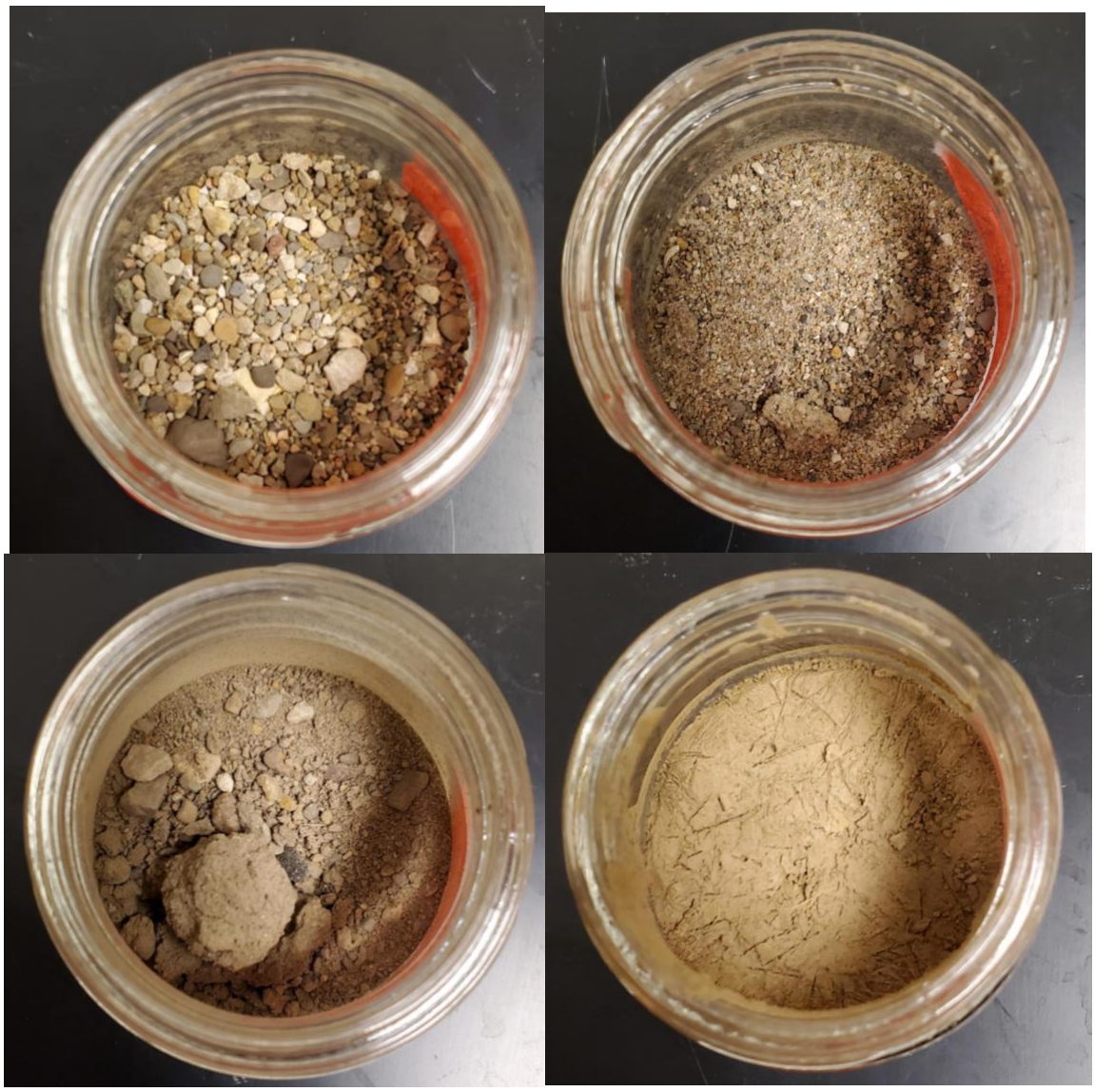

**Figure D2 Freeze dried sediment from CCJKR-C (top left), CCJKR-LBANK (top right), IRJKR-C (bottom left), and LCFR-RBANK (bottom right).**

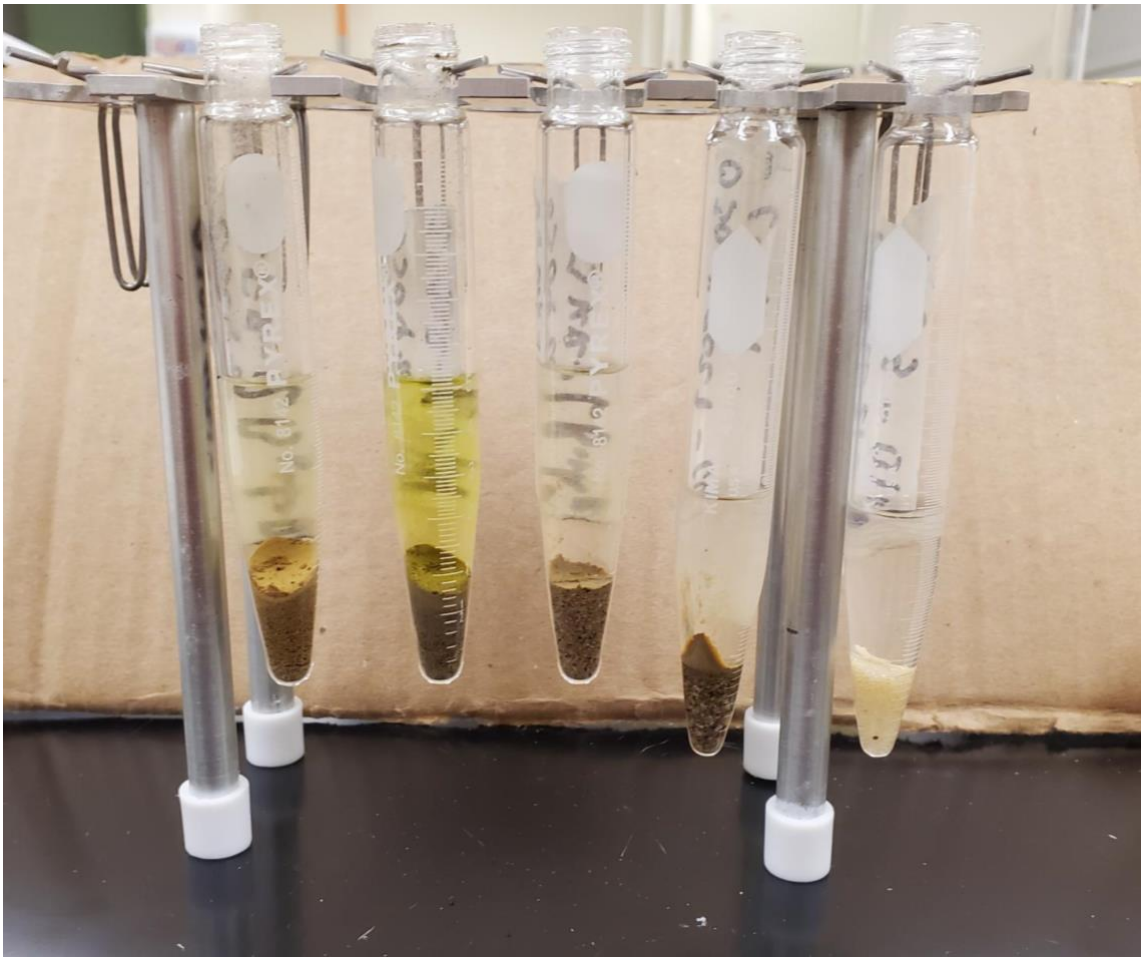

**Figure D3 Sediment samples during methanol extraction, left to right: LCFR-RBANK, IRJKR-C, CCJKR-LBANK, CCJKR-C, RGS-BLK.**

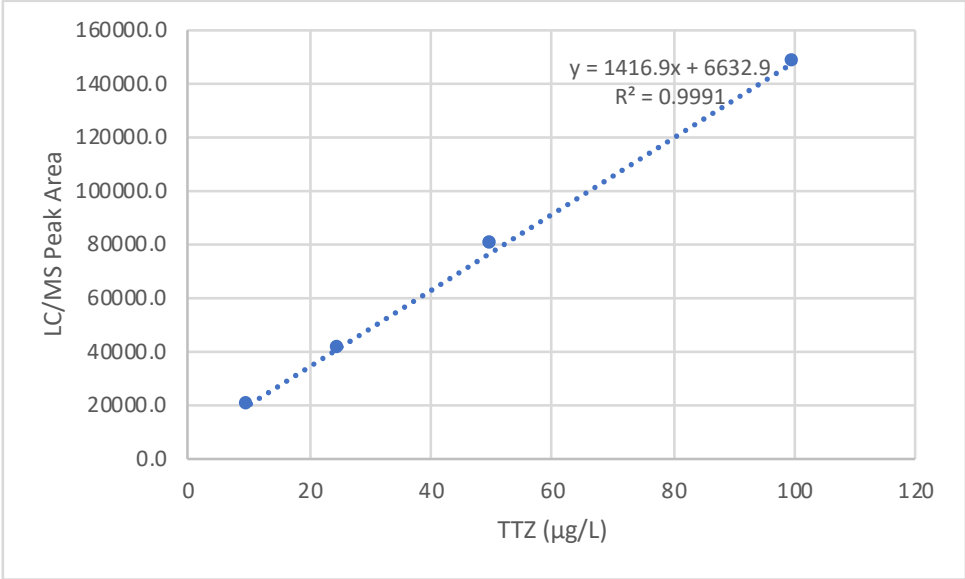


**Figure D4 LC/MS calibration curve for TTZ in sediment sample**

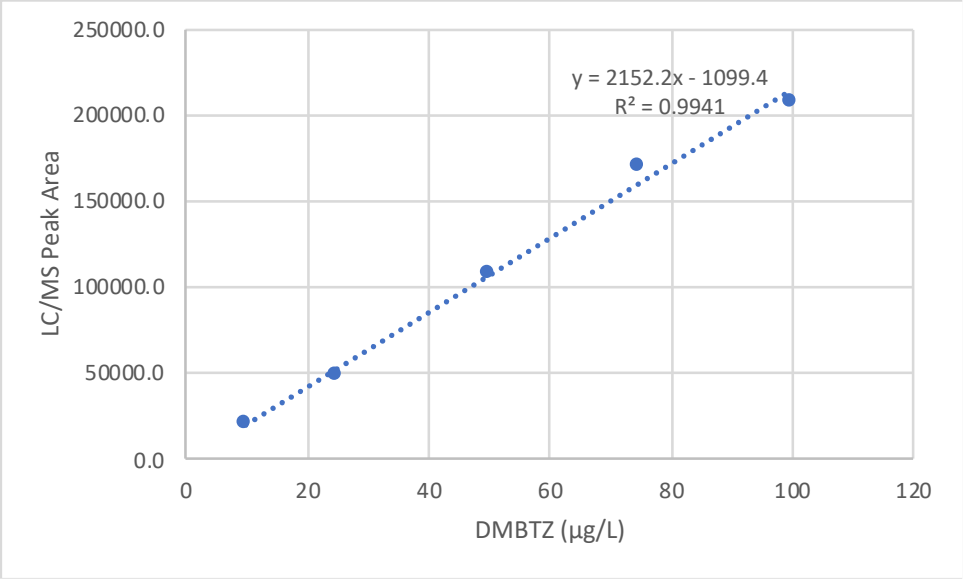


**Figure D5 LC/MS calibration curve for DMBTZ in sediment sample**

**Calculation for ng TTZ/ g sediment at IRJKR:**

TTZ concentration from the LC/MS: 0.065 µg/L

The sample volume was 1 mL, and the µg must be converted to mg:

$$\frac{0.065\ \mu g\ TTZ}{L} \times 0.001\ L \times \frac{1000\ ng}{1\ \mu g} = 0.0650\ ng\ TTZ$$

The mass of sediment weighed was 1.0312 g

$$\frac{0.0650\ ng\ TTZ}{1.0312\ g\ sediment} = \mathbf{0.0630\ ng/g}$$

**Calculation for µg TTZ in L water in sediment at IRJKR**:

The total µg TTZ in the sediment sample was found by multiplying ng TTZ/g sediment by the mass of the entire sediment sample (42.728 g) and then converting ng to µg

$$\frac{0.0630\ ng\ TTZ}{g\ sediment} \times 42.728\ g\ sediment \times \frac{1\ \mu g}{1000\ ng} = 2.69 \times 10^{-3}\ \mu g\ TTZ$$

The volume of water was found by the difference of the wet sediment sample and the dry sediment sample; the density of water was assumed to be 1 g/mL

42.728 g wet sediment – 26.506 g dry sediment = 16.222 g water or 16.222 mL

The µg TTZ in L sediment water was calculated

$$\frac{2.69 \times 10^{-3}\ \mu g\ TTZ}{0.016222\ L\ water} = \mathbf{0.166\ \mu g/L}$$

Appendix E LC/MS AnalysisStandard 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 C18 (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.66 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".

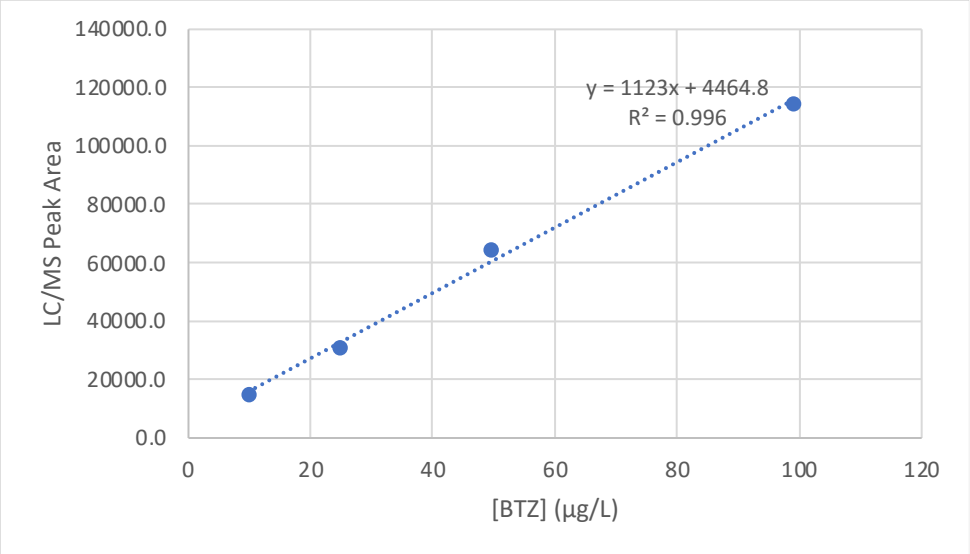


**Figure E1: Sample LC/MS calibration curve for BTZ from Sample Analysis 12092021-R2**

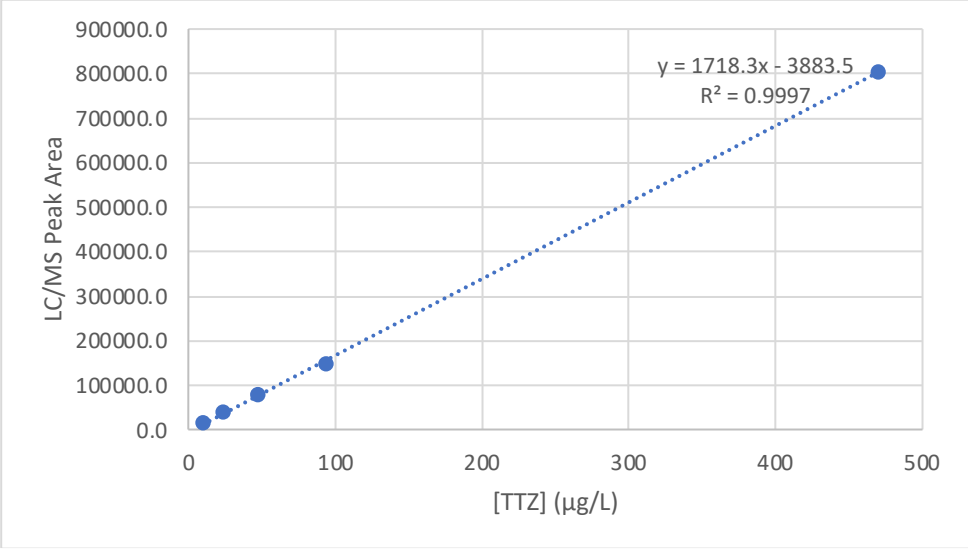


**Figure E2: Sample LC/MS calibration curve for TTZ from Sample Analysis 12092021-R2**

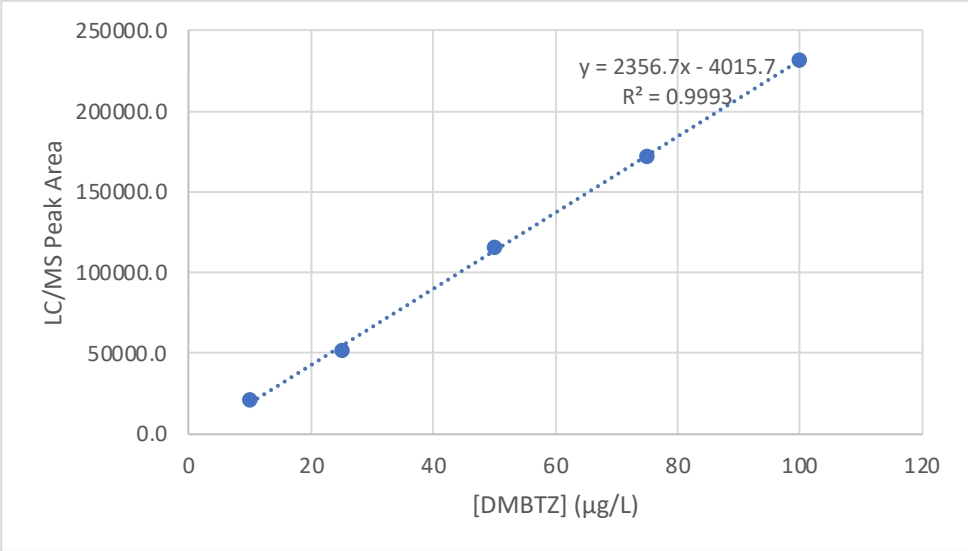


**Figure E3: Sample LC/MS calibration curve for DMBTZ from Sample Analysis 12092021-R2**

**Table E1: Analyte Concentrations and Recoveries for 12/9/2020**

| Sample Code | BTZ (µg/L) | Recovery Corrected BTZ (ppb) | TTZ (µg/L) | Recovery Corrected TTZ (ppb) | DMBTZ (ppb) | Recovery (%) |
|---|---|---|---|---|---|---|
| CCJKR-R1-T1 | ND | ND | ND | ND | 36.34 | 60.57 |
| CCJKR-R1-T2 | ND | ND | ND | ND | 30.60 | 50.99 |
| CCJKR-R1-T3 | ND | ND | 0.0640 | 0.0.0985 | 27.64 | 46.07 |
| CCJKR-R2-T1 | ND | ND | ND | ND | 27.44 | 45.73 |
| CCJKR-R2-T2 | ND | ND | 0.085 | 0.124 | 31.79 | 52.99 |
| CCJKR-R2-T3 | ND | ND | 0.079 | 0.102 | 42.06 | 70.11 |
| IRJKR-R1-T1 | ND | ND | 0.487 | 0.675 | 36.80 | 61.34 |
| IRJKR-R1-T2 | ND | ND | 0.440 | 0.606 | 37.41 | 62.35 |
| IRJKR-R1-T3 | ND | ND | 0.499 | 0.658 | 40.98 | 68.30 |
| IRJKR-R2-T1 | ND | ND | 0.464 | 0.653 | 35.63 | 59.39 |
| IRJKR-R2-T2 | ND | ND | 0.404 | 0.596 | 31.42 | 52.37 |
| IRJKR-R2-T3 | ND | ND | 0.435 | 0.588 | 38.90 | 64.84 |
| LCFR-R1-T1 | 0.0006 | Below LOD | 1.650 | 2.250 | 38.16 | 63.60 |
| LCFR-R1-T2 | 0.026 | Below LOD | 1.835 | 2.369 | 42.55 | 70.91 |
| LCFR-R1-T3 | 0.024 | Below LOD | 1.720 | 2.235 | 77.98 | 130.0 |
| LCFR-R2-T1 | 0.053 | 0.069 | 1.684 | 2.168 | 42.75 | 71.26 |
| LCFR-R2-T2 | 0.056 | 0.070 | 1.768 | 2.215 | 44.83 | 74.73 |
| LCFR-R2-T3 | 0.046 | 0.052 | 1.781 | 1.996 | 52.78 | 87.96 |

**Table E2: Analyte Concentrations and Recoveries for 1/13/2021**

| Sample Code | BTZ (µg/L) | Recovery Corrected BTZ (µg/L) | TTZ (µg/L) | Recovery Corrected TTZ (µg/L) | DMBTZ (µg/L) | Recovery (%) |
|---|---|---|---|---|---|---|
| CCJKR-R1-T1 | ND | ND | ND | ND | 15.43 | 25.72 |
| CCJKR-R1-T2 | ND | ND | ND | ND | 14.66 | 24.42 |
| CCJKR-R1-T3 | ND | ND | ND | ND | 16.89 | 28.14 |
| CCJKR-R2-T1 | ND | ND | ND | ND | 34.61 | 57.69 |
| CCJKR-R2-T2 | ND | ND | ND | ND | 40.07 | 66.79 |
| CCJKR-R2-T3 | ND | ND | ND | ND | 45.37 | 75.61 |
| IRJKR-R1-T1 | ND | ND | 0.257 | 0.395 | 27.96 | 46.60 |
| IRJKR-R1-T2 | ND | ND | 0.216 | 0.329 | 28.44 | 47.40 |
| IRJKR-R1-T3 | ND | ND | 0.270 | 0.320 | 48.79 | 81.30 |
| IRJKR-R2-T1 | ND | ND | 0.255 | 0.346 | 38.46 | 64.09 |
| IRJKR-R2-T2 | ND | ND | 0.274 | 0.344 | 44.65 | 74.40 |
| IRJKR-R2-T3 | ND | ND | 0.282 | 0.339 | 47.99 | 79.98 |
| LCFR-R1-T1 | 0.010 | 0.013 | 1.234 | 1.748 | 34.98 | 58.30 |
| LCFR-R1-T2 | 0.013 | 0.019 | 1.159 | 1.680 | 33.02 | 55.04 |
| LCFR-R1-T3 | 0.012 | 0.017 | 1.190 | 1.685 | 35.02 | 58.37 |
| LCFR-R2-T1 | 0.022 | 0.030 | 1.267 | 1.741 | 37.56 | 62.61 |
| LCFR-R2-T2 | 0.011 | 0.015 | 1.292 | 1.677 | 42.15 | 70.25 |
| LCFR-R2-T3 | 0.029 | 0.036 | 1.354 | 1.667 | 46.14 | 76.90 |

**Table E3: Analyte Concentrations and Recoveries for 2/24/2021**

| Sample Code | BTZ (µg/L) | Recovery Corrected BTZ (µg/L) | TTZ (µg/L) | Recovery Corrected TTZ (µg/L) | DMBTZ (µg/L) | Recovery (%) |
|---|---|---|---|---|---|---|
| CCJKR-R1-T1 | ND | ND | ND | ND | 25.25 | 42.07 |
| CCJKR-R1-T2 | ND | ND | ND | ND | 39.25 | 65.41 |
| CCJKR-R1-T3 | ND | ND | ND | ND | 42.62 | 71.03 |
| CCJKR-R2-T1 | ND | ND | 0.046 | 0.057 | 45.82 | 76.37 |
| CCJKR-R2-T2 | ND | ND | 0.008 | Below LOD | 29.60 | 49.34 |
| CCJKR-R2-T3 | ND | ND | 0.017 | Below LOD | 34.27 | 57.12 |
| IRJKR-R1-T1 | ND | ND | 1.111 | 1.599 | 33.67 | 56.12 |
| IRJKR-R1-T2 | ND | ND | 1.191 | 1.704 | 34.16 | 56.94 |
| IRJKR-R1-T3 | ND | ND | 1.173 | 1.672 | 34.45 | 57.42 |
| IRJKR-R2-T1 | ND | ND | 1.269 | 1.860 | 32.06 | 53.43 |
| IRJKR-R2-T2 | ND | ND | 1.379 | 1.939 | 35.63 | 59.39 |
| IRJKR-R2-T3 | ND | ND | 1.418 | 1.936 | 38.07 | 63.46 |
| LCFR-R1-T1 | 0.137 | 0.176 | 41.77 | 54.76 | 41.33 | 68.89 |
| LCFR-R1-T2 | 0.161 | 0.210 | 42.86 | 54.32 | 43.96 | 73.27 |
| LCFR-R1-T3 | 0.134 | 0.164 | 43.13 | 52.24 | 47.31 | 78.86 |
| LCFR-R2-T1 | 0.085 | 0.104 | 40.79 | 49.84 | 46.68 | 77.80 |
| LCFR-R2-T2 | 0.048 | 0.058 | 42.12 | 51.27 | 46.96 | 78.27 |
| LCFR-R2-T3 | 0.163 | 0.197 | 40.94 | 48.80 | 48.48 | 80.80 |

**Table E4: Analyte Concentrations and Recoveries for 3/4/2021**

| Sample Code | BTZ (µg/L) | Recovery Corrected BTZ (µg/L) | TTZ (µg/L) | Recovery Corrected TTZ (µg/L) | DMBTZ (µg/L) | Recovery (%) |
|---|---|---|---|---|---|---|
| CCJKR-R1-T1 | ND | ND | 0.058 | 0.089 | 26.85 | 44.74 |
| CCJKR-R1-T2 | ND | ND | ND | ND | 30.40 | 50.66 |
| CCJKR-R1-T3 | ND | ND | ND | ND | 28.74 | 47.90 |
| CCJKR-R2-T1 | ND | ND | ND | ND | 37.74 | 62.91 |
| CCJKR-R2-T2 | ND | ND | ND | ND | 43.61 | 72.69 |
| CCJKR-R2-T3 | ND | ND | ND | ND | 42.39 | 70.65 |
| IRJKR-R1-T1 | ND | ND | 0.660 | 0.991 | 29.92 | 49.87 |
| IRJKR-R1-T2 | ND | ND | 0.619 | 0.914 | 31.37 | 52.28 |
| IRJKR-R1-T3 | ND | ND | 0.663 | 0.986 | 30.81 | 51.35 |
| IRJKR-R2-T1 | ND | ND | 0.480 | 0.765 | 24.39 | 40.66 |
| IRJKR-R2-T2 | ND | ND | 0.658 | 0.932 | 35.05 | 58.42 |
| IRJKR-R2-T3 | ND | ND | 0.686 | 0.958 | 36.18 | 60.31 |
| LCFR-R1-T1 | 0.072 | 0.098 | 11.89 | 16.23 | 37.93 | 63.21 |
| LCFR-R1-T2 | 0.069 | 0.093 | 11.91 | 16.16 | 38.55 | 64.25 |
| LCFR-R1-T3 | 0.083 | 0.103 | 14.12 | 17.40 | 46.09 | 76.82 |
| LCFR-R2-T1 | 0.048 | 0.056 | 13.40 | 15.73 | 49.58 | 82.64 |
| LCFR-R2-T2 | 0.081 | 0.093 | 13.34 | 15.29 | 51.26 | 85.42 |
| LCFR-R2-T3 | 0.029 | Below LOD | 13.44 | 16.70 | 45.48 | 75.80 |

**Table E5: Analyte Concentrations and Recoveries for 6/24/2021**

| Sample Code | BTZ (µg/L) | Recovery Corrected BTZ (µg/L) | TTZ (µg/L) | Recovery Corrected TTZ (µg/L) | DMBTZ (µg/L) | Recovery (%) |
|---|---|---|---|---|---|---|
| CCJKR-R1-T1 | ND | ND | 0.021 | Below LOD | 34.097 | 56.828 |
| CCJKR-R1-T2 | ND | ND | 0.103 | 0.138 | 38.902 | 64.837 |
| CCJKR-R1-T3 | ND | ND | 0.020 | Below LOD | 38.663 | 64.438 |
| CCJKR-R2-T1 | ND | ND | ND | ND | 36.673 | 61.122 |
| CCJKR-R2-T2 | 0.081 | **Below LOD | 0.102 | 0.132 | 42.186 | 70.309 |
| CCJKR-R2-T3 | 0.158 | **Below LOD | 0.136 | 0.173 | 43.704 | 72.839 |
| IRJKR-R1-T1 | 0.034 | Below LOD | 0.699 | 0.970 | 36.824 | 61.373 |
| IRJKR-R1-T2 | 0.028 | Below LOD | 0.712 | 0.953 | 39.712 | 66.187 |
| IRJKR-R1-T3 | 0.043 | Below LOD | 0.752 | 0.978 | 42.022 | 70.037 |
| IRJKR-R2-T1 | 0.198 | **Below LOD | 0.700 | 1.005 | 33.957 | 56.596 |
| IRJKR-R2-T2 | 0.157 | **Below LOD | 0.697 | 0.956 | 37.662 | 62.770 |
| IRJKR-R2-T3 | 0.199 | **Below LOD | 0.800 | 1.003 | 42.664 | 71.107 |
| LCFR-R1-T1 | 0.142 | 0.178 | 7.111 | 8.918 | 44.756 | 74.594 |
| LCFR-R1-T2 | 0.130 | 0.165 | 6.632 | 8.423 | 43.787 | 72.978 |
| LCFR-R1-T3 | 0.108 | 0.131 | 6.982 | 8.475 | 47.163 | 78.605 |
| LCFR-R2-T1 | *0.339 | **0.215 | 6.866 | 8.618 | 47.983 | 79.971 |
| LCFR-R2-T2 | *0.049 | Below LOD | 5.929 | 8.304 | 35.970 | 59.950 |
| LCFR-R2-T3 | *0.048 | Below LOD | 5.311 | 7.846 | 31.363 | 52.271 |

*****Samples excluded from final calculations due to contamination/variability

******Average of 0.16 µg/L found in methanol blanks, average subtracted to report value

Appendix F GC/MS Analysis
Standard Operating Procedure

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

February 10, 2022

Clara Leedy
Jessica Wiese, MS
Audrey McGowin, PhD

Approved: _____
Audrey McGowin, Ph.D.

## 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 (BTZ) compounds and their analogs tolytriazoles (TTZ) are polar and thermally labile. In addition, BTZs and TTZs are toxic to certain aquatic organisms, and have the potential for impacting the health of creeks, rivers, and ground water reservoirs in which BTZ and TTZ analogs are deposited. The procedures outlined in this SOP were created for the qualitative and quantitative determination of BTZ, and separation of TTZ into 4-methyl- and 5-methyl-1H-benzotriazole isomers by Gas Chromatography – Mass Spectrometry (GC-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 GC-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 7890B GC and 240 Ion Trap GC/MS system that includes the following components:
    a. Agilent (25 μm I**.D 32** x 30-m) column
    b. Autosampler
    c. OpenLAB CDS ChemStation Software

2. Several autosampler vials with Teflon caps.

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

4. Chemicals & Reagents
    a. GC-grade Methanol (MeOH, CAS #67-56-1)
    b. ASTM Type 1 Water
    c. 1H-benzotriazole (BTri, CAS # 95-14-7)
    d. 4-methyl-1H-benzotriazole (4-Me-BTri, CAS #249-921-1)
    e. 5-methyl-1H-benzotriazole (5-Me-BTri, CAS #136-85-6)
    f. 5,6-dimethyl-1H-benzotriazole (5,6-dimethyl-BTri, CAS #4184-79-6)

## F. PROCEDURE – *STANDARD SOLUTION PREPARATION*

1. Weigh out 0.00500 g of BTZ and dissolve it in 50 mL MeOH to create the 100-ppm standard solution.

2. Take 5.0 mL of the 100-ppm solution and dilute to 25 mL with MeOH to create the 20-ppm standard solution.

3. Take 2.5 mL of the 100-ppm solution and dilute to 25 mL with MeOH to create the 10-ppm standard solution.

4. Take 5.0 mL of the 100-ppm solution and dilute to 10 mL with MeOH to create the 5.0-ppm standard solution.

5. Take 4.0 mL of the 100-ppm solution and dilute to 10 mL with MeOH to create the 4.0-ppm standard solution.

6. Take 3.0 mL of the 100-ppm solution and dilute to 10 mL with MeOH to create the 3.0-ppm standard solution.

7. Take 2.0 mL of the 100-ppm solution and dilute to 10 mL with MeOH to create the 2.0-ppm standard solution.

8. Take 1.0 mL of the 100-ppm solution and dilute to 10 mL with MeOH to create the 1.0-ppm standard solution.

9. Take 1.0 mL of the 5.0-ppm solution and dilute to 10 mL with MeOH to create the 500-ppb standard solution.

10. Take 1.0 mL of the 500-ppb solution and dilute to 10 mL with MeOH to create the 50-ppb standard solution.

6. Repeat steps 1-9 for both 4-m-BTZ and 5-m-BTZ. Mixed TTZ standards can be made by adding 5.0 mL of each isomer stock to 25 mL in step 2, then substituting step 3 with 12.5 mL of the mixed 20-ppm diluted to 25 mL to create a mixed 10-ppm standard for further dilutions.

7. Label each standard with initials, date, analyte, and concentration.

8. Store all standard solutions in amber glass vials/jars in the freezer (-20°C).

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

1. Weigh out 0.00025 g of 5,6-dimethyl-BTri and dissolve it in 50 mL of MeOH to create the 5.0-ppm standard solution.

2. Take 8.0 mL of the 5.0-ppm solution and dilute to 10 mL with MeOH to create the 4.0-ppm standard solution.

3. Take 6.0 mL of the 5.0-ppm solution and dilute to 10 mL with MeOH to create the 3.0-ppm standard solution.

4. Take 4.0 mL of the 5.0-ppm solution and dilute to 10 mL with MeOH to create the 2.0-ppm standard solution.

5. Take 1.0 mL of the 5.0-ppm solution and dilute to 10 mL with MeOH to create the 1.0-ppm standard solution.

6. Take 500 µL of the 1.0-ppm solution and dilute to 10 mL with MeOH to create the 50-ppb standard solution.

7. Label each standard with initials, date, analyte, and concentration.

8. Store all standard solutions in amber glass vials/jars in the freezer (-20°C).

**I. PROCEDURE – *GC-MS ANALYSIS***

1. Make sure the helium tank is has adequate pressure (80 psi). If empty, contact Joseph Solch or Garrett Vanness to replace as soon as possible.

2. If the GC-MS has not been used in a while, it is important to check that it is tuned properly.

    a. Under the “Show MS” tab, select “Auto Tune” and then “Start Auto Tune” and check that the tune passes.

3. Under “File” across the top of the System Control window, choose “Activate Method”. Select the desired method from the dropdown list or choose “Start with this method.mth” to create a new method. After the method is selected, choose “open”.

4. To edit the method, select the “Method” button in the “GC Operation” box. If there is an existing method, skip to step 7.

5. Start by naming the new method. On the “Method Builder” window, select “File” then “Save as”. Name the method with the user/group’s initials, date, and identifying information. Ex: CLL-01292022-SIM.
    a. In the “Method Builder” window, on the left-hand side choose “7890 Method” to edit GC method parameters.
    b. Under the “ALS” icon set the injection volume and wash sample volumes.
    c. Under the “Inlets” icon, set the check “Heater” and set the desired temperature. Select split/splitless mode based on analysis.
    d. Under the “Columns” icon, check the column flow rate.
    e. Under the “Oven” icon, set initial oven temperature and desired temperature program.

6. To set up SIM, select the “MS Acquisition Method” on the left-hand side of the “Method Builder” window.
    a. Change “Scan Type” from “full scan” to “uSIS” for selected ion scanning.
    b. Under the “MS/MS Parameters” tab, enter the desired ion (m/z) un “Precursor Ion (m/z)”

7. Set up a sequence by going to the "File" tab and clicking "New Sample List".
    a. Name the sequence with initials, run date, and identifying information. For sample analysis, use the date samples were taken (MMDDYYYY) as the sequence date and “Sample Analysis” as the identifying information.
    b. Enter the sequence of your samples, denoting the name, number of injections, and vial position.
    c. To add lines for more samples, click "Insert". To remove sample lines, click "Cut".
    d. Check that the data is being saved in the correct directory by selecting “Data Files” at the bottom right of the sequence box. If the directory is

incorrect, double click the “data” folder, then double click the correct directory folder.

8. To start the run, select “Begin” at the bottom of the sequence box. Check that the correct method name and data directory are listed. Confirm to begin the run, or cancel to change the method/directory based on above steps.

9. To view the data, go to the "Review/Process MS Data” icon on the “System Control” window sidebar. A chromatogram may be viewed once the run for the desired injection is complete.

    a. Click the sample name to view the chromatogram.
    b. To search for specific ions, type the desired m/z in the bottom right “Ions” box.
    c. On the top toolbar, use the seventh icon from the left on “Zoom Chromatograms” to zoom in, or “Integrate” to integrate peaks. To delete integration, right click, choose “Delete Labels”, “Plot 1”, then “Integration”.
    d. Right click on a selected peak and choose “Library Match” to see possible compound matches based on the mass spectrum of the selected peak.

10. Take screen shots of desired chromatograms or library matches using the green circular icon at the bottom of the screen. Save screen shots in a folder on the desktop with names identifying the sequence name and sample.

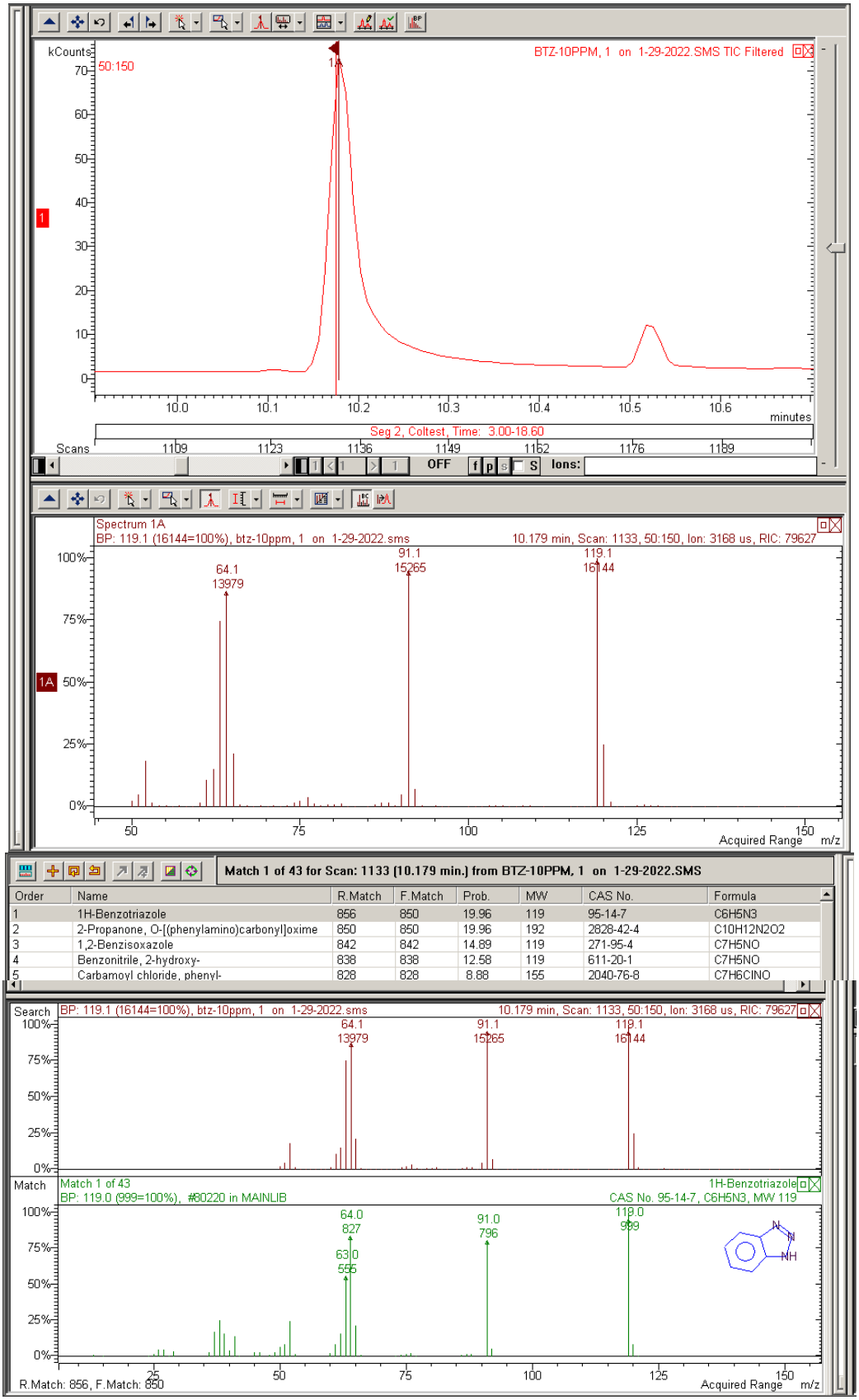


**Figure F1** depicts the GC/MS library match for BTZ

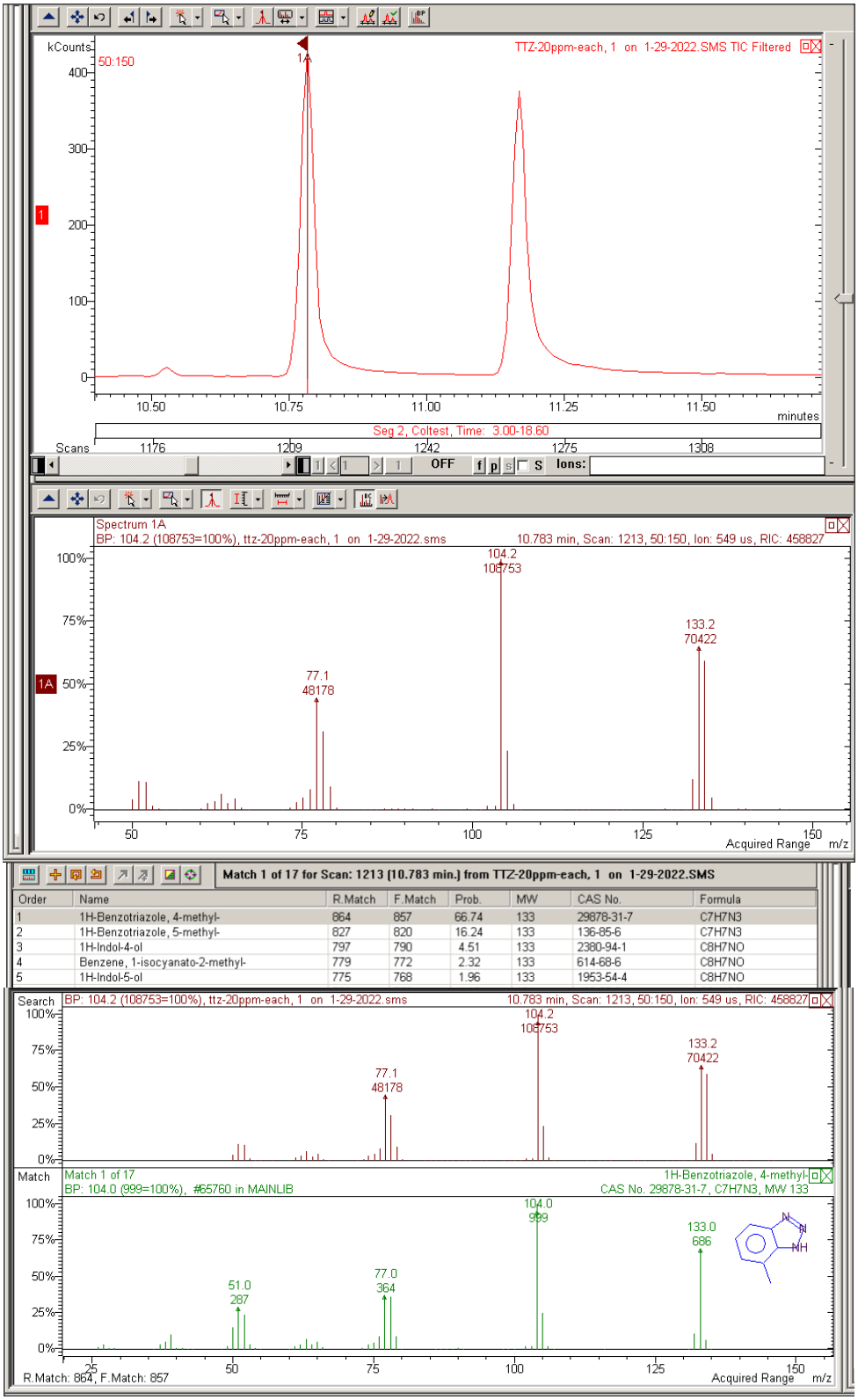


**Figure F2 depicts the GC/MS library match for 4m-BTZ**

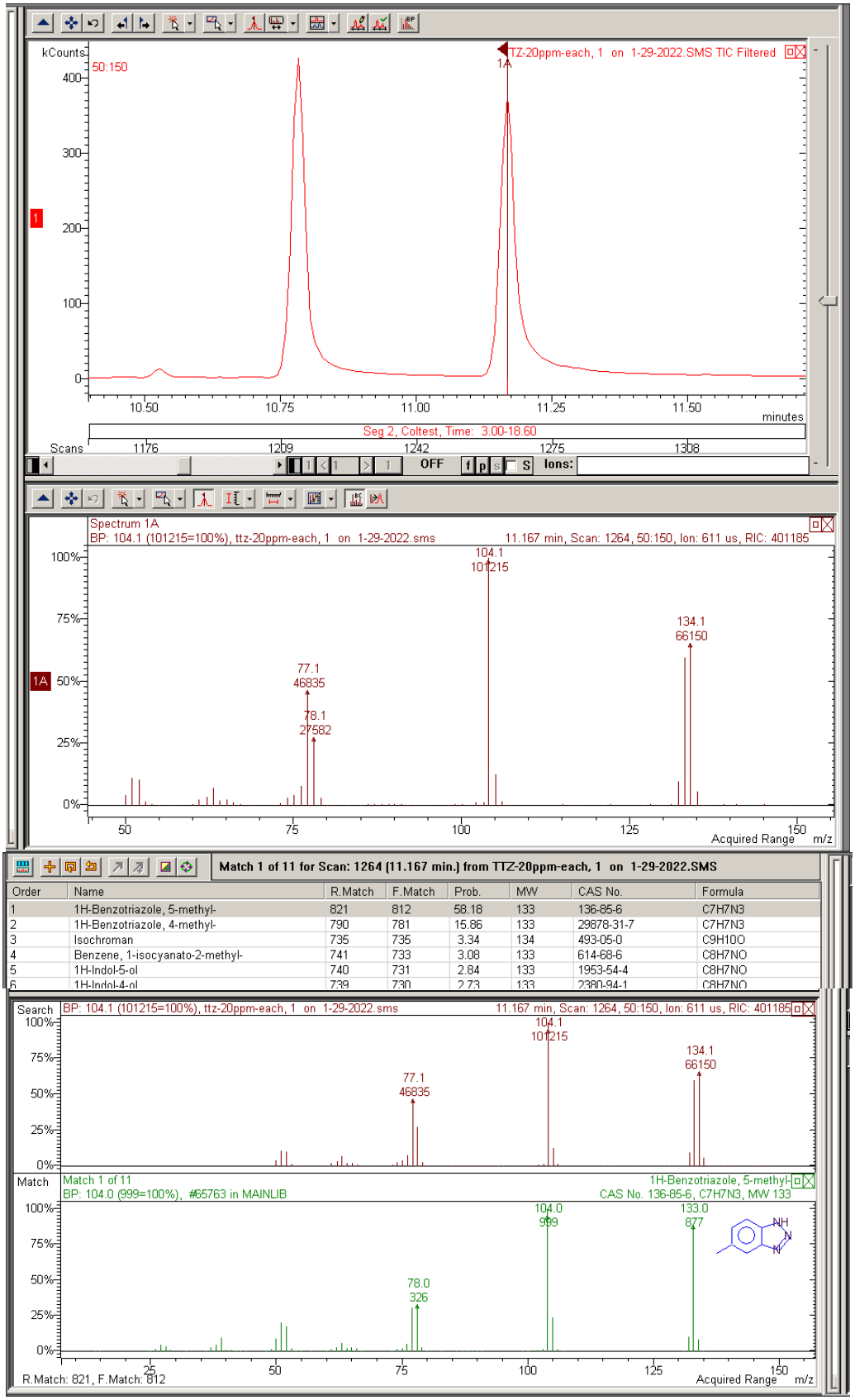


**Figure F3 depicts the GC/MS library match for 5m-BTZ**

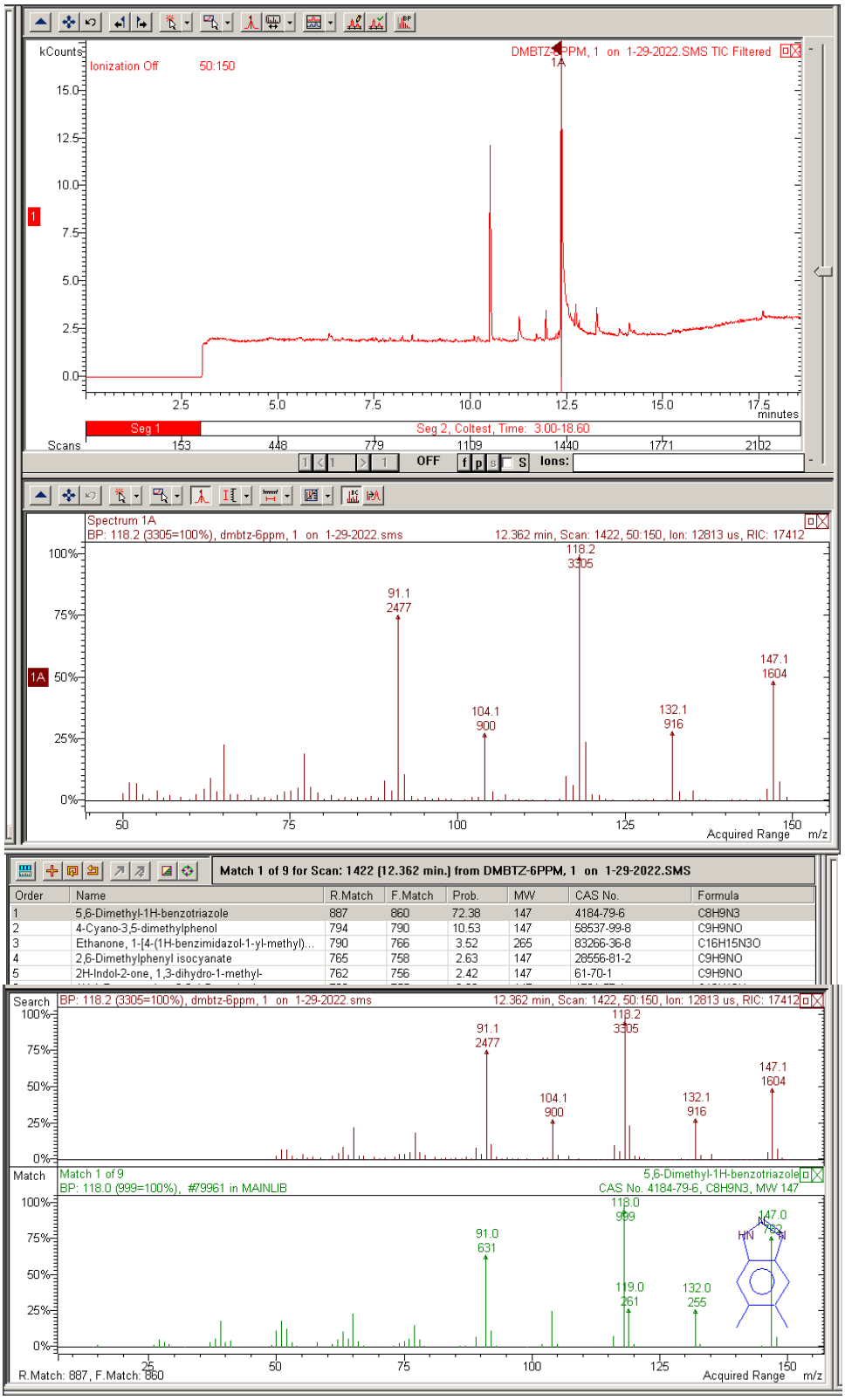


**Figure F4 depicts the GC/MS library match for DMBTZ**

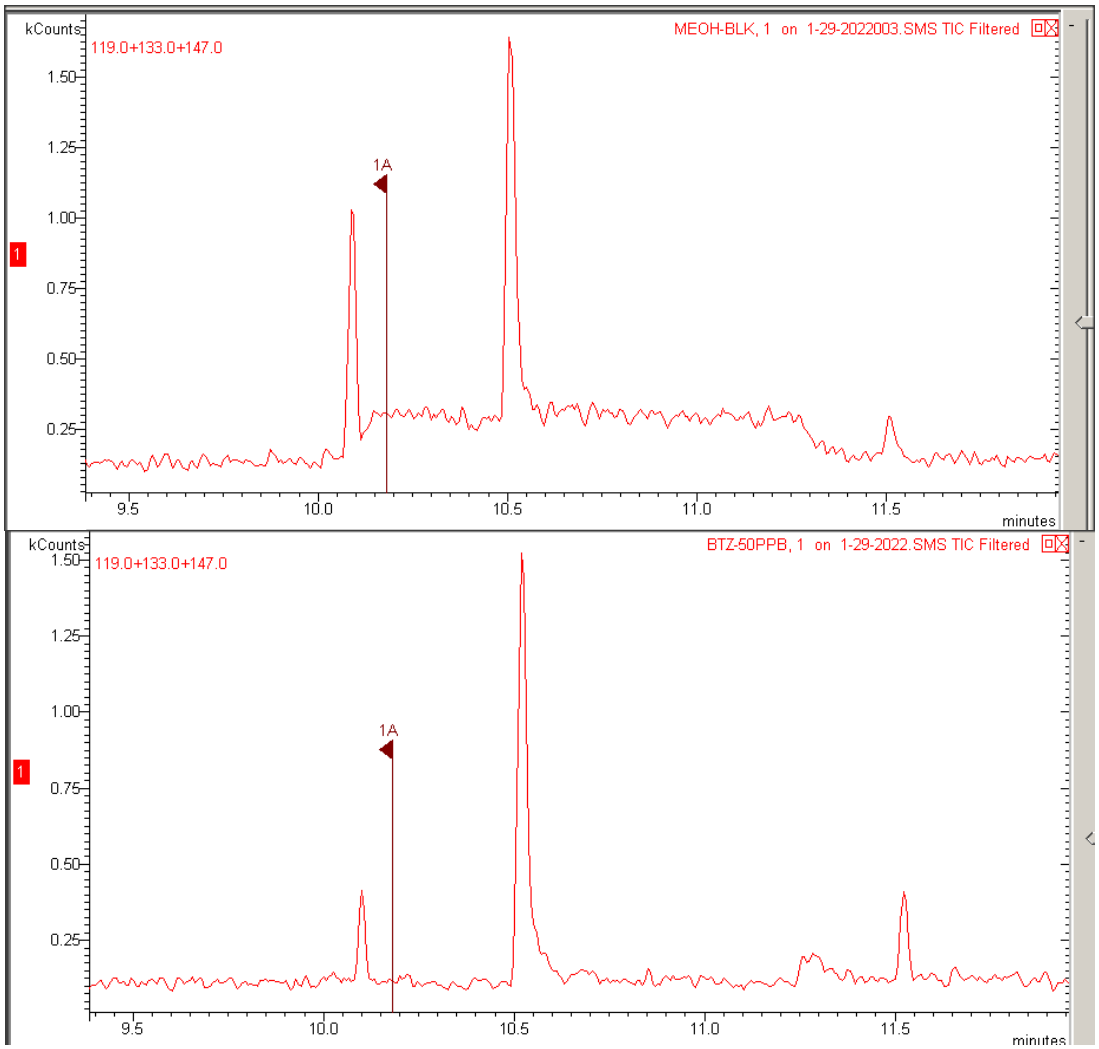


**Figure F5 Depicts GC/MS chromatograms for a methanol blank (top) and a 50-µg/L BTZ standard (bottom), both with the BTZ retention time highlighted for comparison.**

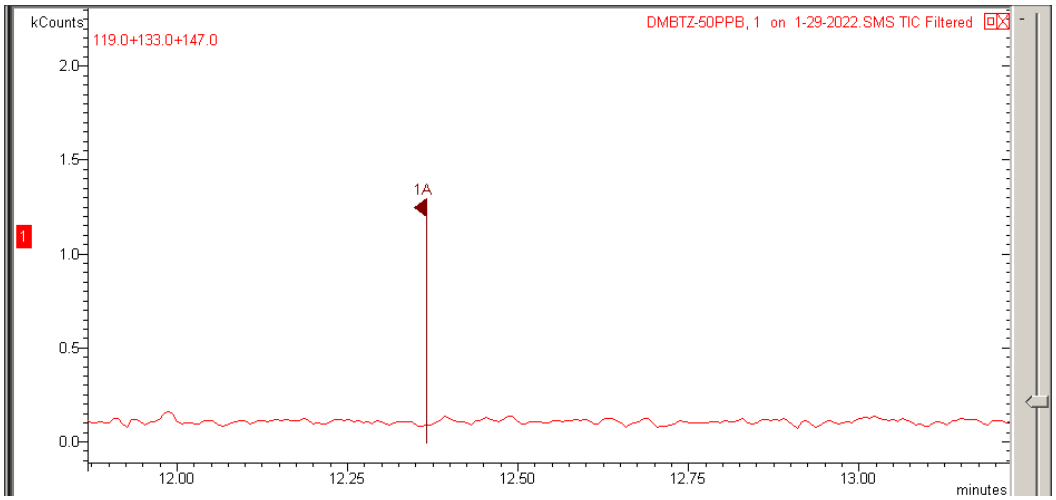


**Figure F6 Depicts a GC/MS chromatogram of 50-µg/L DMBTZ standard with retention time highlighted.**

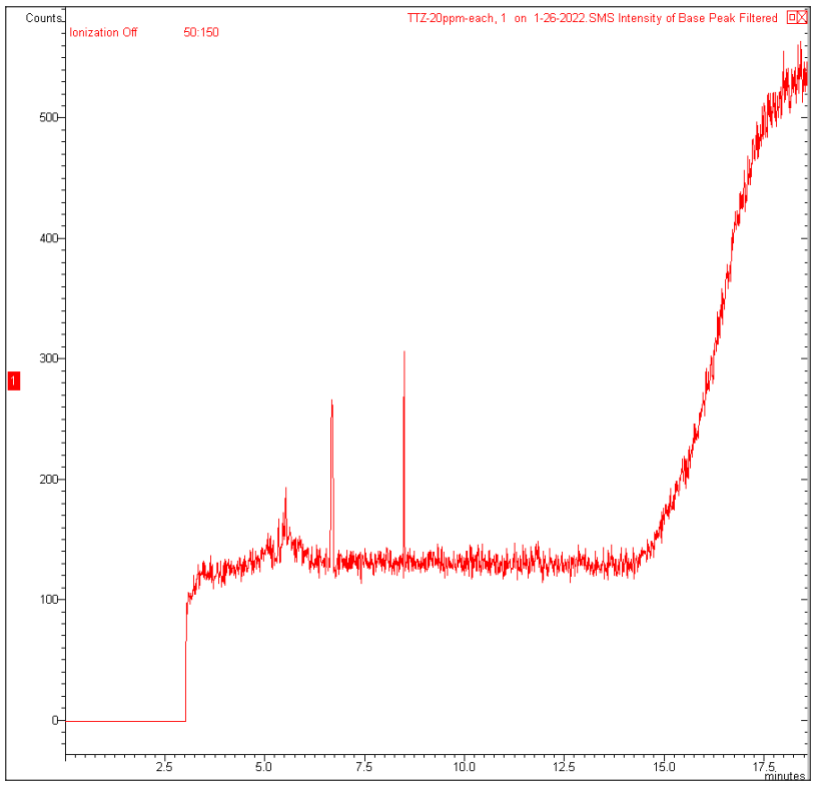


**Figure F7 GC/MS original method with inlet temp set to 120°C**

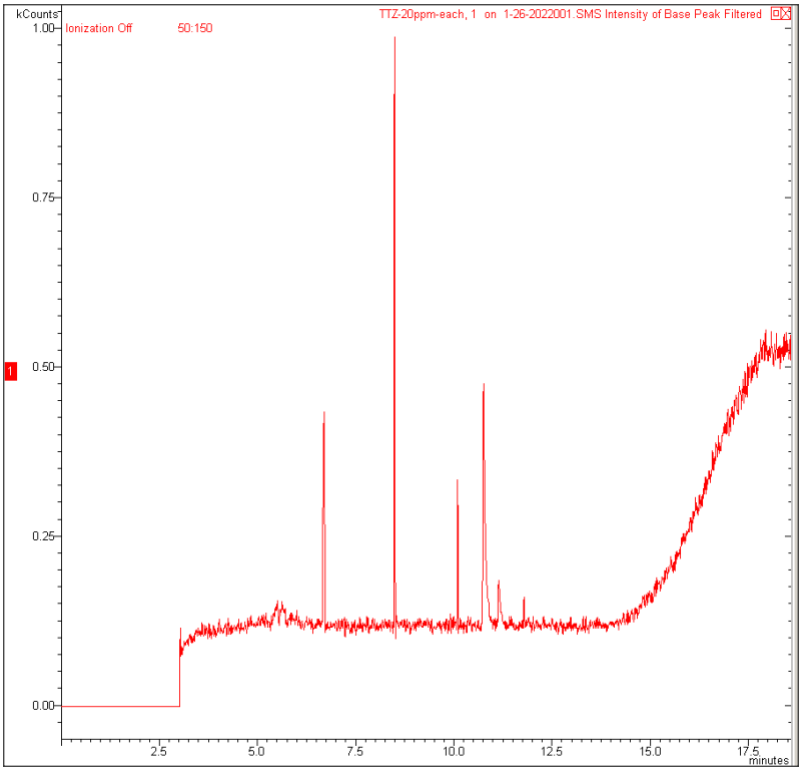


**Figure F8 GC/MS original method with inlet temp set to 170°C**

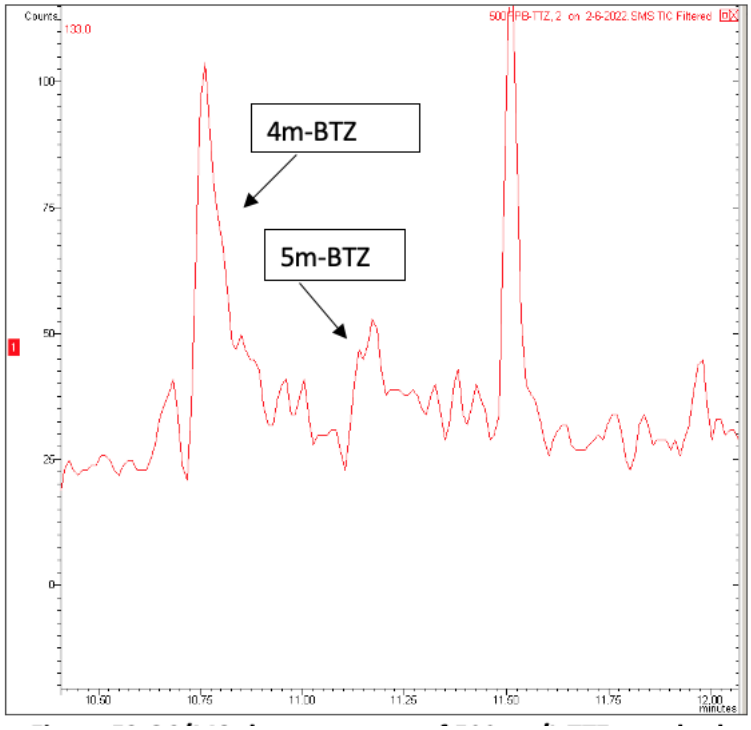


**Figure F9 GC/MS chromatogram of 500-µg/L TTZ standard**

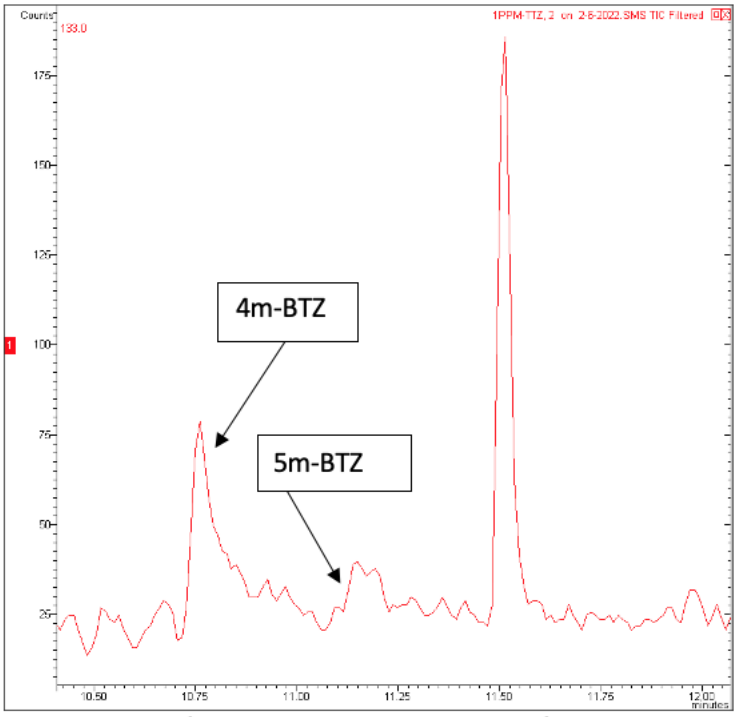


**Figure F10 GC/MS chromatogram of a 1-mg/L TTZ standard**

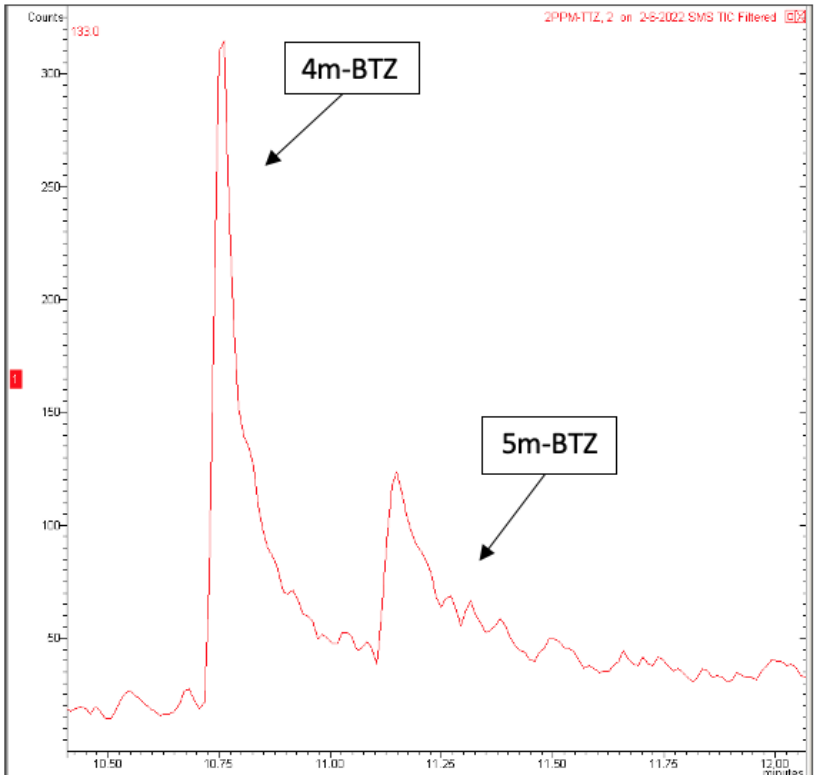


**Figure F11 GC/MS chromatogram of a 2-mg/L TTZ standard**

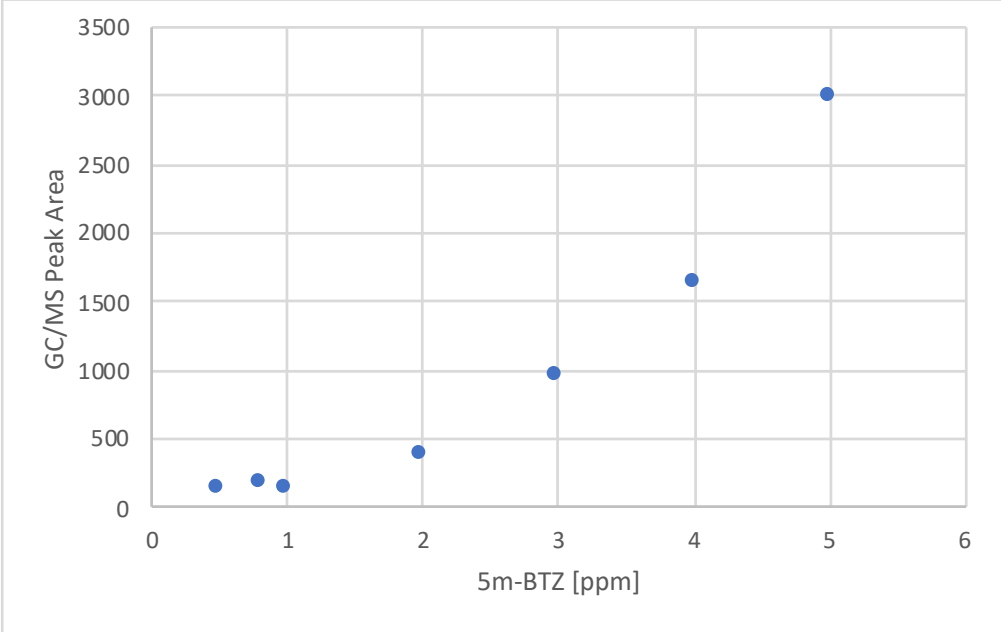


**Figure F12 Depicts the 5m-BTZ standards plotting GC/MS peak area vs standard concentration (for LOD determination).**

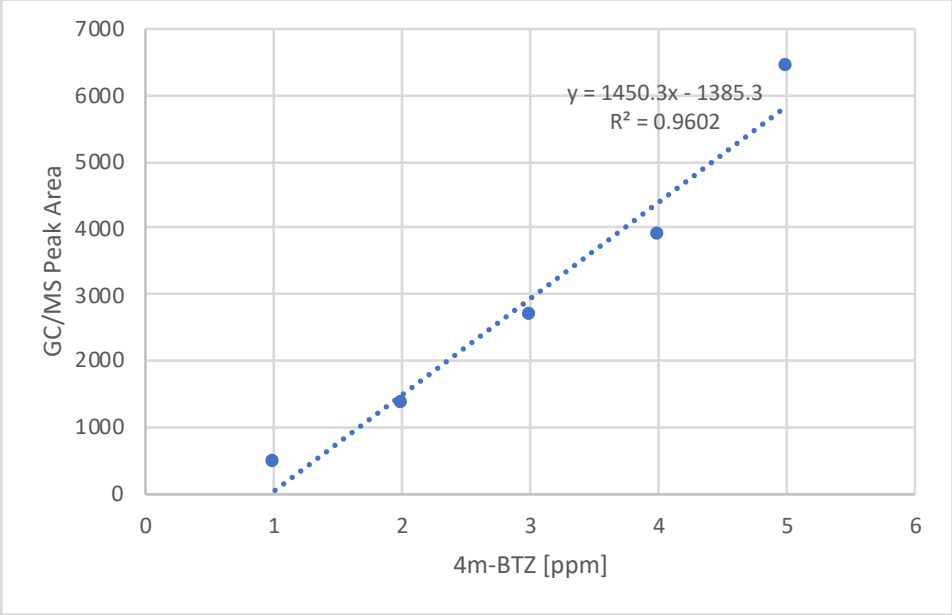


**Figure F13 GC/MS calibration curve for 4m-BTZ**

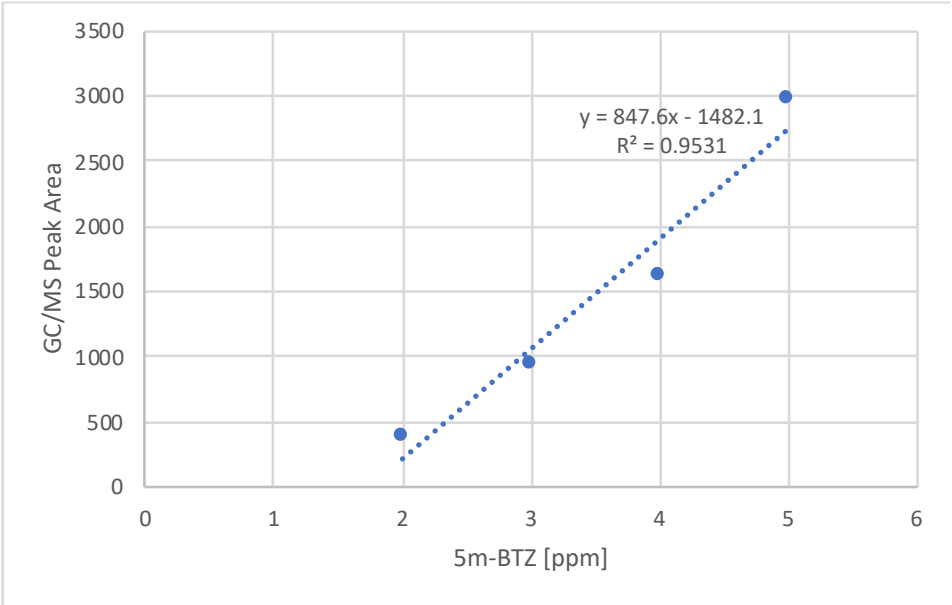


**Figure F14 GC/MS calibration curve for 5m-BTZ**

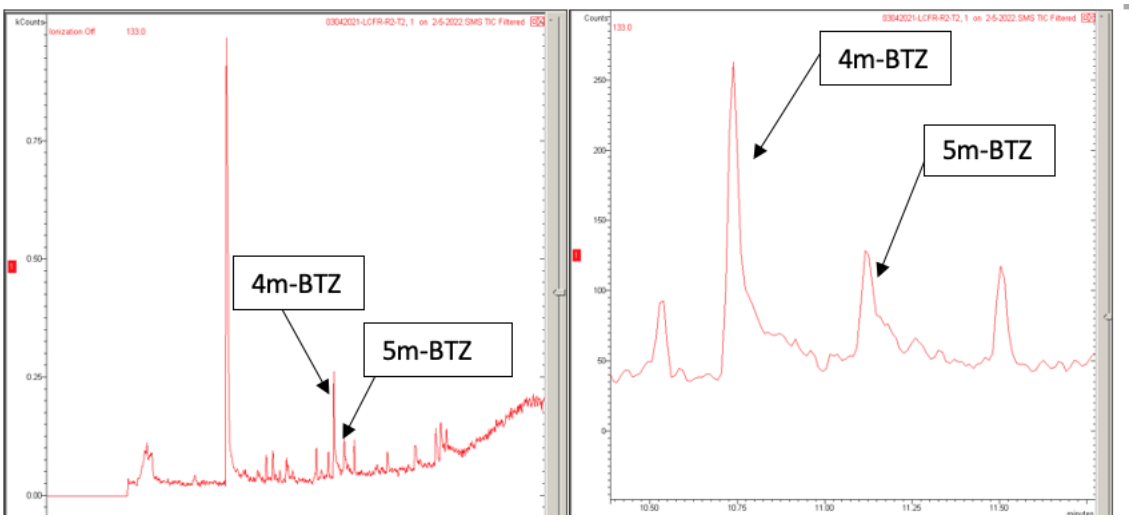


**Figure F15 GC/MS chromatogram of 03042021-R2-T2 full chromatogram (right) and zoomed in (left)**

Appendix G Additional Information

**Table G1** Average concentrations for benzotriazoles during 2019 season from LC/MS[23]

| Sample Date | IRJKR BTZ (µg/L) | IRJKR TTZ (µg/L) | LCFR BTZ (µg/L) | LCFR TTZ (µg/L) |
|---|---|---|---|---|
| 2/1/2019 | Below LOD | 0.869±0.10 | Below LOD | 2.724±0.13 |
| 2/6/2019 | ND | 0.111±0.04 | ND | 0.822±0.18 |
| 2/13/2019 | ND | 0.112±0.04 | Below LOD | 1.731±0.13 |
| 2/22/2019 | ND | 0.204±0.04 | Below LOD | 1.714±0.13 |
| 2/28/2019 | ND | 0.596±0.04 | Below LOD | 1.660±0.12 |

**Table G2** Average concentrations for benzotriazoles during 2020 season from LC/MS[26]

| Sample Date | IRJKR TTZ (µg/L) | LCFR BTZ (µg/L) | LCFR TTZ (µg/L) |
|---|---|---|---|
| 11/13/2019 | 5.649±0.45 | 0.625±0.022 | 11.943±0.24 |
| 11/20/2019 | 1.679±0.71 | 3.47±0.24 | 5.058±0.38 |
| 1/14/2020 | 0.670±0.05 | 0.211±0.02 | 1.698±0.10 |
| 1/23/2020 | 1.660±0.65 | Below LOD | 1.648±0.06 |
| 2/11/2020 | 0.760±0.08 | Below LOD | 2.757±0.16 |
| 2/25/2020 | 0.214±0.01 | Below LOD | 1.350±0.13 |
| 3/10/2020 | 1.655±0.09 | ND | 0.725±0.06 |

**Table G 3** Temperature and Precipitation from 2019 season[29]

| Sample Date | Day of Sampling | 1 Day Before | 2 Days Before | 3 Days Before |
|---|---|---|---|---|
| 2/1/2019 | Avg. Temp: -9.7°C<br>Precipitation: 0.43 cm | Avg. Temp: -16.7 °C<br>Precipitation: 0.13 cm | Avg. Temp: -15.3 °C<br>Precipitation: 0.05 cm | Avg. Temp: -6.9°C<br>Precipitation: 0.07 cm |
| 2/6/2019 | Avg. Temp: 9.4 °C<br>Precipitation: 3.2 cm | Avg. Temp: 7.5 °C<br>Precipitation: 0.46 cm | Avg. Temp: 9.4 °C<br>Precipitation: 0.25 cm | Avg. Temp: 8.1 °C<br>No precipitation |
| 2/13/2019 | Avg. Temp: -1.4 °C<br>Precipitation: No data | Avg. Temp: 5.8 °C<br>Precipitation:2.7 cm | Avg. Temp: 1.9 °C<br>Precipitation: 1.6 cm | Avg. Temp: -3.6 °C<br>Precipitation: 0.33 cm |
| 2/22/2019 | Avg. Temp: 1.9 °C<br>No precipitation | Avg. Temp: 4.2°C<br>No precipitation | Avg. Temp: 4.2 °C<br>Precipitation: 3.8 cm | Avg. Temp: -3.3 °C<br>No precipitation |
| 2/28/2019 | Avg. Temp: -0.3 °C<br>Precipitation: 0.13 cm | Avg. Temp: 4.2 °C<br>No precipitation | Avg. Temp: 0.3 °C<br>No precipitation | Avg. Temp: -1.9 °C<br>No precipitation |

**Table G4** Temperature and Precipitation from 2020 season[26,29]

| 3 Days Prior | 2 Days Prior | 1 Day Prior | Day of Sampling | Sample Date |
|---|---|---|---|---|
| Avg. -6.7 ℃; Almost completely Overcast, Fog; 0.46 cm of precipitation | Avg. -6.7 ℃; Overcast, Wind, Fog and Haze; 0.05 cm of precipitation | Avg. -8.3 ℃; Clear skies, some fog | Cold; slight wind; small amount of snow on ground | 11-13-2019 |
| Avg. 2.8 ℃; Clear Sky, Fog and Haze | Avg. 3.3 ℃; Mostly Overcast, Fog and Haze | Avg. 6.1 ℃; Overcast, Fog; NEPTM | Cool; Cloudy; Very Muddy | 11-20-2019 |
| Avg. 3.3 ℃; Overcast, Fog, Windy; 1.24 cm of precipitation | Avg. 6.7 ℃; Very Overcast, Fog, Windy; NEPTM | Avg. 6.1 ℃; Partly Overcast | Cold; Very Muddy: Fog | 01-14-2020 |
| Avg. -6.7 ℃; Pretty Overcast; NEPTM | Avg. -6.7 ℃; Little Cloud cover | Avg. -2.8 ℃; Clear | Overcast; Mostly Dry; little mud; 0.03 cm of precipitation | 01-23-2020 |
| Avg. -1.1 ℃; Completely Overcast, Fog; 0.15 cm of precipitation | Avg. 0 ℃; Very Overcast, Thick Fog, Haze; 0.20 cm of precipitation | Avg. 0 ℃; Completely Overcast, Fog, Windy; 0.25 cm of precipitation | Very Overcast, Fog, Haze; 1.02 cm of precipitation | 02-11-2020 |
| Avg. 0.56 ℃; Clear, Windy | Avg. 3.9 ℃; Partly Cloudy | Avg. 3.9 ℃; Pretty Overcast, Fog; 1.16 cm of precipitation | Very Overcast, Thick Fog; 0.76 cm of precipitation | 02-25-2020 |
| Avg. 3.9 ℃; Some Clouds | Avg. 7.8 ℃; Clear | Avg. 12 ℃; Clear, Windy NEPTM | Actively raining during sampling; Very overcast; 1.12 cm of precipitation | 03-10-2020 |