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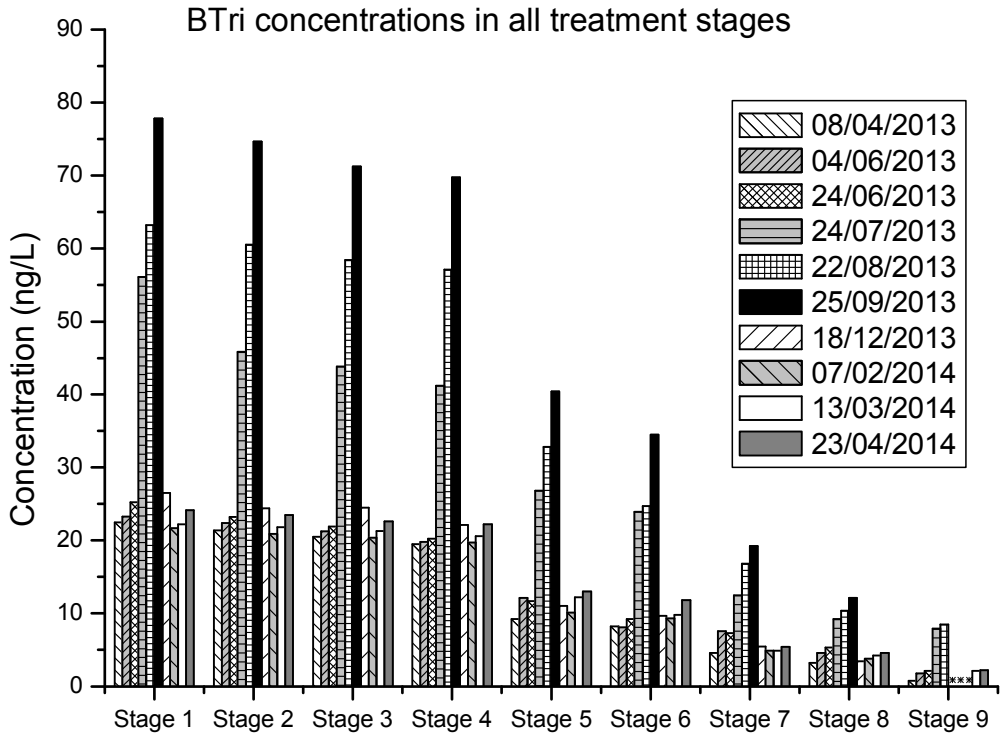
Graphical and textual abstract

A simplified analytical method was developed and used to assess the occurrence of benzotriazole and 5-methyl benzotriazole and removal rates in various Western Australian environmental water samples.



Environmental impact

To assess the environmental impact of benzotriazole and 5-methyl benzotriazole contamination in various Western Australian environmental waters, the source and removal rates of these compounds from environmental waters are required. Here we report (i) the development of a simplified analytical method for detecting the occurrence benzotriazole and 5-methyl benzotriazole in water samples at ultra-low (ng L^{-1}) concentrations; (ii) the occurrence of these compounds in wastewater and surface water samples and; (iii) assess their removal rates through an advanced water recycling plant.

Benzotriazole and 5-methylbenzotriazole in recycled water, surface water and dishwashing detergents from Perth, Western Australia: Analytical method development and application

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ABSTRACT

A simplified and sensitive liquid chromatography mass spectrometry (LC-MS) method without requiring sample pre-concentration was successfully developed for detecting the occurrence of ultra-low (ng L^{-1}) concentrations of benzotriazole (BTri), and its derivative 5-methyl benzotriazole (5-MeBT) in various Western Australian environmental water samples. The method detection limit was 2 ng L^{-1}, providing similar detection limits to other more process intensive methods where pre-concentration using solid phase extraction (SPE) was employed.

The method was used to assess the occurrence of BTri and 5-MeBT in wastewater and surface water samples. Over a period of 12 months, BTri and 5-MeBT concentrations in secondary treated wastewater were measured, with the highest BTri and 5-MeBT concentrations observed during winter months at 78 ng L^{-1} and 21 ng L^{-1}, respectively. The method was also used to assess the removal efficiency of BTri and 5-MeBT through an advanced water recycling plant (AWRP). While BTri was more persistent than 5-MeBT, both compounds were removed from the AWRP to < 10 ng L^{-1} (BTri) and < 2 ng L^{-1} (5-MeBT), with reverse osmosis (RO) providing the most effective treatment process for their removal.

Keywords: wastewater; occurrence; dishwashing detergents; analysis; LC-MS.

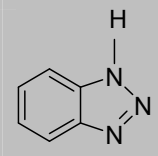
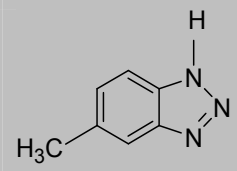
1. Introduction

Benzotriazole (BTri) and its derivative 5-methylbenzotriazole (5-MeBT) are widely used in various industrial applications such as ultraviolet light stabilisers, photographic agents, anti-fogging agents, corrosion inhibitors in aircraft de-icing/anti-icing fluids (ADAFs), and household dishwasher detergents[1,2,3,4]. Due to BTri and 5-MeBT being non-flammable and having anti-corrosion properties, these compounds are highly concentrated within ADAFs and increasingly applied to aircraft runways[5,6,7]. Additionally, BTri and 5-MeBT (BTs) are incorporated into dishwasher tablets for their polishing and silver protection properties[1]. BTs have been considered as the second most abundant water contaminants following ethylenediaminetetraacetate (EDTA)[8]. The Australian government water guidelines state that 7 ng L^{-1} is the permissible concentration of 5-MeBT in drinking water[9], while a health guideline of 20 µg L^{-1} has been suggested for BTri [10].

The chemical properties of BTs (Table 1) make it difficult to remove them through conventional wastewater treatment plants (WWTP) and may lead to their entrance into waterways through waste discharge systems[1,11,12,13]. Collectively, these point to why BTs have been found in the environment samples throughout the world including river water, groundwater, drinking water, wastewater, soil, as well as human urine samples[1,12,13,14].

Many studies from various nations indicate that sewer systems and dishwashing detergents are possible sources of BTri and 5-MeBT contamination of natural water bodies[1,2,4,7,17,19,20]. Studies conducted across Western Europe found the averge BTri concentration of 2.9 µg L^{-1} in effluents of municipal WWTP[7]. Likewise, BTri and 5-MeBT have been recently detected in a municipal WWTP in South Australia, with concentrations ranging between 928 and 5,706 ngL^{-1} (BTri) and 1,438 and 6,758 ng L^{-1} (5-MeBT)[19]. However, as Australia imports less than 100 tonnes of BTs annually[10], only limited studies have been undertaken to determine the occurrence of BTs in Australian WWTPs[10,19]. Nonetheless, the frequent usage of these compounds in detergents is believed to lead to the wider distribution of BTs throughout the environment.

Table 1– Chemical structure and relevant properties of BTri and 5-MeBT.

Common name	**Benzotriazole (BTri),** **1H-Benzotriazole**	**5-Methylbenzotriazole (5-MeBT),** **5-Tolylitriazole (5-Trri)**	**References**
Chemical structure			
CAS No.	95_14_7	136_85_6	
Log K_{ow}	1.23	1.89	15
Log K_{oc}	1.02	1.68	15
Melting point (°C)	98-99°C	76-87°C	16
pK_a (conjugated acid)	8.2	8.5	15
Vapour pressure (°C)	0.04 mmHg	0.03 mmHg	16
Solubility in water	28 g L^{-1}	7 g L^{-1}	17
Solubility in methanol	1.33 g L^{-1}	1.16 g L^{-1}	16
Refractive index	1.73	1.68	18
Henry's low coefficient (at 25 °C)	3.17×10^{-7} m^3/mol	3.14×10^{-7} m^3/mol	16

To efficiently assess the distribution of BTs in surface waters and wastewaters, rapid and reliable analytical methods with low detection limits are required. However, to achieve low level detection of BTri and 5-MeBT, pre-concentration of environmental water samples using solid phase extraction (SPE), have been previously used, prior to sample analysis by liquid chromatography mass spectrometry (LC-MS)[7,10,17,21]. The lowest detection level to date with pre-concentration was Loi et al.[10] who achieved a detection limit of 4 ng L^{-1} for BTri and 5 ng L^{-1} for 5-MeBT. Without pre-concentration, the lowest detection level achieved was 33 ng L^{-1} by Weiss and Reemtsma[13].

In this paper, we describe a simplified and sensitive LC-MS method, without sample pre-concentration, that was successfully developed to detect BTri and 5-MeBT in environmental water samples at low ng L^{-1} concentrations. The LC-MS method was selected for use in this study because of its benefits over GC-MS[22,23]. First, LC-MS can determine polar analytes without the need for derivatization, and is not restricted by compound-of-interest specific limitations that hinder GC-MS[24,25]. Conversely, GC-MS is restricted to detection based solely on volatility and molecular weight[26]. When BTs are analysed using GC, it often results in irreversible absorption, smearing, and/or possible complete peak disappearance. Thus, LC-MS has broader analytical applications for detecting polar contaminants such as BTs[3]. The LC-MS method was selected on the basis of its advantages over GC-MS.

Using this developed method, the occurrence of BTs in wastewater, recycled water and surface water samples from Perth Western Australia were investigated. Also, the removal efficiency of BTs from the various treatment processes of an advanced water recycling plant (AWRP) was investigated over a 12 month period.

2. Materials and methods

2.1. Chemicals and materials

The compounds, 1-H-benzotriazole (BTri, 99%), 5-methyl-benzotriazole (5-MeBT, 98%) and the reference standards, Leucine (Leu) enkephalin acetate salt hydrate (Leu-enkephalin, 95%) were purchased from Sigma-Aldrich (Sydney, Australia). 1H-benzotriazole-D4 (4d-BTri, 97%) was purchased from Toronto Research Chemicals (Toronto, Canada). Triethylamine (99%) was purchased from Merck Schuchardt OHG, (Hohenbrunn, Germany). Milli-Q water (10 mΩ) was obtained from ultrapure water system (Sydney, Australia). High performance liquid chromatography (HPLC) grade methanol (MeOH) was obtained from Merck (Darmstadt Germany) and formic acid (98%) analytical grade from purchased from Ajax Finechem (Taren Point, Australia). A 0.45 µm Supor® membrane syringe filter was obtained from Pall Corporation (Port Washington, USA). Dishwashing products (10) were bought from local supermarkets in Perth, Western Australia.

2.2. Analytical method

2.2.1. LC-MS analysis

Analysis of the target compounds, BTri and 5-MeBT, was performed using a Waters Alliance e2695 HPLC system (Waters, Milford, MA, USA). The mobile phase was a water methanol mixture (70:30) modified with 0.1% formic acid. The separation of BTs was achieved using a 3.5 µm 2.1×100 mm XBridge Phenyl column with 3.5 µm particle size (Waters, Milford, MA, USA). The column temperature was 30 °C and 50 µL of sample volume was injected into the HPLC with a flow rate of 0.3 mL min^{-1}.

Note, commercially available methyl-benzotriazole consists of a mixture of both the 5-MeBT and the 4-methyl-benzotriazole (4-MeBT) isomer, and the analytical method used could not separate 5-MeBT from the 4-MeBT isomer. Therefore, while 5-MeBT results of field (wastewater, recycled water and surface water) and detergent analysis are given in this paper, the 5-MeBT data are likely a mixture of both 4-MeBT and 5-MeBT isomers.

2.2.2. Mass spectrometry

A LCT Premier XE time-of-flight (TOF) mass spectrometer (Waters MS Technologies, Manchester, UK) equipped with an electrospray interface (ESI) operated in the positive ion mode. The operating system of the spectrometer was Mass Lynx. Pure nitrogen gas supplied from a nitrogen generator (Parker Domnick Hunter) was used as a nebulising and drying gas. The temperature of desolvation was 300 °C. The source temperature was 80 °C with a desolvation gas flow of 750 L h^{-1}, and the cone gas flow of 10 L h^{-1}. The capillary voltage was 3 kV and the sample voltage was 60 V.

2.2.3. Accurate mass

Instrument calibration was achieved using a solution of sodium formate. The solution consisted of (i) 0.5 mL of 10% (v/v) formic acid in MQ-water; (ii) 0.5 mL of 0.05 M sodium hydroxide; and (iii) 9 mL of 9:1 (v/v) 2-propanol in MQ-water. This sodium formate solution

was modified with triethylamine (10 µL in 10 mL of sodium formate solution). A standard sample of Leu-enkephalin reagent was used as an external calibrant at a concentration of 200 pg μL^{-1}.

2.2.4. Mobile phases selection for LC-MS analysis

To optimise the separation of the BTs and the sensitivity of the analysis, a range of varying compositions of the mobile phase were examined. The HPLC conditions were tested using two different ion sources (APCI and ESI) in positive and negative ion modes. The solvent mixtures were prepared from acetonitrile (ACN), MeOH and water with and without formic acid addition. The mobile phases examined included : 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 80:20 and 90:10 of (i) 0.1% formic acid in water + ACN ; (ii) 0.1% formic acid in water + MeOH; (iii) water + ACN; and (iv) water + MeOH. Several samples of the BTs were injected directly into the mass spectrometer for testing optimum instrumental conditions (without HPLC column). The HPLC separation column (XBridge Phenyl column - Waters) was thereafter used for all experiments. The testing concentration was 500 µg L^{-1} and the injection volume was 10 µL. Further, different mixtures and solvent ratios of mobile phases were examined.

2.2.5. Method validation: stability and reproducibility

BTri and 5-MeBT stock solutions were prepared in MQ-water. A set of calibration standards prepared with concentrations of 2, 6, 10, 20, 40, 60, 80 and 100 ng L^{-1} were stored at 4 °C and analysed within 24 hours. A pure sample of MQ-water was analysed as a blank to determine if there was any contamination occurring during sample preparation. Consequently, calibration curves of the nine samples were obtained by plotting the concentration against the peak area.

Three different fresh stock solution standards of BTs samples were prepared in three different time periods as well as in different instrument set-ups and calibrations. Samples from these different stock solutions were analysed in eleven replicates to ensure that the method was stable and produced consistent results when using similar aquatic

environmental samples (Table S1 and Table S2). Additionally, five replicates at the same concentrations mentioned above were analysed at three different injection volumes 30 µL, 50 µL and 70 µL to confirm that the method was reproducible.

2.2.6. BTri and 5-MeBT Stability

The stability of numerous standards containing each compound (BTri and 5-MeBT) was examined at -28 °C, 4 °C, 25 °C and analysed at 0, 30, 60 and 90 days. The BTs were dissolved in water at 5, 10, 50 and 100 ng L^{-1} and analysed in five replicates to investigate whether the BTs analytical standards were stable for analysis in a workable timeframe.

2.3. Sample collection and preparation

2.3.1. Surface water

Surface water samples were collected from a small urban lake in Perth, Western Australia three times throughout 2013. Water samples were collected from a stream feeding the lake and the lake itself. The samples were collected at different times between March and July from different locations around the lake perimeter. These samples were collected in 1 L glass-bottles, which were then immediately sealed and transported to the laboratory. On arrival, samples were immediately filtered using a 0.45 µm syringe filter before storage at 4 ^{o}C and analysed within 24 hours.

2.3.2. Wastewater and recycled water samples

Water samples were collected monthly for 12 months from the Beenyup AWRP that used secondary treated wastewater from the Beenyup Wastewater Treatment Plant (WWTP) in Perth, Western Australia, as feed water. The treatment processes within the AWRP consisted of ultrafiltration (UF), reverse osmosis (RO), and ultraviolet (UV) disinfection. Prior to UF, the water was chlorinated to prevent biofouling of treatment

infrastructure. The highly treated water from the AWRP was then to be used as influent water managed aquifer recharge (MAR). A schematic for the AWRP processes and sampling locations are illustrated in Figure. 1. Water samples were collected from the following locations within the AWRP: S1, raw feed (secondary treated wastewater); S2, feed water after ammonia and hypochlorite dosing; S3, after UF treatment; S4, after sulphuric acid dosing; S5, after RO treatment; S6, after degassing; S7, after stage 1 UV disinfection and sodium hydroxide dosing; S8, after stage 2 UV disinfection; and S9, recycled water prior to aquifer recharge. Recycled water samples were collected in 1 L glass-bottles. The samples were kept cold during transport to the laboratory and then filtered using a 0.45 µm Supor® Membrane syringe filter. Samples were then stored at 4 ^{o}C prior to analysis.

2.3.3. Dishwashing detergent samples

To determine if the source of BTri and 5-MeBT in Australian environments could be from dishwasher soaps/detergents, as has been previously suggested in the literature[9,10,19], 10 commercial soaps/detergents from local markets around Perth, Western Australia were tested. Two mL of liquid and 2 g of powdered soap detergents were dissolved in 1 L of MQ-water. For these samples a more intensive sample preparation was required. Non-dissolved material was removed by filtration and interfering compounds were removed from the filtrate using a SPE clean-up step. All samples (10 mL) were filtered using a 0.45 µm syringe filter prior to clean-up. Following filtration, all samples were spiked with 4d-BTri before SPE clean-up step.

For sample clean-up, Oasis HLB cartridges were used (6 mL polypropylene, 500 mg; Waters, Milford, MA, USA) after conditioning with 10 mL of MeOH, then 10 mL of acidified water (pH 2.95 ± 0.05). A water sample (5 mL) was then passed through the cartridge, and the cartridge dried under vacuum (20 minutes). The cartridge was then washed with 2 x 5 mL acidified water (pH 2.9 ± 0.05) /MeOH (95:5% v/v), and then dried under vacuum (20 minutes). Dry residues were then eluted from the cartridge with 10 mL of MeOH/ACN (50:50), and the extract was evaporated to near-dryness under nitrogen and transferred into a HPLC vial for LC-MS analysis.

3. Results and Discussion

3.1. LC-MS conditions and signal optimisation

The best separation of BTs was achieved using a mobile phase with a 70:30 mixture of formic acid (0.1%) in MQ-water and MeOH. This optimum LC mobile phase condition was subsequently used in order to test MS parameters for optimum detection limits. The analysis of BTs by MS was examined with both APCI and ESI ion sources tested in positive and negative ion modes. Ion source parameters such as capillary voltage or corona conditions, sample cone, desolvation temperature, ion source temperature, flow rate and gas flow were trialled to optimise peak area signals for BTs. The positive ion mode of both ESI and APCI was found to have higher sensitivity than the negative mode. The optimal ion source conditions for the BTri and 5-MeBT peak area signals are shown in Table 2.

Table 2- Tabulated data and optimised HPLC conditions with testing different ion sources (APCI and ESI).

Ion Source Conditions	APCI	ESI	Optimum BT detection parameters
Positive/negative mode	Both	Both	Positive ion mode
Corona or Capillary V	6-20 (uA)	1000-3000V	3000 V (ESI) or 7 (uA) (APCI)
Sample cone	40-100V	40-100V	60 V
Source Temp.	60-120 ^{o}C	60-120 ^{o}C	80 ^{o}C (ESI) 60 ^{o}C (APCI)
Desolvation Temp.	200-350 ^{o}C	200-350 ^{o}C	300 ^{o}C or 350^{o}C (ESI) 250 ^{o}C (APCI)
Gas Flow	250-750 L hr^{-1}	250-750 L hr^{-1}	750 (ESI) 750 (APCI)
Flow Rate	0.5-0.2 mL min^{-1}	0.5-0.2 mL $^{-1}$	0.3 mL min^{-1}

Using the optimum mobile phase and ESI conditions, a detection limit for BTri and 5-MeBT of 2 ng L^{-1} was achieved. BTri and 5-MeBT were detected at *m/z* 120.055 and *m/z* 134.069; and the internal standard 4d-BTri was determined at *m/z* 124.083. The lowest detection limit accomplished using APCI was 700 ng L^{-1}. The optimised LC conditions allowed good separation of all the analytes and internal standard (Figure 2).

3.2. Reproducibility and Linearity

The reproducibility of the LC-MS method was confirmed using 11 freshly prepared replicate standards. The combined experimental errors for the standards solution analysis and the dilution errors were calculated and shown in the supporting information (Figure S1). The standard errors of BTri and 5-MeBT were 0.0021 ng L^{-1} and 0.0020 ng L^{-1}, respectively. The normalised results show good reproducibility. The calibration curve for all normalised results and errors are shown in Figure S1. Fitting was performed using the York algorithm (2004) for linear least squares regression[27].

A nine-point calibration curve of BTri and 5-MeBT was prepared separately in concentrations of 2, 4, 6, 10, 20, 40, 60, 80 and 100 ng L^{-1} (Table S1 and Table S2) to test the linearity of the analysis as shown in Figure S1. The correlation coefficients (R^2) were 0.999 for 5-MeBT and 0.995 for BTri. The greater variability of BTri (as shown in Figure S1.) was assumed to be due to dilution errors of the sample standards in one of the 11 replicates.

3.3. BTri and 5-MeBT stability and method variability

The results (Table 3) illustrated a negligible amount of loss of the BTs at -28°C. Loss was observed at 4 °C and 25 °C, with substantial loss between 60 to 90 days. Greatest loss was observed at 25°C (Table 3). Earlier sampling times showed minor losses of both compounds at 25°C, and negligible loss at 4°C and -28°C. At -28°C the compounds concentration remained stable for at least 90 days.

Table 3- BTri and 5-MeBT stability at different concentrations and times, for different temperatures.

Target compounds	Conc. (ng L^{-1})	Day (30)			Day (60)			Day (90)		
		25 °C	4 °C	-28 °C	25 °C	4 °C	-28 °C	25 °C	4 °C	-28 °C
BTri	5	4.7	5	4.9	3.8	4.6	4.8	nd	3.9	5
	10	9.3	9.9	10	7.3	9.1	9.8	4.8	8	9.9
	50	46.5	49.8	49.9	37.1	45.6	50	24.6	39.8	49.8
	100	92.7	100	99.9	74.2	92.4	100	49.4	79.6	99.9
5-MeBT	5	4.6	4.9	5	3.3	4.2	4.9	nd	3.5	5
	10	8.9	9.8	9.9	6.6	8.2	9.9	3.9	7.1	9.9
	50	45.1	49.7	49.8	33.3	41.8	49.8	19.7	34.8	49.8
	100	90.7	99.8	99.8	67.8	83.6	99.9	38.8	70.5	100

nd= not detected

3.4. Comparison to previous research

Previously published research using LC-MS for the analysis of BTri and 5-MeBT in water samples is shown in Table 4. Various LC-MS systems have been used to analyse BTs from environmental water samples. ESI ion source has been widely applied to measuring concentration of BTs in aqueous solutions, compared to APCI. There are a number of studies that have utilised different mass analysers, with the TOF mass analyser frequently used. The advantages of the LC/TOF/MS as an analytical technique for detecting BTs is its capacity for providing contaminant quantification in complex environmental samples[28].

Without pre-concentration, the lowest detection level achieved using LC-MS was 33 ng L^{-1} by Weiss and Reemtsma[13]. Here, through optimisation of instrument parameters and chromatographic conditions, a detection limit of 2 ng L^{-1} for BTri and 5-MeBT was achieved, with a limit of quantification of 6 ng L^{-1}. This detection limit was comparable to the lowest pre-concentration method of Loi et al.[10] who achieved a detection limit of 4 ng L^{-1} for BTri and 5 ng L^{-1} for 5-MeBT.

Table 4 -Previously published research conducted using LC-MS for the analysis of BTri and 5MeBT in water samples.

Matrix	LC-MS system	Type of Mass Analyser	Limit of detection	SPE Cartridge Type	Reference
Without pre-concentration					
Wastewater and surface waters	LC-ESI-MS + ion	TOF	2 ng L^{-1}	-	This study
Municipal wastewater and groundwater	LC-ESI-MS/MS + ion	TOF	100 ng L^{-1}	-	7
Untreated wastewater and Treated wastewater	LC-ESI-MS/MS + ion	TOF	33 ng L^{-1}	-	13
With pre-concentration					
Municipal wastewater and groundwater	LC-ESI-MS/MS + ion	TOF	100 ng L^{-1}	Oasis HLB (60 mg)	7
Drinking water surface water Ultrapure water	LC-ESI-MS/MS + ion	Orbitrap	100 ng L^{-1}	Oasis HLB (5mL glass cartridges, 200 mg) Oasis HLB (6mL polypropylene, 500mg)	21
Lakes River waters	LC-ESI-MS/MS + ion	Quadrupole	20 ng L^{-1}	Oasis HLB (60 mg, 3 mL)	17
Tank water	LC-ESI-MS/MS + ion	Quadrupole	>1000 ng L^{-1}	Oasis HLB (60 mg, 3 mL)	29
Municipal wastewater River water	LC-ESI-MS/MS + ion	Quadrupole	>1000 ng L^{-1}	Oasis HLB (60 mg, 3 mL)	8
Wastewater	LC-ESI-MS/MS + ion	Trap-Orbitrap	BTri= 4 ng L^{-1} 5-MeBT= 5 ng L^{-1}	Oasis HLB (500 mg, 6 mL)	10

279

280

3.5. Detection of BTri and 5MeBT in Surface water samples

Water samples were collected from a small lake in Perth, Western Australia to assess if BTs were present in Australian surface water environments. BTs were detected at concentrations ranging between 11 to 79 ng L^{-1} for BTri and 2 to 46 ng L^{-1} for 5-MeBT (Table 5). Concentrations of both compounds increased in the stream and lake water samples from March to July 2013, mirroring the increased rainfall patterns for this area. In March, and prior to annual rainfall, ultra-low concentrations were detected, with 5-MeBT down to 2 ng L^{-1} and BTri at 11 ng L^{-1} (see Table 5). Temporal variations in concentration were large for all BTs (see Table 5), and may indicate seasonal contamination during periods of more rapid stream flow as BTs are flushed from contaminant sources into the Lake. Concentration differences over time are not unusual, as Giger et al.[17] reported similar variations in BT concentrations across several Swiss lakes.

Table 5- BTri and 5-MeBT concentrations in lake and stream samples analysed in three different times.

Target compounds	Lake (ng L^{-1})						Stream (ng L^{-1})					
	March 2013	SD	May 2013	SD	July 2013	SD	March 2013	SD	May 2013	SD	July 2013	SD
BTri	11	1	14	0.8	27	0.9	21	0.2	33	1.4	79	2.1
5-MeBT	2	0.4	8	0.9	24	1.3	16	1.1	28	1.2	46	1.9

SD= standard deviation

The observed concentrations of both compounds were higher in the colder month of July (2013), compared to the warmer month of March (2013) which is similar to findings in the United States and Germany, where BTri and 5-MeBT have been more abundant in the colder months[30,31,32]. Note, these previous studies were conducted in areas with possible ADAF input, which could explain the BTs contamination[33]. However, the climatic conditions in our study area are mild and ADAFs are not used. Other potential BTs sources could include detergents, antifreeze formulation used in cars, and corrosion inhibitors. Variations in temporal concentrations of BTri and 5-MeBT could indicate (i) variable emission sources with differing compositions and/or (ii) different removal processes, such as

photodegradation and/or sorption depending on compound properties. Additionally, BTri concentrations were higher than 5-MeBT across all time points. This could reflect source contamination product formulation. For example, detergents (see Table 7) have greater proportion of BTri compared to 5-MeBT.

The stream samples contained a much higher concentration of BTri and 5-MeBT than the lake (Table 5). Thus, the disparity between the stream and the lake suggests that dilution and/or the photo-transformation of lake water was occurring. The source of BTs in the stream was not identified and an extended investigation at different sampling times and sampling locations would be required to identify potential inputs.

3.6. Detection of the BTri and 5MeBT in wastewater and recycled water samples

Over the period of one year, from April 2013 to April 2014, wastewater and recycled water samples were collected 10 times from the Beenyup AWRP in Perth, Western Australia. Samples were collected approximately monthly, however times were varied to coincide with periods of prolonged stable operation. As such two sampling events were unable to be conducted due to scheduled maintenance of the AWRP. Each time, nine samples were collected from different sampling locations within the AWRP (Figure 1). Higher concentrations were observed for BTri than for 5-MeBT in all water samples (Figures 3 and 4). The highest concentration of both compounds were detected in S1 (raw feed - secondary treated wastewater) during September 2013 at a concentration of 78 ng L^{-1} and 21 ng L^{-1} for BTri and 5-MeBT, respectively. Average removal of BTs for each treatment process as a percentage of the AWRP feed water (S1) is given in Table 6. These results showed that the most effective treatment for BTs removal was the RO process with 38% (BTri) and 52% (5-MeBT) removal.

The concentrations of BTri and 5-MeBT in the secondary treated wastewater used as the feed water to the AWRP were comparable to concentrations determined in a previous study conducted in Greece[34]. A strong seasonal trend was observed (Figures 3 and 4) with substantially higher feed water concentrations in July, August and September, being the winter months of the year. The minimum feed water concentrations were 22 ng L^{-1} for BTri and 6.5 ng L^{-1} for 5-MeBT in February (2014) while maximum feed water concentrations

were 78 ng L^{-1} (BTri) and 21 ng L^{-1} (5-MeBT), in September and August (2013). The lower influent concentrations of BTs into the AWRP during the summer season may have resulted from the increased temperature of wastewater within the WWTP, enhancing evaporation or degradation during wastewater treatment, such as the aerobic treatment stage[35,36].

Table 6-Removal efficiency of BTri and 5-MeBT for each wastewater treatment process.

Monitoring stage	Treatment processes and dosing steps	Percentage of Initial Concentration Removed	
		BTri	5-MeBT
S1 to S2	Ammonia and hypochlorite dosing	6	11
S2 to S3	Ultrafiltration Treatment	3	8
S3 to S4	Sulphuric acid dosing	4	17
S4 to S5	Reverse osmosis (RO)Treatment	38	52
S5 to S6	Degassing after RO	8	7
S6 to S7	Stage 1 UV treatment, sodium hydroxide dosing and pH modification	16	5
S7 to S8	Stage 2 UV Treatment	7	nc
S8 to S9	Storage in post-treatment tank and prior to aquifer recharge	10	nc

nc = not calculated as concentrations were below the detection limit.

BTri and 5-MeBT concentrations showed substantial removal through the AWRP (see Figures 3 and 4). The BTri concentrations decreased by 87%, while 5-MeBT concentrations were removed to below detection level (<2 ng L^{-1}), except during the winter months of July, August and September when feed water concentrations were higher.

The AWRP was consistent at efficiently removing BTri and 5-MeBT from the secondary treated wastewater to < 10 ng L^{-1}, and < 2 ng L^{-1} respectively. BTri removed to < 2 ng L^{-1} was observed on 4 out of the 10 sampling occasions. These results suggest that the recycled water produced from the AWRP would be suitable for groundwater replenishment.

The removal efficiency of each treatment process or dosing step within the AWRP was calculated based on the concentration difference between the sampling locations prior and after the treatment process/dosing step, and expressed as a percentage of feed water concentration into the AWRP (i.e. S1). As expected, there were substantial differences in

BTri and 5-MeBT reduction for the treatment processes (see Table 6) compared to the dosing steps, as the dosing steps were not designed to remove BTs, but provide infrastructure protection (minimising biofouling and scaling) and pH amendment. The dominant treatment process in removing BTs was RO (S4 to S5), with BTri and 5-MeBT concentrations being reduced by 38% and 52%, respectively. The improved removal of 5-MeBT compared to BTri was likely a reflection of the slightly higher hydrophobic properties of 5-MeBT (log K_{ow} of 1.89 for 5-MeBT compared to log K_{ow} of 1.23 for BTri).

The combined treatments of UF and RO (S1 to S4) resulted in a reduction of 51% and 88% respectively. The reduction of 5-MeBT was consistent with a previous study by Loi et al.[10], where 85% removal of 5-MeBT was observed for combined UF and RO treatment[10]. A reduction of ~ 60% for BTri and 5-MeBT between influent wastewater to RO permeate was reported during a 1.5 year investigation of an upgraded municipal wastewater plant[37].

3.7. Detection of the BTri and 5M-eBT in soap detergent samples

To test whether commercial cleaning agents could be the source of the BTs contamination observed in wastewater, 10 commercially available soaps and detergents were screened for BTs. The soaps and detergents analysed were recommended for the use of cleaning dishes, clothing and the human body. As shown in Table 7, BTri was detected in four liquid soap samples (40%), whereas 5-MeBT was only detected in two (20%). The highest concentrations were detected in liquid dishwashing detergent S2 at 4600 ng L^{-1} (BTri) and 2600 ng L^{-1} (5-MeBT). Samples S1 and S2 contained both compounds, while S3 and S4 only contained BTri. The lowest BTri concentration found was 680 ng L^{-1} in S4, which was a clothing detergent. The other six washing detergents/soaps (S5 to S10) did not show the presence of BTs. Note, both powdered soaps did not show the presence of either compound. The variation in product formulations of the different manufactures is the likely reason for the variation in BTri and 5-MeBT concentrations within the different detergents.

Table 7- BTri and 5-MeBT concentrations in washing products samples.

Target compounds	Washing products (ng L^{-1})				
	S1	S2	S3	S4	S5 to S10
BTri	2800	4600	1500	680	nd
5-MeBT	2000	2600	nd	nd	nd

nd*= below the detection limit.

In South Australia, BTs have been reported in municipal WWTPs and drinking water samples[9,19]. The frequent use of detergents may largely underpin the widespread distribution of BTs throughout Australian environments. Prior to this study, there was no data available assessing BTri and 5-MeBT in commercially available Australian dishwashing detergents.

This study indicated that one possible cause of BTri and 5-MeBT accumulation in Australian environments is the use of household soaps and detergents, and their subsequent discharge to wastewater. This is a likely given Australia's use of ADAFs is minimal, unlike other counties where ADAFs are significant contributors to BT contamination[9,19]. As indicated in Table 7, each liquid washing detergent contained a seemingly low mean BT concentration of 958 ng L^{-1} (BTri) and 460 ng L^{-1} (5-MeBT). However, taking into consideration the potential collective amount of washing detergents that are being used in Australia, the combined environmental discharge could be substantial. This aligns with previous studies implicating dishwashing products as potential wastewater contaminants[1,2,4,7,17,19,20]. Vetter and Lorenz[4] also showed that 99% of BTs will travel into WWTPs via dishwasher effluents. It could also be inferred that similar collective accumulation as a result of dishwashing products, coupled with the use of ADAF's in Europe and North America are attributing to the higher concentrations in the environment[1, 38,39].

The properties of both BTri and 5-MeBT such as oxidative resistance, UV resistance, and high solubility would facilitate their longevity under environmental conditions and

perhaps accumulation to toxic levels, if treatment processes are ineffective in attenuating wastewater concentrations.

4. Conclusions

This paper discusses the development of a simplified and sensitive liquid LC-MS method without sample pre-concentration was successfully developed for detecting the occurrence of ultra-low (ng L^{-1}) concentrations of BTri and 5-MeBT in various Western Australian environmental water samples. Through optimization of instrument parameters and chromatographic conditions, a detection limit of 2 ng L^{-1} was achieved for BTri and 5-MeBT, which was similar to other more process intensive methods where pre-concentration using solid phase extraction (SPE) was employed.

This method was subsequently used to: (i) assess the effectiveness of an AWRP for the removal of BTs from wastewater, and (ii) investigate BTri and 5-MeBT concentrations in a surface water environment. The removal of BTs from wastewater was successful using the AWRP, where BTri and 5-MeBT concentrations were reduced to < 2 ng L^{-1}, and 10 ng L^{-1} respectively. RO treatment was the most effective removal process within the AWRP.

In a surface water environments, a Perth lake and in the stream water feeding the lake, BTs were present. The higher stream water concentrations suggested the lake contamination was a result of the contaminated stream water. However, the source of BTs in the stream was not identified. A potential source of BTri and 5-MeBT in wastewater and possibly surface waters could be washing detergents as BTs were detected in 40% of the washing detergent samples analysed.

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437 **REFERENCES**

438

439 1. H. Janna, M. D. Scrimshaw, R. J. Williams, J. Churchley and J. P. Sumpter. From
440 Dishwasher to Tap? Xenobiotic Substances Benzotriazole and Tolyltriazole in the
441 Environment. *J. Environ. Sci. Technol.* 2011, 45, 3858-3864.
442 2. A. Kiss and E. Fries. Seasonal source influence on river mass flows of benzotriazoles.
443 *J. Environ. Monit.* 2012, 14, 697-703.
444 3. M. Pervova, V. Kirichenko and V. Saloutin. Determination of 1,2,3-benzotriazole in
445 aqueous solutions and air by reaction-gas-liquid chromatography. *J. Anal. Chem.*
446 2010, 65, 276-279.
447 4. W. Vetter and J. Lorenz. Determination of benzotriazoles in dishwasher tabs from
448 Germany and estimation of the discharge into German waters. Environ. Sci. Pollut.
449 Res. 2012, 1-6.
450 5. A. Kiss and E. Fries. Occurrence of benzotriazoles in the rivers Main, Hengstbach, and
451 Hegbach (Germany). *Environ. Sci. Pollut. Res. Int.* 2009, 16, 702-710.
452 6. A. M. Sulej, Ż. Polkowska and J. Namieśnik. Pollutants in Airport Runoff Waters. Crit.
453 Rev. *Env. Sci. Technol.* 2011, 42, 1691-1734.
454 7. S. Weiss, J. Jakobs and T. Reemtsma. Discharge of Three Benzotriazole Corrosion
455 Inhibitors with Municipal Wastewater and Improvements by Membrane Bioreactor
456 Treatment and Ozonation. *J. Environ. Sci. Technol.* 2006, 40, 7193-7199.
457 8. D. Voutsa, P. Hartmann, C. Schaffner and W. Giger. Benzotriazoles, Alkylphenols and
458 Bisphenol A in Municipal Wastewaters and in the Glatt River, Switzerland. *Environ.*
459 *Sci. Pollut. Res.* 2006, 13, 333-341.
460 9. NRMMC, 2008. Australian Guidelines for Water Recycling Augmentation of Drinking
461 Water Supplies, Natural Resource Management Ministerial Council, Environment
462 Protection and Heritage Council, and National Health and Medical Research Council,
463 Adelaide.
464 10. C. H. Loi, F. Busetti, K. L. Linge and C. A. Joll. Development of a solid-phase extraction
465 liquid chromatography tandem mass spectrometry method for benzotriazoles and

benzothiazoles in wastewater and recycled water. *J. Chromatogr. A* 2013, 1299, 48-57.

11. E. Kadar, S. Dashfield and T. Hutchinson. Developmental toxicity of benzotriazole in the protochordate, Ciona intestinalis (Chordata, Ascidiae). *Anal. Bioanal.Chem.* 2010, 396, 641-647.
12. T. Reemtsma, S. Weiss, J. Mueller, M. Petrovic, S. González, D. Barcelo, , F. Ventura and T. P. Knepper. Polar Pollutants Entry into the Water Cycle by Municipal Wastewater: A European Perspective. *J. Environ. Sci. Technol.* 2006, 40, 5451-5458.
13. S. Weiss and T. Reemtsma. Determination of Benzotriazole Corrosion Inhibitors from Aqueous Environmental Samples by Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry. *Anal. Chem.* 2005, 77, 7415-7420.
14. A. G. Asimakopoulos, L. Wang, N. S. Thomaidis and K. Kannan. Benzotriazoles and benzothiazoles in human urine from several countries: A perspective on occurrence, biotransformation, and human exposure. *Environ. int*. 2013b, 59, 274-281.
15. D. S. Hart, L. C. Davis, L. E. Erickson and T. M. Callender. Sorption and partitioning parameters of benzotriazole compounds. *Microchem. J.* 2004, 77, 9-17.
16. S. Castro, L. C. Davis and L. E. Erickson. Natural, cost-effective, and sustainable alternatives for treatment of aircraft deicing fluid waste. *Environ. Prog.* 2005, 24, 26-33.
17. W. Giger, C. Schaffner and Kohler, H. P. E. Benzotriazole and Tolyltriazole as Aquatic Contaminants. 1. Input and Occurrence in Rivers and Lakes. *J. Environ. Sci. Technol.* 2006, 40, 7186-7192.
18. R. N. Malhas, N. A. Al-Awadi and O. M. E.El-Dusouqui. Kinetics and mechanism of gas-phase pyrolysis of N-aryl-3-oxobutanamide ketoanilides, their 2-arylhydrazono derivatives, and related compounds. *Int. J. Chem. Kinet.* 2007, 39, 82-91.
19. Y. S. Liu, G. G. Ying, A. Shareef and R. S. Kookana. Occurrence and removal of benzotriazoles and ultraviolet filters in a municipal wastewater treatment plant. *Environ. Pollut.* 2012, 165, 225-232.
20. T. Reemtsma, U. Miehe, U. Duennbier and M. Jekel. Polar pollutants in municipal wastewater and the water cycle: Occurrence and removal of benzotriazoles. Water Res. 2010, 44, 596-604.

21. J. A. van Leerdam, A. C. Hogenboom, M. M. E. van der Kooi and P. de Voogt. Determination of polar 1H-benzotriazoles and benzothiazoles in water by solid-phase extraction and liquid chromatography LTQ FT Orbitrap mass spectrometry. *Int. J. Mass spectrom.* 2009, 282, 99-107.
22. F. Hernández, Ó. J. Pozo, J. V. Sancho, F. J. López, J. M. Marín and M. Ibáñez. Strategies for quantification and confirmation of multi-class polar pesticides and transformation products in water by LC-MS2 using triple quadrupole and hybrid quadrupole time-of-flight analyzers. *TrAC, Trends Anal. Chem.* 2005, 24, 596-612.
23. G. Hopfgartner, E. Varesio, V. Tschäppät, C. Grivet, E. Bourgogne and L. A. Leuthold. Triple quadrupole linear ion trap mass spectrometer for the analysis of small molecules and macromolecules. *J. Mass Spectrom.* 2004, 39, 845-855.
24. W. Giger. Hydrophilic and amphiphilic water pollutants: using advanced analytical methods for classic and emerging contaminants. *Anal. Bioanal.Chem.* 2009, 393, 37-44.
25. J. M. Halket and V. G. Zaikin. Derivatization in mass spectrometry - 1. Silylation. *Eur. J. Mass Spectrom.* 2003, 9, 1-21.
26. M. J. L. de Alda and D. Barcelo. Review of analytical methods for the determination of estrogens and progestogens in waste waters. Fresenius J. Anal. Chem. 371, 437-447.
27. D. York, N. M. Evensen, M. L. Martınez and J. D. B. Delgado. Unified equations for the slope, intercept, and standard errors of the best straight line. *Am. J. Phys.* 2004, 72, 367-375.
28. I. Ferrer and E. M. Thurman. Liquid chromatography/time-of-flight/mass spectrometry (LC/TOF/MS) for the analysis of emerging contaminants. *TrAC, Trends Anal. Chem.* 2003, 2001, 22, 750-756.
29. C. A. Harris, E. J. Routledge, C. Schaffner, J. V. Brian, W. Giger and J. P. Sumpter. Benzotriazole is antiestrogenic in vitro but not in vivo. *Environ. Toxicol. Chem.* 2007, 26, 2367-2372.
30. B. Herzog, H. Lemmer, B. Helmreich, H. Horn and E. Müller.Monitoring benzotriazoles: a 1 year study on concentrations and removal efficiencies in three different wastewater treatment plants. *Water Sci. Technol.* 2014, 69, 710-717.

528 31. V. Matamoros, E. Jover and J. M. Bayona. Occurrence and fate of benzothiazoles and
529 benzotriazoles in constructed wetlands. *Water Sci. Technol.* 2010, 61, 191-198.
530 32. D. A. Pillard. Comparative toxicity of formulated glycol deicers and pure ethylene and
531 propylene glycol to Ceriodaphnia dubia and Pimephales promelas. *Environ. Toxicol.*
532 *Chem.* 1995, 14, 311-315.
533 33. K. S. McNeill and D. A. Cancilla. Detection of triazole deicing additives in soil samples
534 from airports with low, mid, and large volume aircraft deicing activities. Bulletin of
535 Environmental Contamination and Toxicology, 2009, 82, 265-9.
536 34. A. G. Asimakopoulos, A. Ajibola, K. Kannan and N. S. Thomaidis. Occurrence and
537 removal efficiencies of benzotriazoles and benzothiazoles in a wastewater treatment
538 plant in *Greece. Sci. Total Environ.* 2013a, 452, 163-171.
539 35. G. D. Breedveld, M. Børresen and R. Roseth. Degradation of airport contaminants in
540 a soil based treatment plant. In: Proceedings of the First European Bioremediation
541 Conference. Chania, Crete, Greece, 2002, pp. 5–20.
542 36. Liu, Y. S., Ying, G. G., Shareef, A., Kookana, R. S., 2011. Biodegradation of three
543 selected benzotriazoles under aerobic and anaerobic conditions. Water Res. 45,
544 5005-5014.
545 37. A. Joss, C. Baenninger, P. Foa, S. Koepke, M. Krauss, C. S. McArdell, K. Rottermann, Y.
546 Wei, A. Zapata and H. Siegrist. Water reuse: >90% water yield in MBR/RO through
547 concentrate recycling and CO2 addition as scaling control. *Water Res.* 2011, 45,
548 6141-6151.
549 38. G. D. Breedveld, R. Roseth, M. Sparrevik, T. Hartnik and L. J. Hem. Persistence of the
550 De-Icing Additive Benzotriazole at an Abandoned Airport. *Water Air Soil Pollut.* 2003,
551 Focus 3, 91-101.
552 39. R. Loos, B. M. Gawlik, G. Locoro, E. Rimaviciute, S. Contini and G. Bidoglio. EU-wide
553 survey of polar organic persistent pollutants in European river waters. *Environ.*
554 *Pollut.* 2009, 157, 561-568.
555

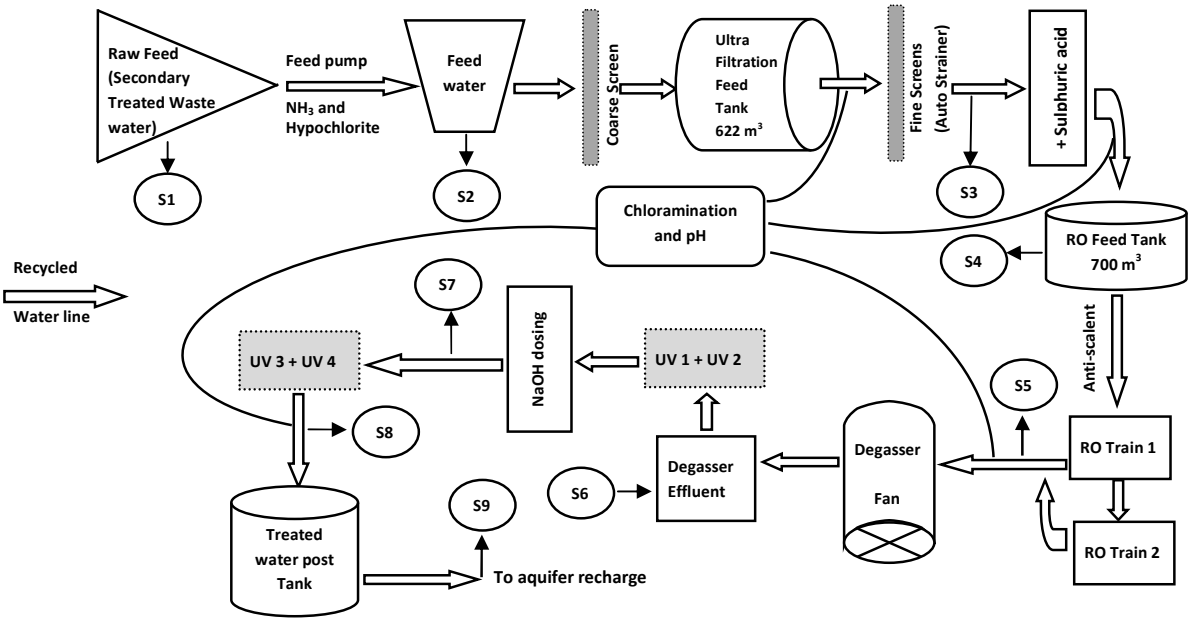

Figure. 1. Schematic of the Beenyup AWRP showing treatment processes and sampling locations.

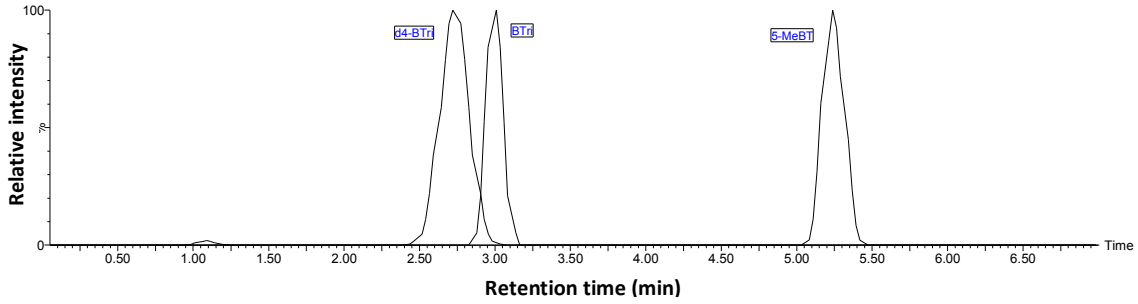

Figure 2. A Combined chromatograms from LC-MS analysis of BTri (*m/z* 120.055), 5-MeBT (*m/z* 134.069) and 4d-BTri (*m/z* 124.083; internal standard).

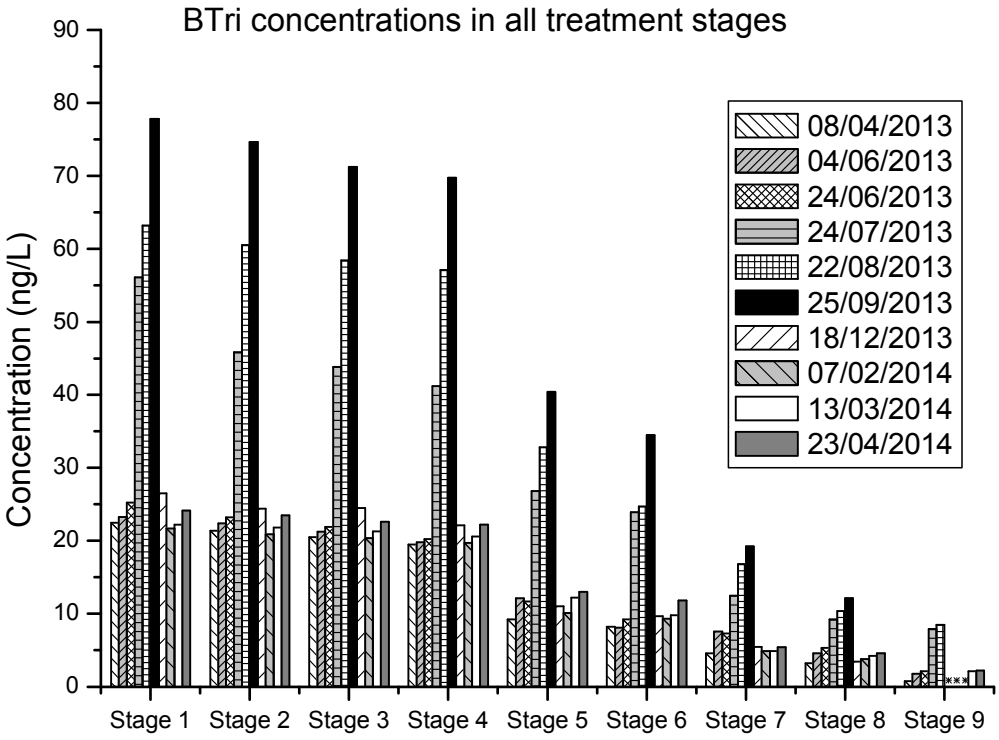

Figure3. BTri concentrations in all AWRP treatment stages. * indicates where concentrations were below detection limit.

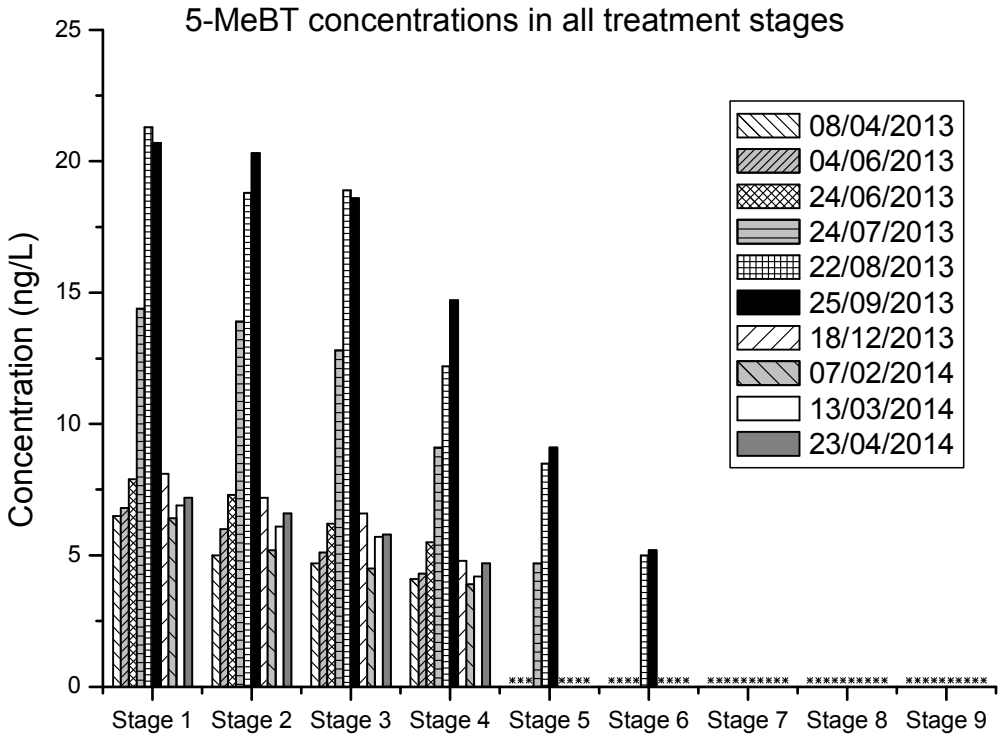

Figure4. 5-MeBT concentrations in all AWRP treatment stages. * indicates where concentrations were below detection limit.