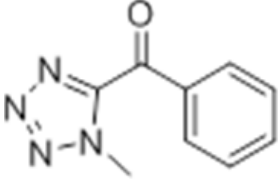
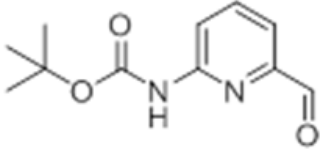
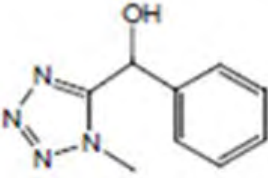


**Part 8.2.2 Analytical methodology (parent compound and transformation products)**

Common Name: Picarbutrazox
Product Name: Picarbutrazox Technical
Submission Number: 2017-5919
PCPA Reg. Number: Not yet assigned
Source Code: PBZ-NIN-2

Chemical structures:

Table 1. Chemical name, code and chemical structure for active and all major transformation products / metabolites		
Chemical name	Code / CAS #	Chemical structure
<i>tert</i> -butyl (6-[[<i>(Z)</i> -(1-methyl-1 <i>H</i> -5-tetrazolyl)(phenyl)methylene]aminooxymethyl]-2-pyridyl)carbamate MW = 409.44 g/mol	NF-171 (parent compound) / 500207-04-5	
<i>tert</i> -butyl (6-[[<i>(E)</i> -(1-methyl-1 <i>H</i> -5-tetrazolyl) (phenyl)methylene]aminooxymethyl]-2-pyridyl)carbamate MW = 409.44 g/mol	TZ-1E / 1253511-94-2	
<i>(Z)</i> -O-[(6-amino-2-pyridyl) methyl] (1-methyl-1 <i>H</i> -5-tetrazolyl) (phenyl)methanone oxime MW = 309.33 g/mol	TZ-2 / 500206-79-1	

Table 1. Chemical name, code and chemical structure for active and all major transformation products / metabolites		
(1-methyl-1 <i>H</i> -tetrazol-5-yl) (phenyl)methanone MW = 188.19 g/mol	TZ-4 / 33452-25-4	
carbamic acid, <i>N</i> -(6-formyl-2-pyridinyl)-, 1,1-dimethylethyl ester (CAS) <i>tert</i> -butyl (6-formylpyridin-2-yl) carbamate (<i>ChemSketch IUPAC</i>) MW = 222.24 g/mol	TY-3 / 956523-98-1	
1 <i>H</i> -tetrazole-5-methanol, 1-methyl- α -phenyl- (CAS) (1-methyl-1 <i>H</i> -tetrazol-5-yl) (phenyl) methanol (<i>ChemSketch IUPAC</i>) MW = 190.20 g/mol	TZ-5 / 33452-21-0	

Data Submission and Review History:

Table 2. Correspondence Dates, Data # and Content for Picarbutrazox Technical			
Date Received	Data #	Content Summary	Reviewer Officer #
2017-12-08	1	8.2.2 data for soil, sediment, water and fish	1160

The purpose of this Category A.3.2 application is to register a new fungicide active ingredient via a joint review between Canada and the U.S.; for the environmental analytical methods, Canada is carrying out the primary review while the U.S. is responsible for the secondary review.

Good Laboratory Practices Compliance Statement:

The studies contained within this report were conducted in accordance with the Good Laboratory Practice Standards as specified in 40 CFR 160 (1989) and/or Japan MAFF (1999) and/or French Regulation Annexe II à l'article D523-8 du Code de l'Environnement (2007).

yes

no

not stated / applicable

8.2.2.3 Water

Reference:

1. PMRA #2809358; MRID 50218708. 2017, Method Validation for the Determination of NF-171, TZ-1E and TZ-2 in surface water by LC-MS/MS. SGS Institut Fresenius, Project ID IF-17/04113959. (Data #1)
2. PMRA #2809359. 2017, Applicant DER - Method Validation for the Determination of NF-171, TZ-1E and TZ-2 in surface water by LC-MS/MS. (Data #1)
3. PMRA #2809360; MRID 50218709. 2017, Validation of Residue Analytical Method for Determination of NF-171 and its Metabolites TZ-1E and TZ-2 in Surface Water. SGS Multilab, Project ID BPL 17-0029. (Data #1)
4. PMRA #2809361. 2017, Applicant DER - Validation of Residue Analytical Method for Determination of NF-171 and its Metabolites TZ-1E and TZ-2 in Surface Water. (Data #1)
5. PMRA #2809502. 2016, Analytical Method Verification for the Determination of NF-171 and TZ-1E in Freshwater and Saltwater. Wildlife International, Project #437C-116. (Data #1)
6. PMRA #2809503. 2016, Applicant DER - Analytical Method Verification for the Determination of NF-171 and TZ-1E in Freshwater and Saltwater. (Data #1)
7. PMRA #2809474; MRID 50218604. 2017, TZ-2 – Validation of the Analytical Method for the Determination of a Test Substance in Aqueous Solutions. Smithers Viscient, Study No. 12681.6162. (Data #1)
8. PMRA #2809475. 2017, Applicant DER - TZ-2 - Validation of the Analytical Method for the Determination of a Test Substance in Aqueous Solutions. Nippon Soda, RD-03963. (Data #1)
9. PMRA #2809476; MRID 50218605. 2017, TZ-4 - Validation of the Analytical Method for the Determination of a Test Substance in Aqueous Solutions. Smithers Viscient, Study No. 12681.6194. (Data #1)
10. PMRA #2809477. 2017, Applicant DER - TZ-4 – Validation of the Analytical Method for the Determination of a Test Substance in Aqueous Solutions. Nippon Soda, RD-03964. (Data #1)
11. PMRA #2809478; MRID 50218606. 2017, TY-3 - Validation of the Analytical Method for the Determination of a Test Substance in Aqueous Solutions. Smithers Viscient, Study No. 12681.6178. (Data #1)
12. PMRA #2809479. 2017, Applicant DER – TY-3 – Validation of the Analytical Method for the Determination of a Test Substance in Aqueous Solutions. Nippon Soda, RD-03965. (Data #1)
13. PMRA #2809480; MRID 50218607. 2017, TZ-5 - Validation of the Analytical Method for the Determination of a Test Substance in Aqueous Solutions. Smithers Viscient, Study No. 12681.6170. (Data #1)
14. PMRA #2809481. 2017, Applicant DER – TZ-5 – Validation of the Analytical Method for the Determination of a Test Substance in Aqueous Solutions. Nippon Soda, RD-10089. (Data #1)

Refs. 1 and 3 represent validations of the same method for the parent and two transformation products (TZ-1E and TZ-2) in surface water, performed at two different labs; Refs. 2 and 4 are the applicant's DERs of these validations. The details in Table 7a below are taken from Ref. 3 but note that the validation results from Ref. 1 (using Burbach stream water in Dietzhöhlztal) are equally acceptable. Ref. 1 also included a freezer storage stability study. Ref. 5 is the validation of a method for the parent and TZ-1E in fresh (i.e. well or groundwater) and saltwater while Ref. 6 is the associated DER from the applicant; details are summarized in Table 7b.

Refs. 7-14 for the determination of TZ-2, TZ-4, TY-3 and TY-5 in aqueous solutions were not initially evaluated but the degradates are residues of concern and the initial review was not supported without an ILV for these degradates of concern. ILV reports are important to demonstrate that analytical methods may be successfully used in support of potential monitoring by states, tribes, and other entities that are not familiar with the development of the method. Table 7c summarizes the validated methods for these analytes in well water, fortified well water, and

Algal Assay Procedure (AAP) medium from Refs. 7, 9, 11 and 13; Refs. 8, 10, 12 and 14 constitute the applicant's DERs of these methods.

Table 7a. Principle of the method for the parent and two products in surface water																																							
Items	Details																																						
Details of sample used	Surface water: sampled at Perrier sur Andelle, in the Andelle river. Characterization in Appendix E of Ref. 1.																																						
Sample preparation	Sample was filtered through a folded filter, and 5 mL was then diluted with 5 mL of acetonitrile. An aliquot was filtered through a nylon syringe filter prior to analysis.																																						
Method for quantitative analysis and identification of parent compound and transformation products	<p><u>HPLC conditions:</u> Column: Kinetex EVO C18 100 × 3.0 mm (2.6 μm)</p> <table border="1"> <tr> <td>Mobile Phase A:</td> <td>10 mM ammonium acetate + 0.1 mL 25 % ammonia + 1000 mL ultra pure water</td> </tr> <tr> <td>Mobile Phase B:</td> <td>Acetonitrile</td> </tr> <tr> <td>Flow Rate:</td> <td>0.6 mL/min</td> </tr> </table> <table border="1"> <thead> <tr> <th>Time (min)</th> <th>Phase A (%)</th> <th>Phase B (%)</th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>99</td> <td>1</td> </tr> <tr> <td>0.5</td> <td>99</td> <td>1</td> </tr> <tr> <td>2.0</td> <td>55</td> <td>45</td> </tr> <tr> <td>5.0</td> <td>10</td> <td>90</td> </tr> <tr> <td>7.0</td> <td>10</td> <td>90</td> </tr> <tr> <td>7.2</td> <td>99</td> <td>1</td> </tr> <tr> <td>9.0</td> <td>99</td> <td>1</td> </tr> </tbody> </table> <p>Retention times: TZ-2 3.4 min TZ-1E 4.6 min parent 4.7 min</p> <p><u>MS/MS conditions:</u> electrospray ionization in positive mode</p> <table border="1"> <thead> <tr> <th>Analytes</th> <th>Mass transition (m/z)</th> </tr> </thead> <tbody> <tr> <td rowspan="2">NF-171 and TZ-1E</td> <td>410.2 → 310.2**</td> </tr> <tr> <td>410.2 → 107.1</td> </tr> <tr> <td rowspan="2">TZ-2</td> <td>310.2 → 107.1**</td> </tr> <tr> <td>310.2 → 80.0</td> </tr> </tbody> </table> <p>** - for quantitation</p>	Mobile Phase A:	10 mM ammonium acetate + 0.1 mL 25 % ammonia + 1000 mL ultra pure water	Mobile Phase B:	Acetonitrile	Flow Rate:	0.6 mL/min	Time (min)	Phase A (%)	Phase B (%)	0.0	99	1	0.5	99	1	2.0	55	45	5.0	10	90	7.0	10	90	7.2	99	1	9.0	99	1	Analytes	Mass transition (m/z)	NF-171 and TZ-1E	410.2 → 310.2**	410.2 → 107.1	TZ-2	310.2 → 107.1**	310.2 → 80.0
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Chromatograms of spiked sample, control sample, blank and standard solution	Chromatograms provided for the reagent blank, matrix control blank, calibration standards and fortified samples show no interferences around the peaks of interest.																																						
Quantitation	By external standards prepared in solvent using a seven-point calibration curve. Standards from Nisso Chemical Analysis were supported with certificates of analysis: parent purity 98.8%, TZ-1E 98.0%, TZ-2 99.7%.																																						
Criteria for setting LOD and LOQ	LOQ was defined as the lowest validated level while LOD was set at 30% of the LOQ.																																						
Stability of parent and transformation products at various stages of analysis	All standard solutions (stock, fortification and calibration solutions) were shown to be stable for 25 days when stored at 2 – 8°C. In addition, stability in refrigerated final volume extracts was proven for 14 days for all 3 analytes.																																						

Items	Details
Special problems encountered and/or precautions to be taken during analysis/handling/storage of samples	Stock solutions and working solutions of the test / reference item were stored refrigerated in capped vials protected from light.
Total time for completion	~15 h including data processing

Items	Details												
Details of sample used	<u>Freshwater</u> : ground water obtained from a well on the Wildlife International site (Easton, Maryland, USA), and characterized in Appendix 3 of Ref. 5. <u>Saltwater</u> : seawater collected at Indian River Inlet, Delaware, USA, and characterized in Appendix 5 of Ref. 5.												
Sample preparation	Fresh (well) water was passed through a sand filter, aerated and filtered at 0.45 µm. Primary dilutions of samples were made with 0.2% formic acid in methanol as necessary and then secondary dilutions with 50:50:0.1 methanol / freshwater / formic acid. Saltwater was diluted to a salinity of ~2% using well water, then passed through a sand filter, aerated and filtered at 0.45 µm. Primary and secondary dilutions of samples were made with 50:50:0.1 methanol / HPLC-grade water / formic acid.												
Method for quantitative analysis and identification of parent compound and transformation products	<u>HPLC conditions</u> : Column: Thermo Betasil C-18 column (50 mm × 2.1 mm, 5 µm particle size) and Thermo Betasil C-18 guard column (10 mm × 2.1 mm, 5 µm particle size) Mobile phase: gradient of Solvent A (0.1% formic acid in HPLC-grade water) and Solvent B (0.1% formic acid in acetonitrile) <table border="1"> <thead> <tr> <th>Time (min)</th> <th>%A</th> <th>%B</th> <th>Flow (µL/min)</th> </tr> </thead> <tbody> <tr> <td>0.00</td> <td>40.0</td> <td>60.0</td> <td>300</td> </tr> <tr> <td>4.50</td> <td>40.0</td> <td>60.0</td> <td>300</td> </tr> </tbody> </table> Retention time (min): TZ-1E 2.2 (fresh and salt) parent 2.5 (fresh) and 2.6 (salt) <u>MS/MS conditions</u> : electrospray ionization in positive mode <u>Analyte</u> <u>Q1/Q3 m/z quantitation</u> parent 410/310 TZ-1E 410/310	Time (min)	%A	%B	Flow (µL/min)	0.00	40.0	60.0	300	4.50	40.0	60.0	300
Time (min)	%A	%B	Flow (µL/min)										
0.00	40.0	60.0	300										
4.50	40.0	60.0	300										
Chromatograms of spiked sample, control sample, blank and standard solution	Chromatograms provided for the reagent blank, matrix control blank, calibration standards and fortified samples show no interferences around the peaks of interest.												

Table 7b. Principle of the method for the parent and one product in fresh- and saltwater
Analyses were performed both by HPLC-UV and by HPLC-MS/MS; only the MS method data are included here.

Items	Details
Quantitation	By external standards prepared in solvent/matrix using a five-point calibration curve. Standards from Nisso Chemical Analysis were supported with certificates of analysis: parent purity 98.8%, TZ-1E 98.0%.
Criteria for setting LOD and LOQ	LOQ was defined as the lowest validated level. LOD was calculated by dividing the low standard concentration by the S/N ratio multiplied by 3 × the dilution factor of the matrix blank samples.
Stability of parent and transformation products at various stages of analysis	Not stated.
Special problems encountered and/or precautions to be taken during analysis/handling/storage of samples	Not stated.
Total time for completion	Not stated.

Table 7c. Principle of the method for four products in lab well water, fortified well water and AAP

Items	Details																												
Details of sample used	Laboratory well water: mixture of unadulterated water from bedrock well and dechlorinated town well water in Wareham, MA. Fortified well water: laboratory well water (above) fortified with a mixture of salts, i.e. sodium bicarbonate, calcium sulfate, magnesium sulfate and potassium chloride. Algal Assay Procedure (AAP) medium: sterile, deionized water formulated with 16 components listed in the cited reports.																												
Sample preparation	Recovery samples were diluted 1:9 with 20:80 acetonitrile:purified reagent water (v:v) for a final composition of 18:10:72 acetonitrile:test matrix:purified reagent water (v:v:v). The high-level recovery samples were further diluted into the calibration range with 18:10:72 acetonitrile:test matrix:purified reagent water (v:v:v).																												
Method for quantitative analysis and identification of parent compound and transformation products	<p>HPLC conditions:</p> <p>Column: TZ-2, TZ-4, TZ-5: XBridge C18 (2.1 × 50 mm, 2.5 μm) TY-3: Synergi Hydro RP C18 (2.0 × 50 mm, 2.5 μm)</p> <p>Column temp: 40°C</p> <p>Mobile Phase A: 0.1% formic acid in water</p> <p>Mobile Phase B: 0.1% formic acid in acetonitrile</p> <p>TZ-2</p> <table border="1"> <thead> <tr> <th>Time (min.)</th> <th>Flow rate (mL/min.)</th> <th>Solvent A (%)</th> <th>Solvent B (%)</th> </tr> </thead> <tbody> <tr> <td>0.10</td> <td>0.350</td> <td>90.0</td> <td>10.0</td> </tr> <tr> <td>0.50</td> <td>0.350</td> <td>90.0</td> <td>10.0</td> </tr> <tr> <td>4.00</td> <td>0.350</td> <td>0.00</td> <td>100</td> </tr> <tr> <td>5.00</td> <td>0.350</td> <td>0.00</td> <td>100</td> </tr> <tr> <td>5.10</td> <td>0.350</td> <td>90.0</td> <td>10.0</td> </tr> <tr> <td>6.50</td> <td>0.350</td> <td>90.0</td> <td>10.0</td> </tr> </tbody> </table> <p>TZ-4</p>	Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)	0.10	0.350	90.0	10.0	0.50	0.350	90.0	10.0	4.00	0.350	0.00	100	5.00	0.350	0.00	100	5.10	0.350	90.0	10.0	6.50	0.350	90.0	10.0
Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)																										
0.10	0.350	90.0	10.0																										
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TZ-5	191.2 / 145.1																																																																																														
Chromatograms of spiked sample, control sample, blank and standard solution	Chromatograms provided for the reagent blank, matrix control blank, calibration standards and fortified samples show no interferences around the peaks of interest.																																																																																														
Quantitation	By external standards prepared in matrix (unacceptable results were obtained using non-matrix-matched standards) using a six-point calibration curve. Standards from Nisso America were supported with certificates of analysis: TZ-2 99.2%, TZ-4 99.7%, TY-3 100%, TZ-5 99.4%.																																																																																														

Items	Details
Criteria for setting LOD and LOQ	LOQ was defined as the lowest validated level. LOD was calculated using three times the signal-to-noise value of the control samples. The Method Detection Limit (MDL) was defined as the lowest concentration in test samples that could be detected based on the concentration of the low calibration standard and the dilution factor of the control solutions.
Stability of parent and transformation products at various stages of analysis	Not stated; however, primary and secondary stock solutions were stored refrigerated and sub-stock solutions were prepared fresh on the day of use.
Special problems encountered and/or precautions to be taken during analysis/handling/storage of samples	Not stated.
Total time for completion	Not stated.

The method validation data for the parent compound and the transformation products are summarized in Tables 8a and b.

Parameter	Surface water			Fresh (well) water		Saltwater	
	Parent	TZ-1E	TZ-2	Parent	TZ-1E	Parent	TZ-1E
% Recovery at LOQ ¹	116 (96 – 150)	101 (95 – 107)	98 (94 – 102)	96.1	101	91.1	106
% Recovery at 10xLOQ at 100xLOQ	102 (96 – 105) --	102 (98 – 105) --	101 (95 – 107) --	-- 98.5	-- 98.4	-- 89.1	-- 84.6
Mean % recovery, 2 levels	109	102	100	not reported	not reported	not reported	not reported
RSD %	15.6	4.0	4.1	4.97, 4.66	1.87, 1.13	8.91, 2.14	4.23, 3.57
Method linearity	7.5 – 375 pg/mL	7.5 – 375 pg/mL	15 – 750 pg/mL	5.0 – 50 µg/L	5.0 – 50 µg/L	1.0 – 10 µg/L	1.0 – 10 µg/L
Correlation coefficient	r ≥ 0.995 sample r = 0.9990	r ≥ 0.995 sample r = 0.9992	r ≥ 0.995 sample r = 0.9993	sample r = 0.9944	sample r = 0.9991	sample r = 0.9982	sample r = 0.9981
LOD	0.015 µg/L	0.015 µg/L	0.03 µg/L	25.8 ng/L	7.56 ng/L	60.4 ng/L	6.4 ng/L
LOQ	0.05 µg/L	0.05 µg/L	0.1 µg/L	15 µg/L	15 µg/L	15 µg/L	15 µg/L

¹ LOQ spiking level was 0.05 µg/L for the parent and TZ-1D in surface water, 0.1 µg/L for TZ-2 in surface water, and 15 µg/L for the parent and TZ-1E in fresh- and saltwater.

Table 8b. Method validation: Transformation products in water

Parameter	Laboratory well water				Fortified well water				Algal assay medium			
	TZ-2	TZ-4	TY-3	TZ-5	TZ-2	TZ-4	TY-3	TZ-5	TZ-2	TZ-4	TY-3	TZ-5
% Recovery at LOQ ¹	96.2	92.1	88.5	89.8	106	99.7	96.5	96.7	83.7	101	98.3	89.2
% Recovery at high spike ¹	106	107	108	91.0	103	108	106	93.6	100	99.6	106	91.9
Mean % recovery, 2 levels	101	99.7	98.3	90.4	105	104	101	95.2	92.0	100	100	90.6
RSD %	10.2	9.71	10.8	4.82	4.39	7.46	5.69	9.67	10.5	6.41	6.41	5.56
Method linearity	5 – 100 ng/L				5 – 100 ng/L				5 – 100 ng/L			
Correlation coefficient	0.9975 4	0.9994 2	0.9990 1	0.9995 7	0.9957 8	0.9941 5	0.9948 1	0.9978 0	0.99805	0.9943 7	0.99643	0.9990 9
LOD (ng/L)	0.7	5	3	7	30	10	30	10	0.3	20	3	10
MDL (µg/L)	0.0500											
LOQ (µg/L)	0.100											
¹ LOQ spiking level was 0.1 µg/L, while the high spike was 30 000 µg/L for TZ-2, TZ-4 and TY-3 and 100 000 µg/L for TZ-5. n=5 replicates were prepared at each spiking level.												

Conclusions/Other Comments: HPLC-MS/MS methods were developed for the determination of the parent and five transformation products in water and were independently validated in two surface waters, fresh (well) water, saltwater, laboratory well water, fortified well water and algal assay medium. The recovery data were acceptable (means between 70-120%). The LOQ was determined to be 0.05 µg/L for the parent and TZ-1E / 0.1 µg/L for TZ-2 in surface water; 15 µg/L for both the parent and TZ-1E in fresh (well) and saltwater; and 0.1 µg/L for TZ-3 / TZ-4 / TY-3 / TZ-5 in laboratory well water, fortified well water and algal assay medium. The reported limit of quantitation (LOQ) was determined as the lowest level of method validation (LLMV). Further work could have been done to explore the actual LOQ. This means that concentrations can be reliably quantified at the LOQ (*i.e.*, LLMV), but whether lower concentrations may also be reliably quantified is uncertain. These methods are acceptable for use in post-registration monitoring.

EPA: This study is classified as **acceptable**.