Final Report, June 30, 2023

Final report for the Interagency Agreement between the Missouri Department of Agriculture, Division of Plant Industries, Pesticide Program (MDA), Boone County and the University of Missouri:

1. Introduction:

This project examined detection and risk assessments of 17 pesticides and related metabolites in Boone County waterways. Additionally, chloride, sulfate, nitrate and phosphate were assessed as tracers for urban and agricultural activities. These data were also correlated with land use and point sources from several geospatial datasets (USDA Crop Space, NPDES permitted discharges, NLCD 2019 Land Cover (CONUS)

Table 1: Priority Herbicides, pesticides and their metabolites:

Compound Name:	Common uses:
2,4-Dichlorophenoxyacetic (2,4 D)	Broadleaf herbicide
Acetochlor	Herbicide, commonly used on corn, soy, beets
Dicamba	Broad-spectrum herbicide
Atrazine	Herbicide for preemergent broadleaf
Hydroxyatrazine	Metabolite of Atrazine
Deethylatrazine (DEA)	Metabolite of Atrazine
Deisopropylatrazine (DIA)	Metabolite of Atrazine
Caramba/ metconazole	Fungicide
Glyphosate	Herbicide
Metolachlor	Herbicide
Metribuzin	Herbicide
Neonicotinoids: Thiamethoxam, Clothianidin, Acetamiprid, Thiacloprid, Dinotefuran, Nitenpyram, Imadacloprid	Neuro-active insecticides
Prothiaconazole	Fungicide
Simazine	Herbicide
Tebuconazole	Fungicide

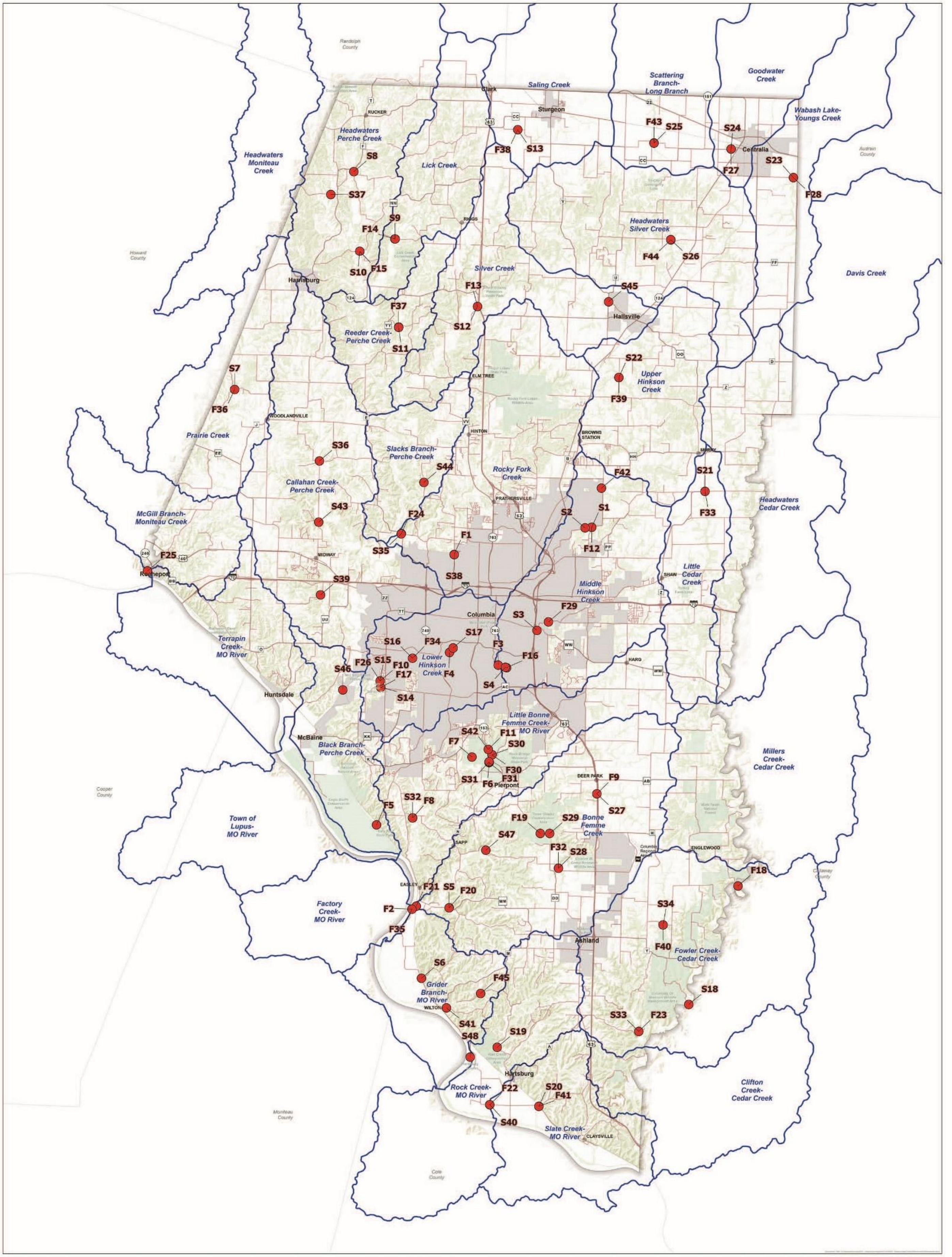
2. Methodology:

2.1 Fieldwork:

For the fall sampling, forty-five grab samples were collected from streams around Boone County and the Missouri River. The number of samples was lower than the estimated forty-eight samples to be collected due to ongoing drought conditions in the County. Forty-eight samples were collected from streams around Boone County and the Missouri River in spring of 2023. The sample collection effort was led by Boone County Hydrologist Lynne Hooper. Sample locations were chosen based on land use, stream order, accessibility, and other watershed characteristics. Collection of fall samples began on September 19, 2022 and ended November 29, 2022. Spring samples were collected from April 2, 2023 through April 17, 2023. Samples for pesticides were collected in 250 mL polypropylene bottles and frozen. Samples for inorganic fertilizers and chloride were filtered with 0.7 µm glass fiber filters into trace clean polypropylene tubes and frozen within 24 hours of collection. Site locations, photos, and precise GPS coordinates for each site were recorded via the Survey123 application to post locations and data. Site locations for fall, 2022 and spring, 2023 are shown in the map below.

Site locations, photos, and precise GPS coordinates for each site were recorded via the Survey123 application to post locations and data on an interactive map on the stormwater page of the Boone County website. https://www.showmeboone.com/stormwater/mda-sampling.asp When the user clicks on sampling points on the map, a subwatershed calculated to the sampling point is displayed. A popup window simultaneously displays land use / land cover data for the subwatershed. Users can also access .pdfs of the chemical sampling data for the sampling point through the popup window.

Figure 1: Sampling Locations, fall, 2022 (denoted F#) and spring, 2023 (S#):



2.2 Chemical analysis for determining the concentrations of 21 pesticides, herbicides, and their metabolites

The Bioanalytical Laboratory (Drs. Chung-Ho Lin) at University of Missouri developed targeted methods seventeen agricultural pesticides, herbicides, and their metabolites (see the list attached) via an ultra-high-performance liquid chromatography coupled with a tandem mass spectrometer (UPLC-MS/MS).
For example, atrazine and metabolites DEA, DIA, and HA were confirmed with limit of detection of 7-10 ppt. This sensitive detection limit is almost 1000-fold less than the atrazine regulation of 3 ppb = 3,000 ppt. This approach yielded high sensitivity, resulting in minimal sample concentration, extraction, and efficient analysis of target pesticides. To our best knowledge, the developed analytical UPLC-MS/MS method in this study is the most sensitive, fully optimized high-throughput direct analysis for the multi-herbicides/pesticides analysis developed for surface water monitoring.

The concentrations of herbicides, pesticides and their degradation products (e.g., atrazine, desethylatrazine DEA, desisopropylatrazine DIA and hydroxyatrazine HA) were determined by a Waters Acquity Ultra-High-Performance Liquid Chromatography coupled with a XEVO TQ-XS tandem mass spectrometer (UPLC-MS/MS). The herbicides, pesticides and their degradation products were separated by a CORTECS® C18 analytical column with 1.6 µm particles size, 100 mm length x 2.1 mm internal diameter. The mobile phase is 0.01% formic acid in water (A) and 100% acetonitrile (B). The gradient conditions are: 0–0.2 min, 2% B; 0.2-1.89 min, 2–80% (linear gradient) B; 1.89-1.92 min, 80–98% (linear gradient) B; 1.92–2.34 min (linear gradient), 98% B; 2.34–6.77 min, 2% B with a flow rate of 0.4 ml/min. The system was conditioned first with 50 % acetonitrile and 50% of 0.01% formic acid, and the column was equilibrated with 2% acetonitrile and 98% of 0.01% formic acid solution. The injection volume is 5 µl. Full spectrum of the protonated $[M+H]^+$ and deprotonated molecular ion $[M - H]^-$ and the spectrum of fragmented product ions were determined by injecting 5 µl of a standard solution containing 1,000 µg/L. The analytes in the samples were confirmed by their retention time, molecular weight, and MS/MS fragmentation. The ionization energy, multi-reaction monitoring (MRM) transition ions (precursor and product ions), capillary and cone voltage (CV), desolvation gas flow and collision energy (CE) were optimized by Waters IntelliStart™ optimization software package. We have completed the method development 21 pesticides, herbicides, and their metabolites. The R^2 values for the developed calibration are greater than 0.999 (**Table 2 and example in the Appendix 1**).

Table 2. The optimized parameters, the limits of the detection (LODs) and limits of quantification (LOQs) for the analysis of the 21 pesticides, herbicides, and their metabolites with the UPLC-MSMS.

No.	Compou0 Name:	Code	MW	ES+/ES-	Parent ion	Daughter Ion	CV	CE	RT std	con (ppb)	S/N	LOD (ppb)	LOQ (ppb)
1	2,4-Dichlorophenoxy	2,4-D	221.03	Negative	220.7	162.87	10	14	2.72	0.1	16.180	0.019	0.062
2	Acetochlor	ATC	269.77	Positive	269.59	148.09	2	20	3.16	0.1	6.570	0.046	0.152
3	Atrazine	ATR	215.68	Positive	215.69	173.89	4	24	2.76	0.1	16.770	0.018	0.060
4	Hydroxyatrazine	HA	197.24	Positive	197.76	155.87	28	22	1.96	0.1	44.200	0.007	0.023
5	Deethylatrazine	DEA	187.63	Positive	187.704	103.905	24	26	2.29	0.1	35.720	0.008	0.028
6	Deisopropylatrazine	DIA	173.6	Positive	173.38	95.85	16	16	2.1	0.1	96.270	0.003	0.010
7	Caramba/ metconaz	CAR	319.83	Positive	320.1	70.1	tune	tune	3.13	0.1	144.73	0.002	0.007
8	Tebuconazole	TEB	307.82	Positive	308.2	70	tune	tune	3.06	0.1	171.83	0.002	0.006
9	Metolachlor	MTC	283.79	Positive	284.1	252.08	14	16	3.16	0.1	221.38	0.001	0.005
10	Metribuzin	MTB	214.29	Positive	215.1	49	4	28	2.62	0.1	82.03	0.004	0.012
11	Thiamethoxam	THI	291.72	Positive	291.79	210.95	tune	14	2.14	0.1	7.77	0.039	0.129
12	Clothianidin	CLO	249.68	Positive	249.5	168.86	6	14	2.23	0.1	46.7	0.006	0.021
13	Acetamiprid	ACE	222.67	Positive	222.68	125.9	8	18	2.32	0.1	16.04	0.019	0.062
14	Thiacloprid	TCP	252.72	Positive	252.79	125.95	30	24	2.43	0.1	45.1	0.007	0.022
15	Dinotefuran	DTF	202.21	Positive	202.92	128.97	20	12	1.91	0.1	3.87	0.078	0.258
16	Imidacloprid	IMI	255.66	Positive	256.05	209.01	tune	16	2.28	0.1	10	0.030	0.100
17	Simazine	SIM	201.66	Positive	201.61	124.02	2	18	2.57	0.1	52.22	0.006	0.019
18	Nitenpyram*	NIT	270.71	Positive	270.85	125.9	22	30	2	0.1	16.76	0.018	0.060
19	prothioconazole*	PTC	344.3	negative	342.1	100.2	52	24	3.1	1	21.51	0.139	0.465
20	Dicamba*	DCB	221.03	Positive	222.57	190	18	24	2.45	10	3.57	8.403	28.011
21	Glyphosate	**GLY**	169.07	ESI-	**168**	**63.0**	**25**	**18**	4.22	**0.1**	**7.23**	**0.041**	0.138

2.3 Urban and Agricultural Ion Determination

Chloride, sulfate, nitrate, and phosphate (Cl^-, SO_4^{2-}, NO_3^-, PO_4^{3-}) on a Dionex Integrion High Pressure Ion Chromatography and methods recommended in EPA 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography. NIST certified multi-anion and single anion standards were utilized to confirm retention times of target anions and confirm presence or absence of non-target anions. Chloride, sulfate, nitrate, and phosphate were well correlated to calibration standards over a large dynamic range (2.5 - 150 mg/L, $R^2 > 0.99$). The detection limits, based on the minimum calibration curve standards were 1.2 mg/L for Cl^-, 5 mg/L for SO_4^{2-}, 1.5 mg/L for PO_4^{3-}, and 1.5 mg/L for NO_3^-. All samples contained Cl^- and SO_4^{2-} greater than detection limits. Sites with elevated NO_3^- and PO_4^{3-} are the focus of this report. Aliquots of these samples remain frozen if there is a need to redetermine concentrations at lower levels. Single anion standards were used for recovery determinations. All ions were recovered at least 90-110% for 10 mg/L targets. Field, filter, and analytical deionized water blanks did not have measurable anions of interest above detection limits.

2.4 Geospatial Analyses

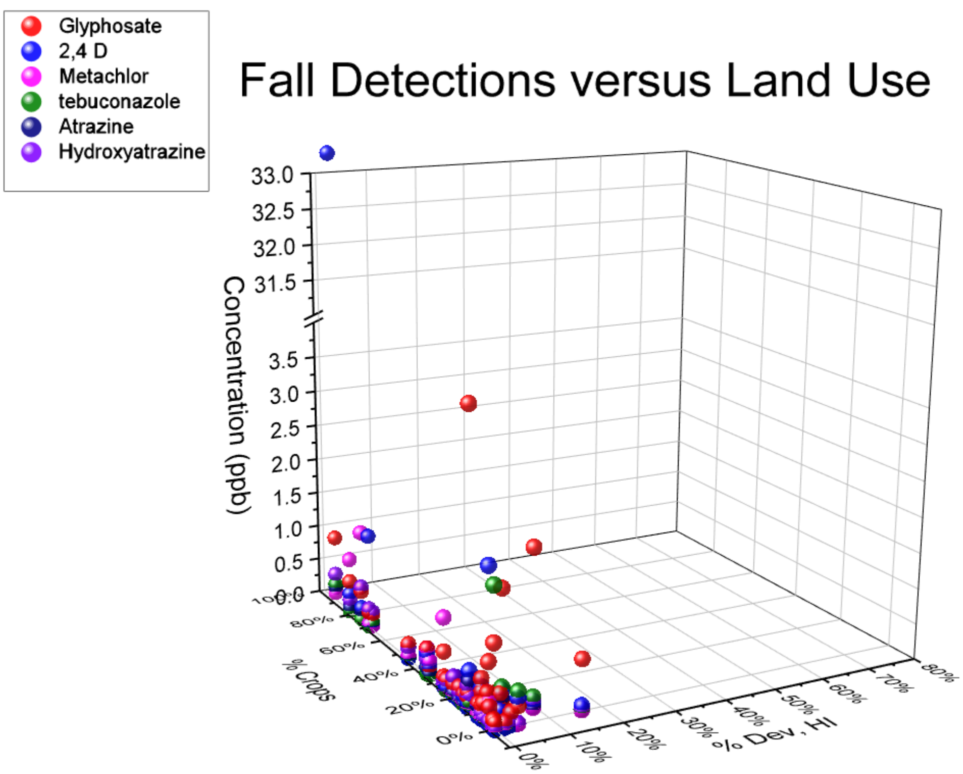
Boone County GIS staff calculated land use / land cover data for subwatersheds to the point of sample collection for all sampling points. The data were taken from the NLCD 2019 Land Cover (CONUS) database (https://www.mrlc.gov/data/nlcd-2019-land-cover-conus). Subwatersheds were calculated using Boone County DEM imagery for locations within the County (2-foot resolution), and a lower resolution DEM dataset where subwatersheds extended outside of County boundaries. Subwatersheds may be viewed by clicking on the sampling points on the interactive map viewer located on the Boone County Stormwater web page (https://www.showmeboone.com/stormwater/mda-sampling.asp).

3 Results:

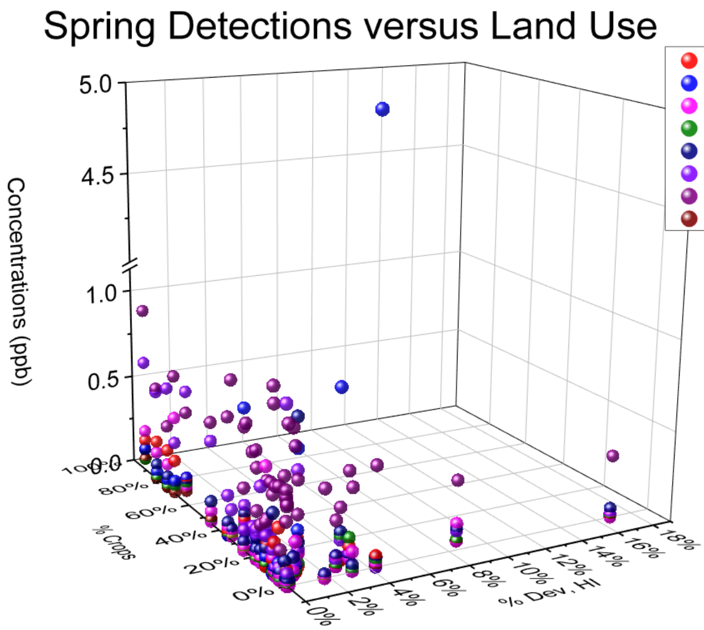
A tabular summary of average pesticides, herbicides, fungicides, metabolites, and anthropogenic ions is below. Data is divided by spring and fall field campaigns. The frequency of a positive chemical detection is also noted. Considering all 48 sites and 17 chemicals of interest, there were **611 total positive detections of pesticide targets in fall, an only 383 detections in spring.** This suggests that pesticide/herbicide detections in streams may be more closely timed with the summer growing season. The most frequently detected compounds present at **>40 sites per field event were atrazine, hydroxyatrazine, glyphosate, tebuconazole ,and metolachlor (fall and spring)**, **and nitenpyram and caramba (spring only). In fall at site 43 – Long Branch Creek (northwest Boone County near Centralia), 2-4 D was detected at 33 ppb, or levels 30 times greater average concentrations.** This elevated concentration may suggest a point source or heavy input of this compound. Chloride is both naturally occurring and elevated due to human activities, and was present in all samples. This area of Scattering Branch/Long Branch Creek also contains several permited discharges/point sources. This site also drained land with ~92% crop coverage.

Compound Name:	Fall Average Concentration, Standard Deviation, (ppb), Frequency of Detection (45 sites)	Spring Average Concentration, Standard Deviation, (ppb) Frequency of Detection (48 sites)
2,4-Dichlorophenoxyacetic (2,4 D)	1.09 ± 5.03 25/45 sites	0.21 ± 0.80 p 16/48 sites
Acetochlor	0.02 ± 0.07 7/45 sites	No detection >0, any site
Dicamba	No detection >0, any site	No detection >0, any site
Atrazine	0.05 ± 0.07 42/45 sites	0.08 ± 0.11 48/48 sites
Hydroxyatrazine	0.13 ± 0.10 45/45 sites	0.16± 0.20 39/48 sites
Deethylatrazine (DEA)	0.02 ± 0.02 25/45 sites	0.02 ± 0.04 18/48 sites
Deisopropylatrazine (DIA)	0.01 ± 0.02 7/45 sites	0.02 ± 0.09 23/48 sites
Caramba/ metconazole	0.01 ± 0.05 12/45 sites	0.01 ± 0.01 44/48 sites
Glyphosate	0.4 ± 0.6 45/45	0.04 ± 0.07 17/48 sites
Metolachlor	0.34 ± 1.54 45/45 sites	0.07 + 0.10 48/48 sites
Metribuzin	0.0 ± 0.01 7/45 sites	0.0 + 0.01 4/48 sites
Neonicotinoids: Nitenpyram (Thiamethoxam, Clothianidin, Acetamiprid, Thiacloprid, Dinotefuran, Imadacloprid)	0.05 ± 0.15 8/45 sites (Other neonicitoids, low detection rate <4 sites at >0 ppb)	0.49 ± 0.21 48/48 sites

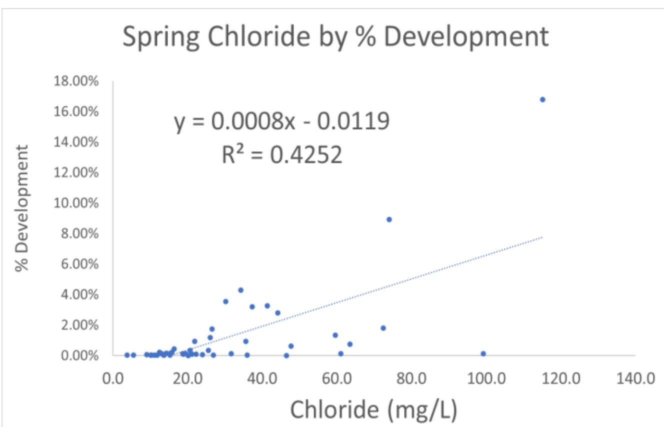
		Other neonicitoids, low detection rate <4 sites at >0 ppb
Prothiaconazole	0.02 ± 0.13 5/45 sites	0.03 ± 0.06 6/48
Simazine	0.01± 0.04 9/45	0.04 ± 0.16 13/48
Tebuconazole	0.08 ± 0.28 45/45 sites	0.02 ± 0.03 48/48 sites
Chloride (ppm)	40.9 ± 39.8 ppm 36/36 sites	30.7 ± 23.5 ppm 48/48 sites
Nitrate (ppm) *Average based on samples above detection limit of > 1.5 ppm	3.4 ± 2.7	4.6 ± 2.6
Phosphate Detection Limit > 1.5 ppm *Average only for 9 sites with detects	4.5 ± 3.9 ppm 9/36	0/48

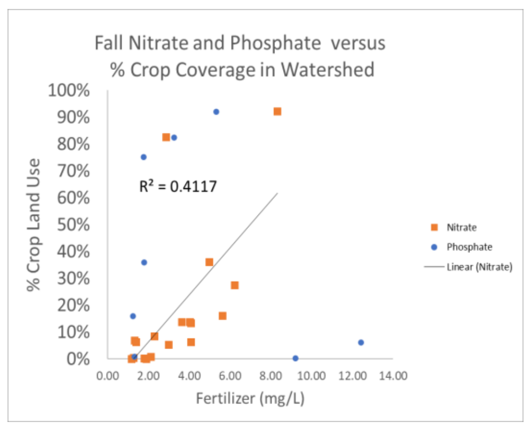


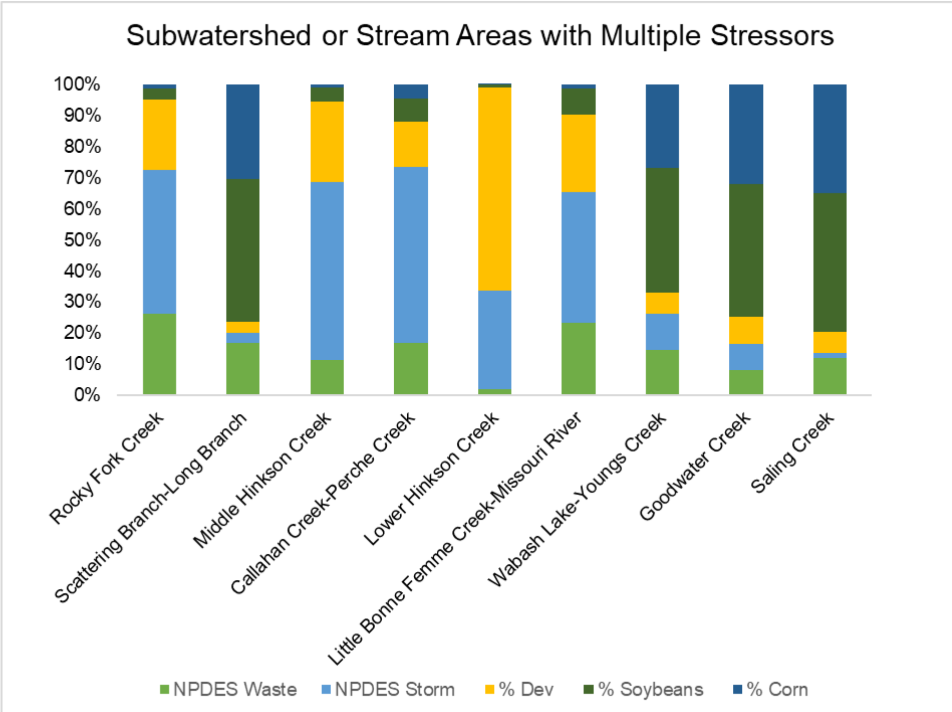
For the six most frequently detected pesticides/herbicides in fall, most detections were more strongly associated with crop land use than development in Boone County. Glyphosate (red) an 2,4 D (blue) also associated with increasing development (graph left).



In spring 2,4 D, nitenpyram, metconazole and atrazine were associated with %crop land coverage and % high development land coverage (graph left). Average chloride was greater in spring than fall and correlated to increasing development in Boone County. Nitrate and phosphate were detected more frequently and at high concentrations in fall. Detection of nitrate and phosphate positively correlated % crop coverage in the Boone County watersheds (graphs below).







Boone County contains areas of high development, crop production, or permitted storm and wastewater discharges (bar graph, above). The top nine subwatersheds/streams areas with high amounts of NPDES discharge, urbanization, or agricultural are presented, left. This geospatial analysis can also help future efforts to target best management practices or water quality interventions in areas of Boone County that may be most impacted by land use stressors. For example, Scattering Branch-Long Branch Creek areas had high levels of 2,4 D, nitrate, phosphate, and glyphosate in the fall, consistent with several water quality stressors.

To evaluate the effectiveness of conservation practices and land use on the degradation of the herbicides, such as atrazine, metabolite to parent ratios (M/P) of each sample were determined. We did not find the direct and strong correlation between the M/P ratios and land use. However, the average M/P ratios are higher in the Fall (24.2%) than the ratios in the Spring (17.5%).

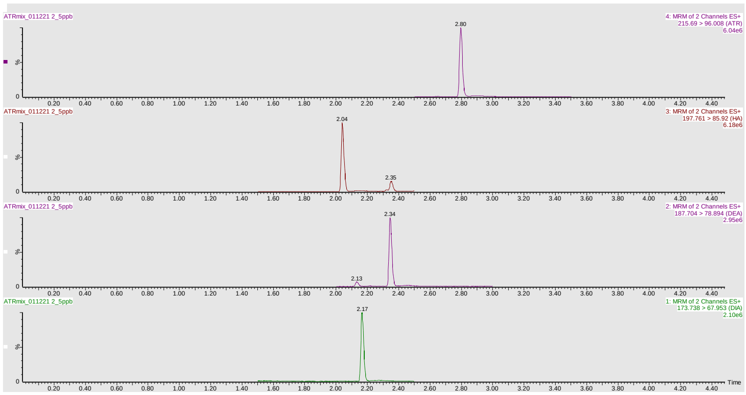
Overall, the pesticide/herbicide, water quality, and geospatial data produced via this project will help future efforts to manage point and non-point sources across Boone County. Detailed data is also submitted to MDA and Boone County with this report. Specific sites with elevated concentrations of pesticide/herbicides/fungicides or anions can be targeted on a stream or sub watershed basis for “hotspots” of highest impact.

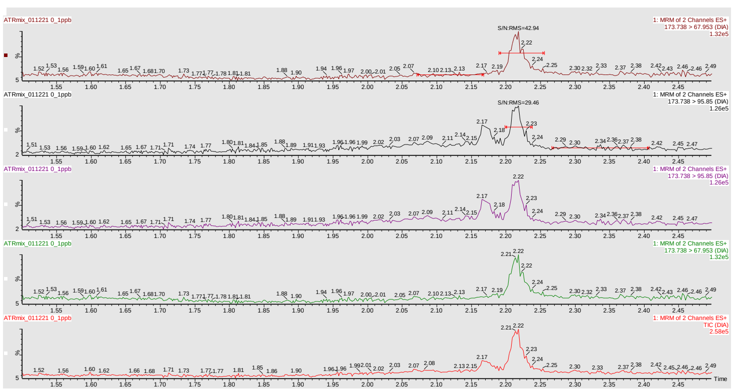
4 Summary and Future Work:

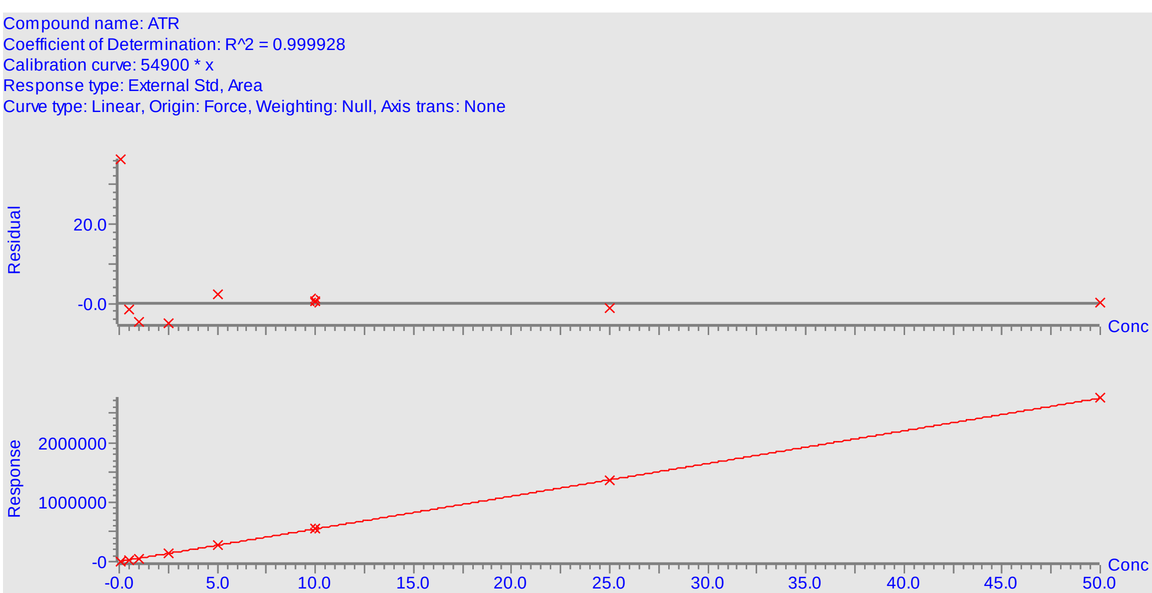
This project provided Boone County and the University of Missouri a unique opportunity to survey streams across a variety of land use types and ecological conditions. The data can be used as a baseline for future work exploring stream conditions and how they are affected by changing climate, land use and/or installation of best management practices (BMPs) in locations across the County. The work ties in well with the interests of Boone County and other local partners in current work to implement BMPs to reduce other pollutants of concern, including *E. coli* and sediment, as recommended BMPs could also mitigate the transport of herbicides and pesticides into local waterways. Additionally, the results from this project have inspired another County-wide project to explore water quality in other streams and tributaries.

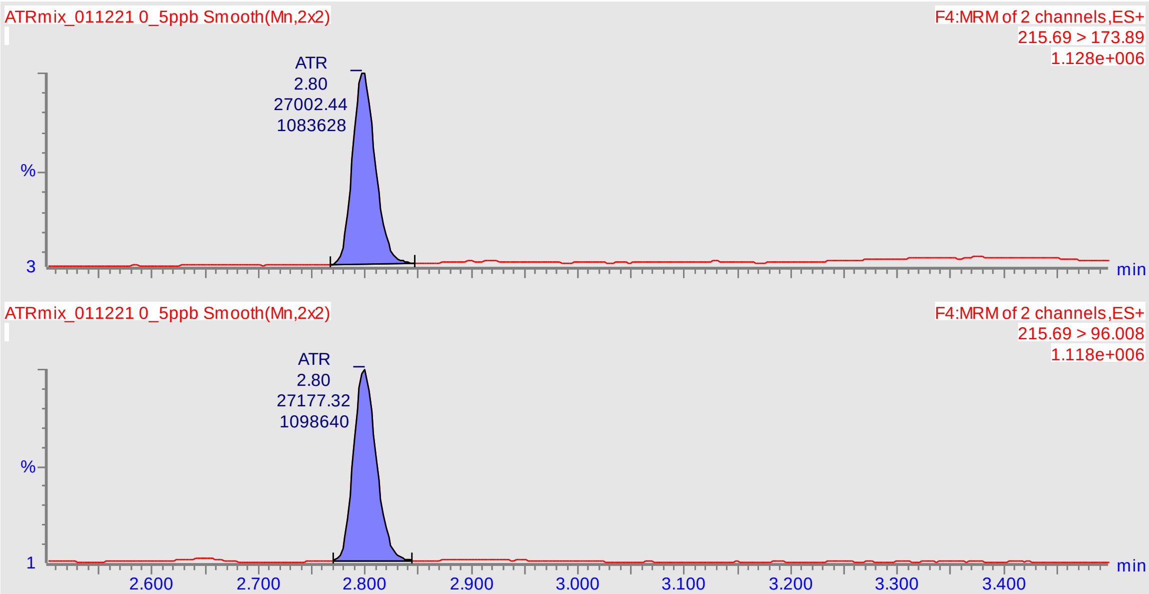
The analytical methods developed by Dr. Chung-Ho Lin and his graduate students will be very useful moving forward for other projects in Boone County and elsewhere. Dr. Lin will be working on a publication to further explore the results from this project including potential toxicity levels of compounds detected in stream samples.

Appendix 1: Chromatographs and Calibration of Low-Level Pesticide and Metabolite Detection

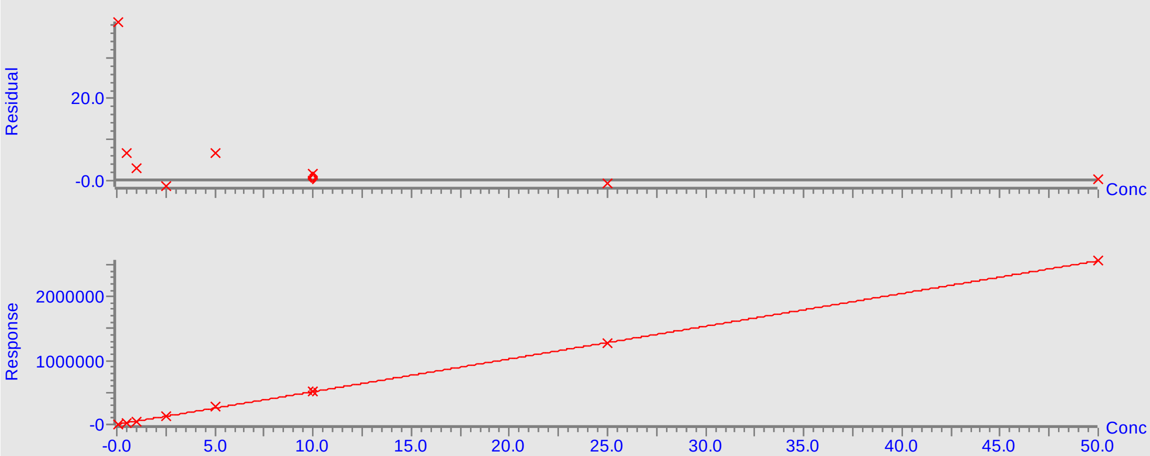


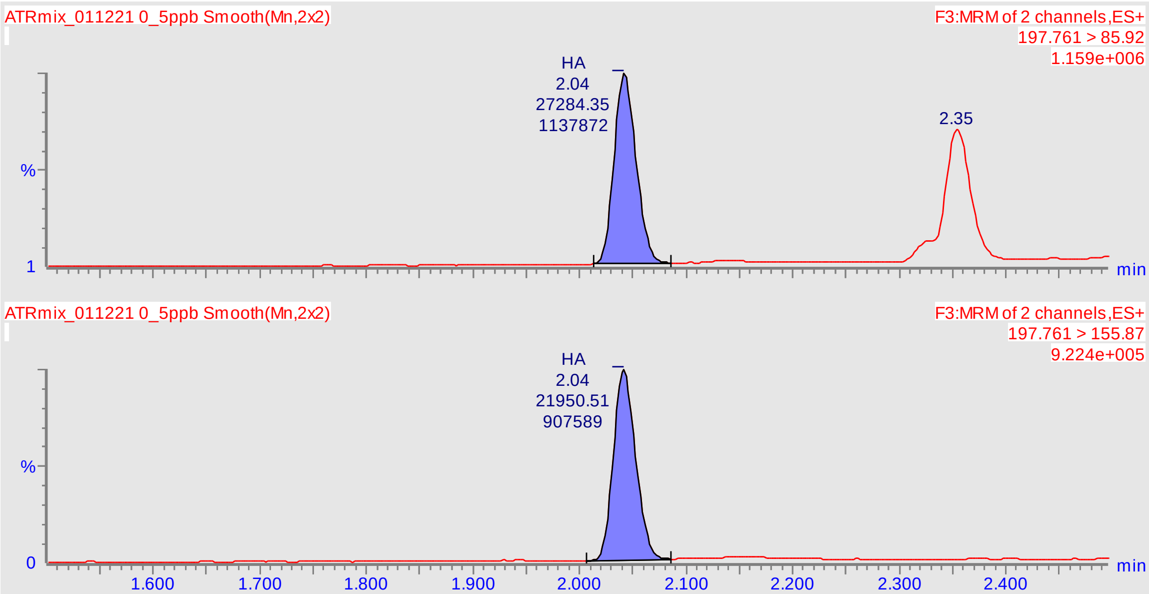




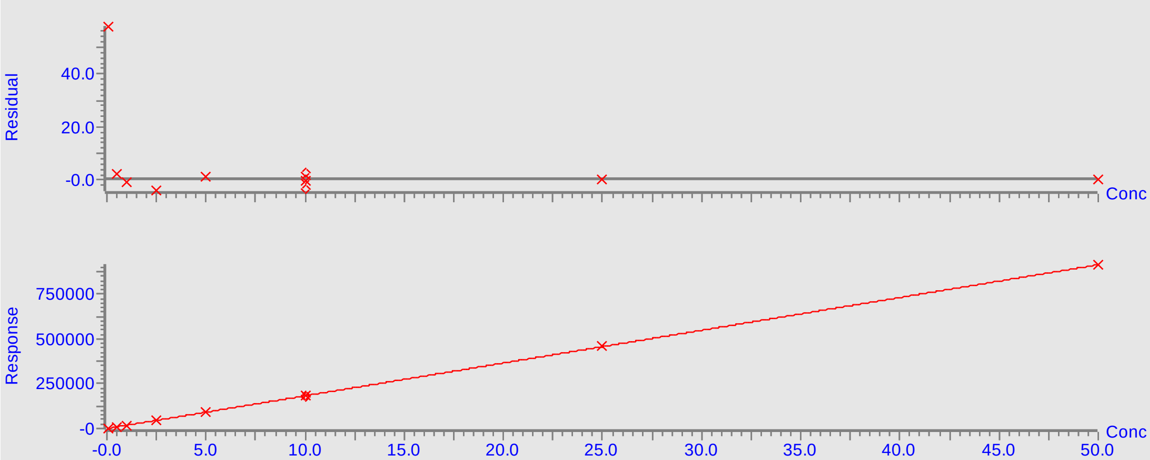


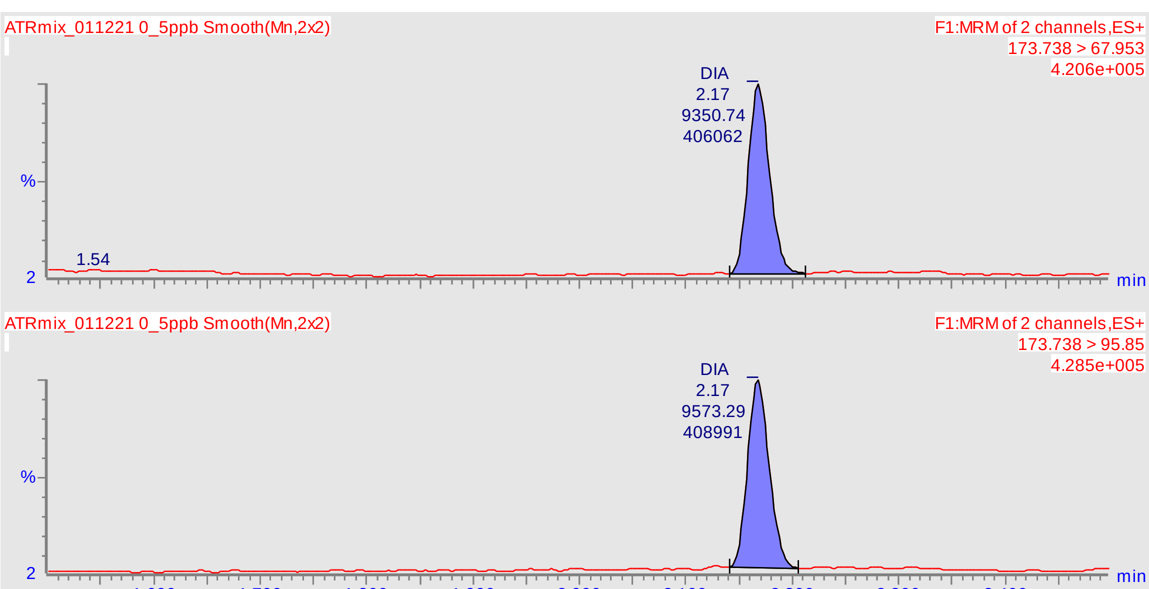
Compound name: HA
Coefficient of Determination: R^2 = 0.999906
Calibration curve: 51207.2 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None



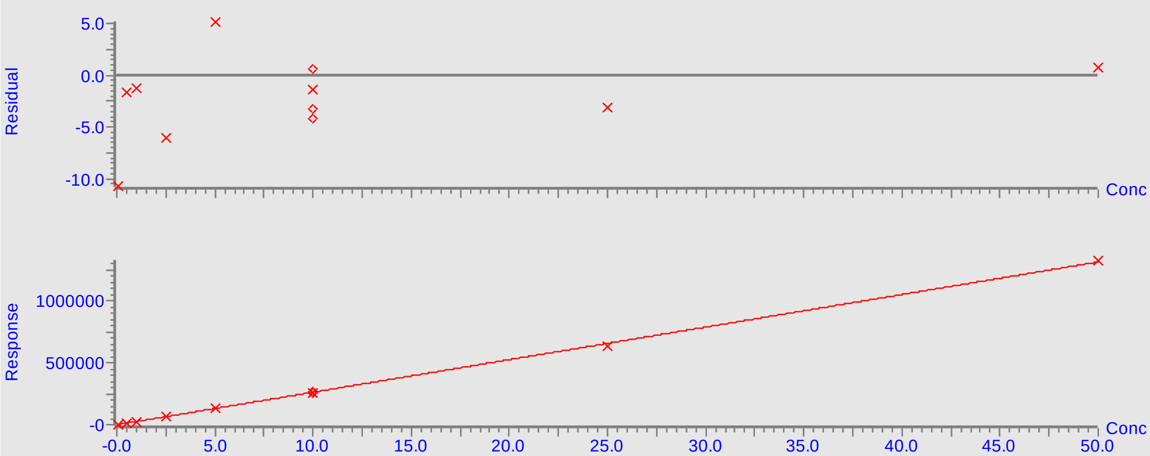


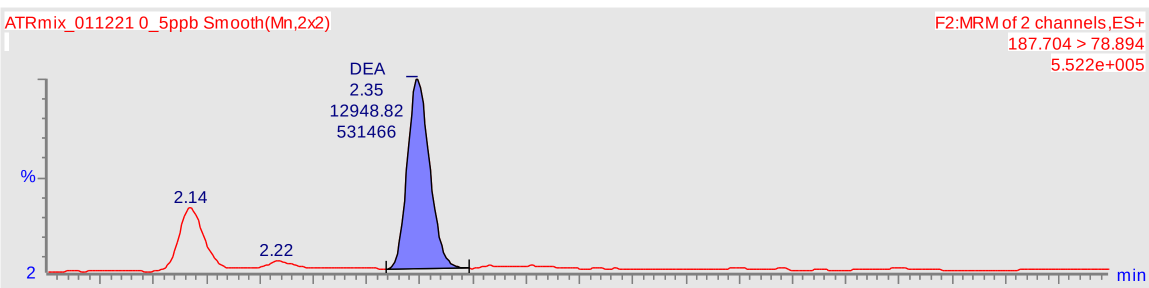
Compound name: DIA
Coefficient of Determination: R^2 = 0.999991
Calibration curve: 18299.5 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None

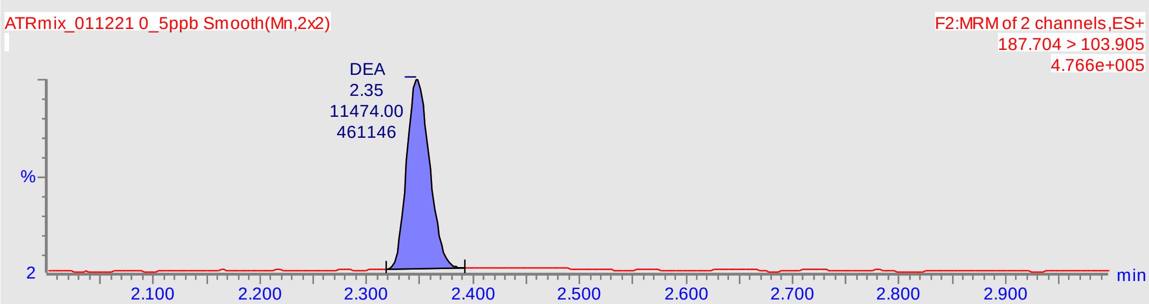




Compound name: DEA
Coefficient of Determination: R^2 = 0.999589
Calibration curve: 26325.7 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None







References:

Crop Scape and Development Data:

1. Han, W., Yang, Z., Di, L., Yue, P., 2014. A geospatial Web service approach for creating on-demand Cropland Data Layer thematic maps. Transactions of the ASABE, 57(1), 239-247. **link**

2. Han, W., Yang, Z., Di, L., Yagci, A., Han, S., 2014. Making Cropland Data Layer data accessible and actionable in GIS education. Journal of Geography, 113(3), 129-138. **link** **full text**

3. Han, W., Yang, Z., Di, L., Mueller, R., 2012. CropScape: A Web service based application for exploring and disseminating US conterminous geospatial cropland data products for decision support. Computers and Electronics in Agriculture, 84, 111–123. **link**

4. Boryan, C., Yang, Z., Willis, P., 2014. US geospatial crop frequency data layers. Third International Conference on Agro-geoinformatics (Agro-geoinformatics 2014), August 11-14, 2014, Beijing, China. **link** **full text**

5. Boryan, C., Yang, Z., Mueller, R., Craig, M., 2011. Monitoring US agriculture: the US Department of Agriculture, National Agricultural Statistics Service, Cropland Data Layer Program. Geocarto International, 26(5), 341–358. **link**

NPDES Geospatial Data:

Watershed Area Data: NLCD 2019 Land Cover (CONUS) https://www.mrlc.gov/data/nlcd-2019-land-cover-conus)

EPA Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography, 1993, https://www.epa.gov/sites/default/files/2015-06/documents/epa-300.1.pdf