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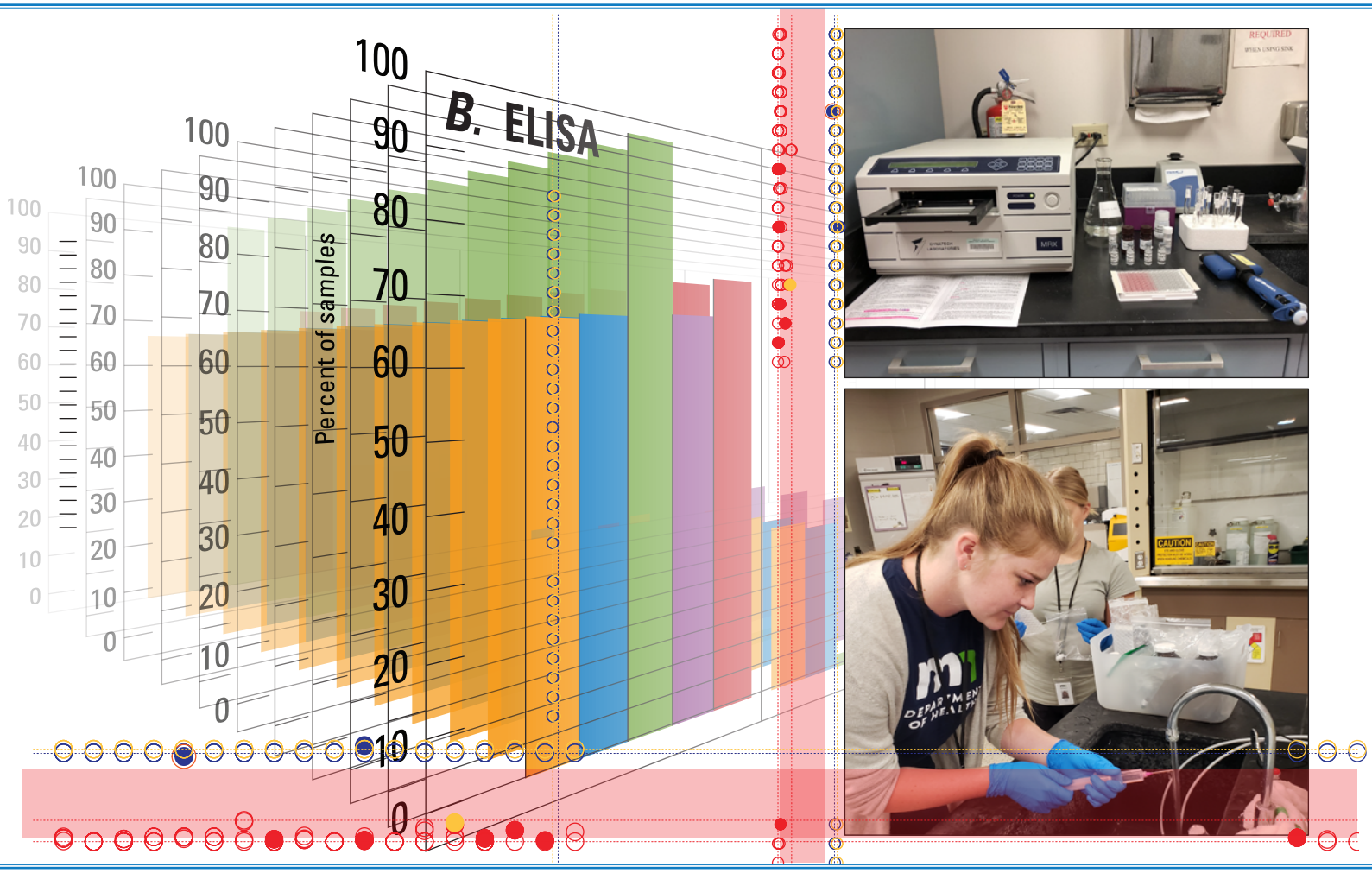
Comparison of the Results of Enzyme-Linked Immunosorbent Assay (ELISA) to Mass-Spectrometry Based Analytical Methods for Six Unregulated Contaminants in Source Water and Finished Drinking-Water Samples



Scientific Investigations Report 2022–5066

U.S. Department of the Interior
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Cover. Arianna Giorgi, Minnesota Department of Health, sampling groundwater from a tap. Photo by Jane De Lambert, Minnesota Department of Health

Enzyme-linked immunosorbent assay analysis at the U.S. Geological Survey, Upper Midwest Water Science Center in Mounds View, Minnesota. Photo by Aliesha Krall, U.S. Geological Survey

Comparison of the Results of Enzyme-Linked Immunosorbent Assay (ELISA) to Mass-Spectrometry Based Analytical Methods for Six Unregulated Contaminants in Source Water and Finished Drinking-Water Samples

By Aliesha L. Krall, Sarah M. Elliott, Jane R. de Lambert, and Stephen W. Robertson

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Acknowledgments

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Conversion Factors

U.S. customary units to International System of Units

Multiply	By	To obtain
	Length	
mile (mi)	1.609	kilometer (km)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows: °F = (1.8 × °C) + 32.

Supplemental Information

Concentrations of chemical constituents in water are given in nanograms per liter (ng/L).

Samples sizes are given in milliliters (mL) or microliters (µL).

Filter sizes are given in micrometers (µm).

Abbreviations

ATZ_{TOT}	atrazine and immunologically similar contaminants
ATZ1	atrazine
ATZ2	ametryn
ATZ3	2-Chloro-4-isopropylamino-6-amino-*s*-triazine
ATZ4	2-Hydroxy-4-isopropylamino-6-ethylamino-*s*-triazine
ATZ5	propazine
ATZ6	simazine
AXYS	SGS AXYS Analytical Services Ltd.
CAF_{TOT}	caffeine and immunologically similar contaminants
CAF1	caffeine
CAF2	1,7-Dimethylxanthine
CAF3	theophylline
CBZ_{TOT}	carbamazepine and immunologically similar contaminants
CBZ1	carbamazepine
CBZ2	amitriptyline
CV	coefficient of variation
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
GW_{Ag}	groundwater influenced by agriculture
GW_{WW}	groundwater influenced by wastewater
$GW_{WW/Ag}$	groundwater influence by both wastewater and agriculture
HPLC/MS/MS	direct aqueous injection with high performance liquid chromatography and tandem mass spectrometry
IMD_{TOT}	imidacloprid and immunologically similar contaminants
IMD1	imidacloprid
LC-MS/MS	direct aqueous injection with liquid chromatography and tandem mass spectrometry
MDH	Minnesota Department of Health
NWQL	National Water Quality Laboratory
PPW	Paired Prentice-Wilcoxon test
PYR_{TOT}	pyrethroids and immunologically similar contaminants
PYR1	*cis*-Permethrin
PYR2	bifenthrin
QC	quality control
R^2	correlation coefficient

RPD	relative percent difference
SMX_{TOT}	sulfamethoxazole and immunologically similar contaminants
SMX1	sulfamethoxazole
SMX2	sulfamethizole
SMX3	sulfadimethoxine
SMX4	sulfamerazine
SMX5	sulfamethazine
SW	surface water
UMID	U.S. Geological Survey, Upper Midwest Water Science Center
USGS	U.S. Geological Survey

Comparison of the Results of Enzyme-Linked Immunosorbent Assay (ELISA) to Mass-Spectrometry Based Analytical Methods for Six Unregulated Contaminants in Source Water and Finished Drinking-Water Samples

By Aliesha L. Krall,[1] Sarah M. Elliott,[1] Jane R. de Lambert,[2] and Stephen W. Robertson[2]

Abstract

Regulatory entities, such as the Minnesota Department of Health, monitor public water systems for conformance with Federal and State monitoring requirements and water-quality standards. Although some contaminants have Federal and (or) State regulations and guidance values, many contaminants, such as pesticides and pharmaceuticals, are unregulated in that only non-enforceable health-based guidance values have been assigned to them. Furthermore, because these contaminants are not regulated, commonly only limited resources are available to public water systems or regulatory entities to monitor them in drinking water. Focused screening efforts on contaminants that are frequently detected in the environment can provide information to help monitoring entities prioritize their sampling efforts.

Here we assess the use of enzyme-linked immunosorbent assay (ELISA) method, a rapid, inexpensive screening method, as an alternative to more expensive methods to analyze source and finished drinking-water samples collected from public water systems throughout Minnesota for three commonly detected pesticides (atrazine, imidacloprid, and pyrethroids) and three commonly detected pharmaceuticals (caffeine, carbamazepine, and sulfamethoxazole). The ELISA results were compared to results provided by more advanced mass-spectrometry analytical methods at the U.S. Geological Survey National Water Quality Laboratory (NWQL) and SGS AXYS Analytical Services Ltd. (AXYS).

Overall, these datasets are highly censored (>80 percent) and contain multiple reporting limits within and between laboratories. To discern agreement between paired contaminant group results (target contaminant plus immunologically similar contaminants) by ELISA and the advanced analytical methods at NWQL and AXYS, presence-absence agreement analysis was coupled with false negative and false positive analysis. Analysis of presence-absence agreement shows that ELISA has generally good agreement (77.9 to 100 percent) with both NWQL and AXYS for all unregulated contaminant groups. Imidicloprid, pyrethroids, and caffeine contaminant groups have relatively low false positivity rates (16, 6, and 5 percent, respectively) when analyzed by ELISA, which indicates the ELISA method, for these contaminant groups, could be experiencing low-level interference attributed to the detection of immunologically similar contaminants. Similarly, sulfamethoxazole has a low false positivity rate (0.8 percent), which indicates ELISA is likely not overestimating results for this contaminant group. Analyses for carbamazepine and sulfamethoxazole by ELISA resulted in low false negativity rates (1.6 and 0.8 percent, respectively), which indicates the ELISA method is likely not underestimating the results for this contaminant group. Conversely, the atrazine contaminant group has a high false negativity rate (84 percent), which indicates the method has a strong negative bias and that ELISA underestimates results for this contaminant. These qualitative results indicate that the ELISA method could potentially serve as a reliable and cost-effective screening method to help drinking water monitoring entities prioritize sampling efforts for analyzing carbamazepine and sulfamethoxazole in source and finished drinking-water samples collected from public water systems. At the same time, although ELISA did not prove to be a good screening method for atrazine, evaluation of ELISA results indicated that its use for screening imidacloprid, pyrethroids, and caffeine could be beneficial for water testing.

Introduction

Given the frequent occurrence of unregulated contaminants (for example, pharmaceuticals, pesticides, personal care products, and so on) in aquatic environments (Noguera-Oviedo and Aga, 2016; Ebele and others, 2017; Glassmeyer

[1]U.S. Geological Survey.

[2]Minnesota Department of Health.

and others, 2017; Wilkinson and others, 2017), and in groundwater and surface water in Minnesota (Erickson and others, 2014; Elliott and others, 2018; Elliott and others, 2017; Lee and others, 2011), it is plausible that these contaminants pose a threat to the quality of drinking water in the State. Furthermore, because these contaminants are unregulated, resources available for monitoring them in drinking water is commonly limited. Rapid, inexpensive screening methods may offer monitoring entities an alternative for assessing the presence of unregulated contaminants in drinking water to fill this gap in knowledge.

More than 75 percent of Minnesotans get their drinking water from a public water system (Minnesota Department of Health, 2020b). The Minnesota Department of Health (MDH) monitors all public water systems in the State (approximately 7,000) for conformance with Federal monitoring requirements and water-quality standards. Currently, the U.S. Environmental Protection Agency (EPA) has primary drinking-water standards and requirements for 88 compounds or chemicals, including disinfectants, disinfection byproducts, metals, microorganisms, radionuclides, and various organic compounds or chemicals (U.S. Environmental Protection Agency, 2020). In addition to the EPA primary drinking-water requirements, EPA and (or) MDH non-enforceable health-based guidance values have been established for another approximately 150 contaminants (Minnesota Department of Health, 2020a). Existing regulations and guidance values represent only a small fraction of the roughly 86,000 chemicals that are currently in use in the United States (U.S. Environmental Protection Agency, 2021). Although it is not feasible to expect drinking-water facilities or monitoring entities to test for every chemical that may be present, focusing screening efforts on particular chemicals that are more frequently used and (or) detected in the environment can provide information in prioritizing sites for more extensive monitoring.

Enzyme-linked immunosorbent assay (ELISA) methods are a rapid, inexpensive way to screen environmental samples. Briefly, ELISA relies on binding of antigens in the environmental sample to chemical-specific antibodies. The unbound antibodies are removed, and an enzyme substrate added. If antigen-antibody binding occurs, the enzyme substrate produces a color change in proportion to the amount of binding. Although ELISA data are semi-quantitative, they have been used successfully in environmental studies as a screening tool and often produce data comparable to those obtained by liquid chromatography or high-performance liquid chromatography methods (Trost and others, 2013; Bradley and others., 2014; Krall and others, 2018). ELISA test kits are commercially available for various current-use chemicals such as pesticides and pharmaceuticals.

To evaluate the use of ELISA as a screening method for monitoring contaminant presence in drinking water, the U.S. Geological Survey (USGS), in cooperation with the Minnesota Environmental and Natural Resources Trust Fund and the MDH, collected both source and finished drinking-water samples from 67 public water systems throughout Minnesota (fig. 1). Samples were analyzed at accredited laboratories for a large suite of pesticides and pharmaceuticals. Samples were also analyzed by ELISA for a subset of the contaminants: three pesticides (atrazine, imidacloprid, and pyrethroids) and (or) three pharmaceuticals (caffeine, carbamazepine, and sulfamethoxazole). These contaminants were chosen on the basis of expected sources from surrounding land use (for example, agriculture or urban), current knowledge of their frequent occurrence in the environment, and based on results from previous monitoring studies by MDH, Minnesota Department of Agriculture, Minnesota Pollution Control Agency, and USGS (Minnesota Department of Agriculture, 2016a; Minnesota Department of Agriculture, 2019; Erickson and others, 2014; Elliott and others, 2018).

Purpose and Scope

The purpose of this report is to present results of the analysis to discern agreement between ELISA and other analytical methods at two accredited laboratories on the prevalence of six unregulated contaminants (atrazine, imidacloprid, pyrethroids, caffeine, carbamazepine, and sulfamethoxazole) in source and finished drinking-water samples collected from public water systems throughout Minnesota. The laboratories used were the USGS National Water Quality Laboratory (NWQL) and SGS AXYS Analytical Services, Ltd (AXYS). The analysis objectives were threefold: (1) to document and describe the ELISA, NWQL, and AXYS results, (2) to statistically compare ELISA concentrations to the NWQL and AXYS concentrations, and (3) to describe implications of the results.

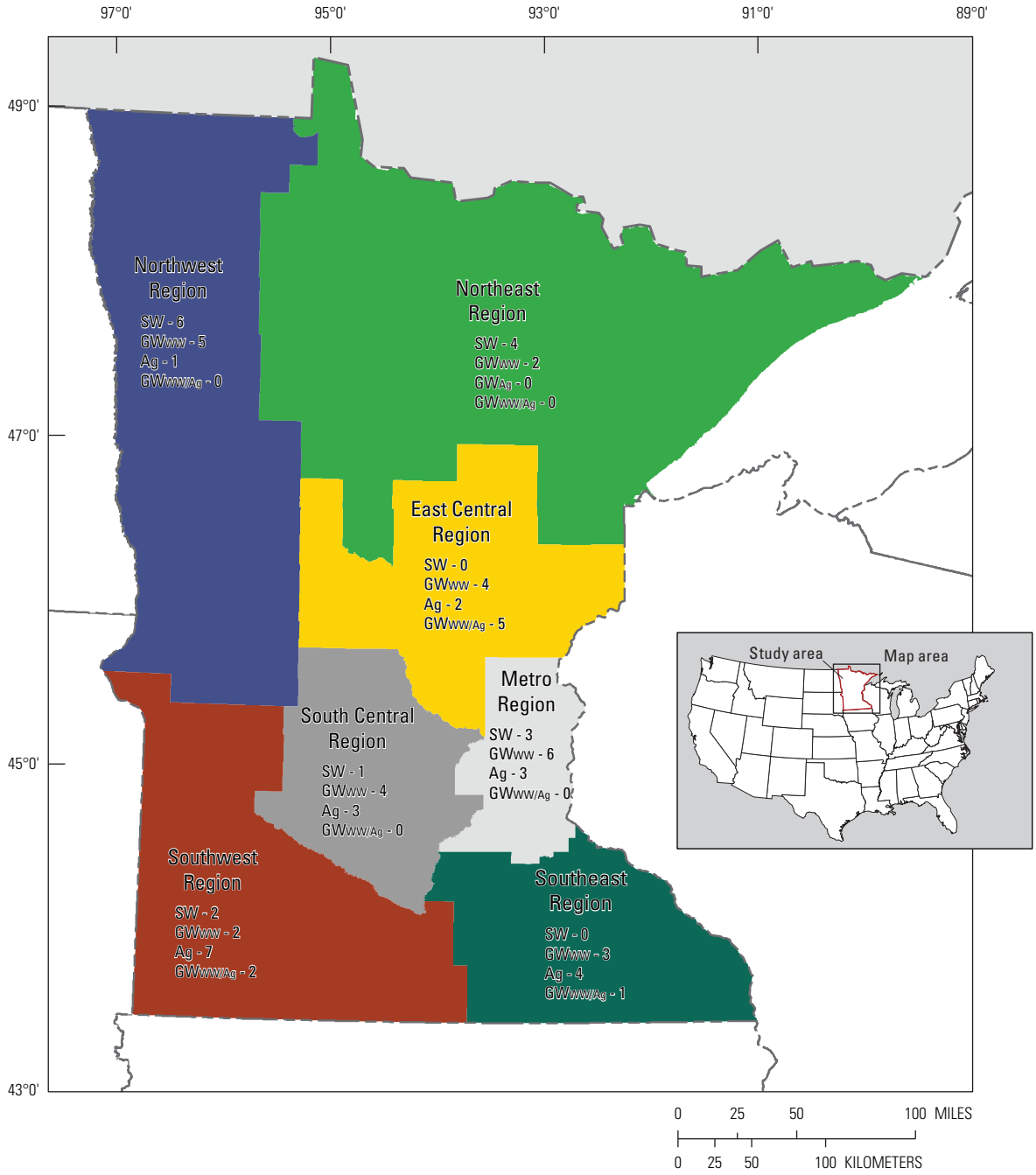

Figure 1. General locations of sampled Minnesota public water systems, 2019. [SW, surface water sourced public water system; GW_{WW}, groundwater sourced public water system influenced by wastewater; GW_{Ag}, groundwater sourced public water system influenced by agricultural activities; $GW_{WW/Ag}$, groundwater sourced public water system influenced by a mix of wastewater and agricultural activities]

Study Area

The data used in this assessment of analytical methods are the results of analyses of samples collected from 67 public water systems throughout Minnesota. To retain anonymity of the sampled facilities, the number and source water type for the public water systems sampled are shown only within geographic regions in figure 1. The public water systems were categorized on the basis of the source of their water, whether surface water or groundwater. Groundwater sources were further categorized by expected sources of contamination—whether influenced by wastewater, agricultural activities, or a combination of those influences. The source waters for the 67 public water systems sampled consisted of 16 surface-water

(SW) sources, 22 groundwater sources influenced by wastewater (GW_{WW}), 21 groundwater sources influenced by agricultural activities (GW_{Ag}), and 8 groundwater sources influenced by both wastewater and agricultural activities ($GW_{WW/Ag}$).

Study Methods

Water samples were collected and handled using methods specified in the USGS National Field Manual for the Collection of Water-Quality Data (USGS, 2006), and analyzed both by the ELISA method at the USGS Upper Midwest Water Science Center (UMID) and by advanced liquid mass-spectrometry based methods at the two accredited laboratories. Quality-assurance and quality-control practices were followed at both field and laboratory analytical phases of sample processing.

Collection of Water Samples

Source water and finished drinking-water samples were collected by MDH staff at public water systems between August and November 2019. Samples were collected once from groundwater sourced public water systems (GW_{WW}, GW_{Ag}, and $GW_{WW/Ag}$) and twice from surface-water-sourced facilities (SW). Source water samples were grab samples collected from raw water taps inside water treatment plants or well houses or from surface-water intakes. Finished water samples were grab samples collected from sampling taps inside the water treatment plant or well house. Prior to sample collection, the water lines were flushed for about 15 minutes and physical or field measurable properties (water temperature, dissolved oxygen concentration, pH, and specific conductance) were monitored and recorded with a YSI ProDSS water-quality meter (YSI Inc., Yellow Springs, Ohio) every three minutes until five consecutive sets of field properties indicated stabilization of those properties. Samples for ELISA analyses were collected in two 40 milliliter (mL) amber vials to about the half-full mark, immediately put on ice, and stored at MDH at 4 degrees Celsius until frozen within 5 days at UMID in Mounds View, Minnesota. For pesticide and pharmaceutical analyses at the NWQL, the water was passed through a 0.7 micrometer (µm) syringe-tip filter, from which 10 mL samples were dispensed into an amber glass vial. For pharmaceutical analyses at AXYS, two samples of approximately 450 mL each were collected. Field (blank and sequential replicate) quality-control (QC) samples were collected and analyzed in the same manner as environmental samples. Samples were stored at MDH at 4 degrees Celsius for up to 5 days prior to shipment to the appropriate analyzing laboratories. Samples for analyses at the NWQL were delivered to UMID for preparation and shipment overnight on wet ice. Samples for analyses at AXYS were shipped to the laboratory on wet ice by MDH personnel.

Laboratory Analyses

Concentrations of three target pesticides (atrazine, ATZ; imidacloprid, IMD; and pyrethroids, PYR) plus several immunologically similar contaminants; and three target pharmaceuticals (caffeine, CAF; carbamazepine, CBZ; and sulfamethoxazole, SMX) plus several immunologically similar contaminants were determined. Target contaminants and immunologically similar contaminants (as defined by ELISA specifications) are referenced by the subscript "TOT" following the target contaminant acronym (for example, ATZ_{TOT}, CBZ_{TOT}) and are referred to as contaminant groups. Note that ATZ_{TOT} and all contaminant group "TOT" results may represent a different set of compounds across analysis methods. Target contaminants are referenced by the number 1 following the acronym (for example, ATZ1, CBZ1). Immunologically similar contaminants are referenced by a number greater than 1 following the acronym (for example, ATZ2, CBZ2). This referencing scheme does not apply to pyrethroids because pyrethroids are a family of contaminants, so there is no single target contaminant. A description of each referenced contaminant is provided in table 1. Samples collected from SW (66 samples) and $GW_{WW/Ag}$ (16 samples) public water systems were analyzed for all six contaminants. Samples collected from GW_{Ag} (40 samples) public water systems were analyzed for the three pesticides, and samples collected from GW_{WW} (40 samples) public water systems were analyzed for the three pharmaceuticals. A total of 162 water-quality samples were analyzed by three different laboratory entities. Analytical results are available in a USGS data release (Krall and Elliott, 2022).

Enzyme-Linked Immunosorbent Assay (ELISA) Methods

Concentrations of six target contaminants plus their immunologically similar contaminants (ATZ_{TOT}, IMD_{TOT}, PYR_{TOT}, CAF_{TOT}, CBZ_{TOT}, and SMX_{TOT}) in 162 samples from 67 public water systems were determined at UMID using commercially available ELISA kits (Eurofins-Abraxis Inc., Warminister, Pennsylvania). The contaminants analyzed in each sample differed depending on the source water used at a public water-supply system. A total of 122 source and finished drinking-water samples collected from 45 public water systems (SW, $GW_{WW/Ag}$, and GW_{Ag}) were analyzed for three pesticide contaminant groups (ATZ_{TOT}, IMD_{TOT}, and PYR_{TOT}). A total of 122 source and finished drinking-water samples collected from 48 public water systems (SW, $GW_{WW/Ag}$, and GW_{WW}) were analyzed for three pharmaceutical contaminant groups (CAF_{TOT}, CBZ_{TOT} and SMX_{TOT}).

The ELISA analytical procedure is described in detail elsewhere (Eurofins-Abraxis Inc., 2019a, 2019b, 2019c, 2019d, 2019e, 2019f). Briefly, all environmental and quality-control (QC) samples were analyzed in duplicate and hereafter are referred to as analysis pairs. Several types of QC

samples were provided by the manufacturer, including five to six calibration standards and up to two control samples of a known concentration. Other QC samples included laboratory reagent blanks, laboratory fortified blanks, and laboratory fortified sample matrix samples. First, the environmental and QC samples were dispensed into separate wells on the microplate. Antibody solution was then added to each well, mixed, and allowed to incubate either at ambient temperature or between 2 and 8 °C for between 30 and 90 minutes, depending on the contaminant being analyzed. After incubation, the contents in the wells were discarded and washed with a 1:5 wash buffer solution. Following the wash, the contents in the wells were discarded, a color solution was added to each well, and the microplate was incubated at ambient temperature or between 2 and 8 °C for 20 to 30 minutes, depending on the contaminant being analyzed. Lastly, stop solution was added to each well and within 15 minutes the plate was placed into a Bio Tek Microplate Reader (Bio Tek Instruments Inc., Winooski, Vermont) to read the absorbance of each well's contents at 450 nanometers. The reporting limits for each contaminant range from 50 to 2,000 nanograms per liter (ng/L) for pesticides, and from 25 to 175 ng/L for pharmaceuticals (table 1).

A four-parameter logistic curve was produced for calibration of each batch assay analysis in Gen5 Microplate Software for Windows (Bio Tek Instruments, Winooski, Vermont). The calibration curve was validated by evaluating the percent coefficient of variation (CV) between absorbance values for each calibration standard pair. To accept the assay calibration, the CV must be less than or equal to 10 percent for each calibration standard pair. However, one calibration standard analysis pair can have less than or equal to 15 percent CV, providing the assay curve correlation coefficient (R^2) is greater than or equal to 0.98. If the calibration fails the percent CV limits of absorbance and R^2 less than 0.98, then the assay analysis is invalid. The percent CV for control and environmental analysis pairs is acceptable at less than 20 percent. When the percent CV exceeds this limit, the individual control or environmental sample analysis is invalid. Acceptable relative percent recovery limits for spiked samples range from 70 to 130 percent of the expected concentration. If recoveries are outside of this range, the results may be matrix biased. The relative percent difference (RPD) of laboratory fortified sample matrix samples should be less than 30 percent, and if RPD exceeds this limit then the precision of the plate analysis may be matrix biased.

Direct Aqueous Injection with Liquid Chromatography and Tandem Mass Spectrometry (LC-MS/MS) Pesticide Analysis at the U.S. Geological Survey National Water Quality Laboratory (NWQL)

A total of 122 source and finished drinking-water samples were collected from 45 SW, GW_{Ag}, and $GW_{WW/Ag}$ public water systems and analyzed at the NWQL for 225 pesticides by LC-MS/MS, using methods described in Sandstrom and others (2016). Briefly, a 100-microliter (µL) sample was directly injected into the LC-MS/MS without any sample preparation. Samples were analyzed in electrospray ionization positive and negative mode using two multiple- reaction monitoring conditions. The target contaminant atrazine (ATZ1) and five immunologically similar contaminants (ametryn [ATZ2], 2-chloro04-isopropylamino-6-amino-*s*-triazine [ATZ3], 2-hydrixy-4-isopropylamino-6-ehtylamino-*s*-triazine [ATZ4], propazine [ATZ5], and simazine [ATZ6]), along with the target contaminant imidacloprid (IMD1), and pyrethroids (*cis*-permethrin [PYR1] and bifenthrin [PYR2]), were included in the analysis. Reporting limits for each contaminant ranged from 3.2 to 250 ng/L (table1).

Direct Aqueous Injection with High Performance Liquid Chromatography and Tandem Mass Spectrometry (HPLC/MS/MS) Pharmaceutical Analysis at the U.S. Geological Survey National Water Quality Laboratory (NWQL)

A total of 122 source and finished drinking-water samples collected from 46 SW, GW_{WW}, and $GW_{WW/Ag}$ public water systems were analyzed at the NWQL for 110 pharmaceuticals by HPLC/MS/MS, using methods described in Furlong and others (2014). Briefly, 100 µL of sample was directly injected into a HPLC/MS/MS. Samples were analyzed using an electrospray ionization source in the positive ion mode. The target contaminant caffeine (CAF1) and two immunologically similar compounds (1,7-dimethylxanthine [CAF2] and theophylline [CAF3]), the target contaminant carbamazepine (CBZ1) and one immunologically similar contaminant (amitriptyline [CBZ2]), and the target contaminant sulfamethoxazole (SMX1) and two immunologically similar contaminants (sulfamethizole [SMX2] and sulfadimethoxine [SMX3]), were included in analysis. Reporting limits for each contaminant ranged from 11 to 200 ng/L (table 1).

Table 1. Unregulated contaminants analyzed in source water and finished drinking-water samples collected from public water systems throughout Minnesota, 2019, and associated laboratory reporting limits and human health-based advisory levels in nanograms per liter.

[CASRN, Chemical Abstracts Services Registry Number; NWIS, National Water Information System; ELISA, enzyme- linked immunosorbent assay analytical method; Min, minimum; Max, maximum; NWQL, U.S. Geological Survey National Water Quality Laboratory; AXYS, SGS AXYS Services Ltd.; ATZ, atrazine; --, no data available; HRL, Minnesota Department of Health health risk limit; MCL, U.S. Environmental Protection Agency enforcable maximum contaminant level; HBV, Minnesota Department of Health health risk limit; HBSL, U.S. Geological Survey cancer health-based screening level; IMD, imidacloprid; PYR, pyrethroids; HHBP, U.S. Environmental Protection Agency carcinogenic or chronic noncancer human health benchmark for pesticide; CAF, caffeine; CBZ, carbamazepine; SMX, sulfamethoxazole; RAA, Minnesota Department of Health risk assessment advice]

Contaminant	Immunologically similar contaminant	Immunologically similar contaminant definition	CASRN	NWIS parameter code	ELISA		
					Reporting limit	Mini	Max
ATZ	ATZTOT	Atrazine and immunologically similar contaminants analyzed by respective laboratory	--	--	50	<50	163
	ATZ1	Atrazine	1912-24-9	65065			
	ATZ2	Ametryn	834-12-8	68533			
	ATZ3	2-Chloro-4-isopropylamino-6-amino-*s*-triazine	6190-65-4	68552			
	ATZ4	2-Hydroxy-4-isopropylamino-6-ethylamino-*s*-triazine	2163-68-0	68660			
	ATZ5	Propazine	139-40-2	68678			
	ATZ6	Simazine	122-34-9	65105			
IMD	IMDTOT	Imidacloprid and immunologically similar contaminants analyzed by respective laboratory	--	--	300	76	<300
	IMD1	Imidacloprid	138261-41-3	68426			
PYR	PYRTOT	Pyrethroids and immunologically similar contaminants analyzed by respective laboratory	--	--	2,000	1,053	2,245
	PYR1	*cis*-Permethrin	61949-76-6	68769			
	PYR2	Bifenthrin	82657-04-3	65067			
CAF	CAFTOT	Caffeine and immunologically similar contaminants analyzed by respective laboratory	--	--	175	<175	384
	CAF1	Caffeine	58-0-2	67440			
	CAF2	1,7-Dimethylxanthine	611-59-6	67446			
	CAF3	Theophylline	58-55-9	67494			
CBZ	CBZTOT	Carbamazepine and immunologically similar contaminants analyzed by respective laboratory	--	--	25	<25	118
	CBZ1	Carbamazepine	298–46–4	67441			
	CBZ2	Amitriptyline	50–48–6	67522			
SMX	SMXTOT	Sulfamethoxazole and immunologically similar contaminants analyzed by respective laboratory	--	--	25	24	27
	SMX1	Sulfamethoxazole	723–46–6	67454			
	SMX2	Sulfamethizole	144–82–1	67476			
	SMX3	Sulfadimethoxine	122–11–2	67503			
	SMX4	Sulfamerazine	127-79-7	--			
	SMX5	Sulfamethazine	57-68-1	--			

Table 1. Unregulated contaminants analyzed in source water and finished drinking-water samples collected from public water systems throughout Minnesota, 2019, and associated laboratory reporting limits and human health-based advisory levels in nanograms per liter.—Continued

[CASRN, Chemical Abstracts Services Registry Number; NWIS, National Water Information System; ELISA, enzyme- linked immunosorbent assay analytical method; Min, minimum; Max, maximum; NWQL, U.S. Geological Survey National Water Quality Laboratory; AXYS, SGS AXYS Services Ltd.; ATZ, atrazine; --, no data available; HRL, Minnesota Department of Health health risk limit; MCL, U.S. Environmental Protection Agency enforcable maximum contaminant level; HBV, Minnesota Department of Health health risk limit; HBSL, U.S. Geological Survey cancer health-based screening level; IMD, imidacloprid; PYR, pyrethroids; HHBP, U.S. Environmental Protection Agency carcinogenic or chronic noncancer human health benchmark for pesticide; CAF, caffeine; CBZ, carbamazepine; SMX, sulfamethoxazole; RAA, Minnesota Department of Health risk assessment advice]

NWQL			AXYS			Human health-based guidance value	Human health-based guidance value type
Reporitng limit	Min	Max	Reporitng limit	Min	Max		
6.80–20.0	1.46	453.06	--	--	--	--	--
6.80–20.0	1.46	363	--	--	--	3,000	HRL, MCL
2.60–25.0	<2.60	<25	--	--	--	--	--
11.0–250	5.45	111	--	--	--	3,000	HRL, MCL
8.0–250	5.81	<250	--	--	--	20,000	HBV
3.2–10.0	1.04	<10	--	--	--	40,000	HBSL
7.2–250	4.26	<250	--	--	--	4,000	HRL, MCL
16.0–250	6.08	<250	--	--	--	--	--
16.0–250	6.08	<250	--	--	--	2,000	HBV
4.2–250	<4.2	<250	--	--	--	--	--
4.2–5.0	<4.2	<5.0	--	--	--	3,340	HHBP
19.0–250	<19.0	<250	--	--	--	70,000	HHBP
91	9.03	<91.0	13.8–85.2	<13.8	<85.2	--	--
91	9.03	<91.0	13.8–85.2	<13.8	<85.2	--	--
88.0–200	<88.0	<200	55.0–291	<55.0	<291	--	--
80.0–140	<80.0	<140	--	--	--	--	--
11	1.27	178	1.38–7.28	<1.38	157	--	--
11	1.27	178	1.38–7.28	<1.38	157	40,000	HRL
37.0–140	<37.0	<140	--	--	--	--	--
20.0–26.0	5.81	101	0.573–6.46	<0.573	86.7	--	--
20.0–26.0	5.81	101	0.573–6.46	<0.573	86.7	100,000	RAA
104–200	<104	<200	0.550–4.32	<0.550	<4.32	--	--
30	2.17	<30.0	0.275–7.85	<0.275	<7.85	--	--
--	--	--	0.572–4.27	<0.572	<4.27	--	--
--	--	--	0.575–9.7	<0.575	<29.5	100,000	HRL

Direct Aqueous Injection with Liquid Chromatography and Tandem Mass Spectrometry (LC-MS/MS) Pharmaceutical Analysis at the SGS AXYS Analytical Services Ltd. (AXYS)

A total of 122 source and finished drinking-water samples collected from 46 SW, GW_{WW}, and $GW_{WW/Ag}$ public water systems were analyzed at AXYS in British Columbia, Canada by LC-MS/MS (SGS AXYS Analytical Services Ltd., 2019). Briefly, samples were filtered and cleaned using solid-phase extraction. Extracts were then analyzed using LC-MS/MS run in multiple reaction monitoring mode. The target contaminant CAF1 and one immunologically similar contaminant (CAF2), the target contaminant CBZ1, and the target contaminant SMX1 and four immunologically similar contaminants (SMX2, SMX3, sulfamerazine [SMX4], and sulfamethazine [SMX5]), were included in analysis. Reporting limits for each contaminant ranged from 0.275 to 291 ng/L (table 1).

Field and Laboratory Quality Assurance/Quality Control

Field and laboratory QC samples were used to validate and interpret the environmental sample data. Field QC samples are used to assess the quality of the sampling process, including the collection, processing, preservation, transportation, and handling of the samples. Laboratory QC samples are used to assess the quality of the analytical procedure. Each laboratory analyzed blanks, reagent spikes, matrix spikes, and surrogates along with each batch of environmental samples. A detailed summary of the QC data for each of the three laboratory entities is available in Krall and Elliott (2022).

Eight field blanks and eight sequential replicates were collected. Field blank sample results were below the detection limit, with one exception. IMD_{TOT} was detected by ELISA in one source and one finished water field blank sample. Many sequential field replicate sample results by each of the three laboratories were reported as below the detection limit, so RPD could not be assessed. For those that could be assessed, the RPD ranged from 2 to 30 percent for ATZ1, ATZ3, and ATZ4 (analyzed by NWQL) and was 51 percent for SMX5 (analyzed by AXYS).

Each laboratory analyzed blanks, reagent spikes, matrix spikes, and surrogates with each batch of environmental samples. CAF1 was detected in two laboratory blanks by AXYS, and SMXtot was detected in one laboratory blank by ELISA at UMID. Reagent and laboratory matrix spike sample recoveries ranged from 50 to 274 percent and from 16 to 211 percent, respectively. Reagent and matrix spike percent recoveries outside acceptable limits (greater than or equal to 70 and less than or equal to 130 percent) indicate the recoveries could be matrix biased.

Data Processing

The datasets from each laboratory were processed individually prior to making comparisons among them. Data generated by the three analytical laboratories are left censored, meaning that the true values of the censored values are unknown and lie between zero and the reporting limit. Each laboratory had different reporting limits for each contaminant, and some had multiple reporting limits for each contaminant (table 1). Appendixes 1 and 2 provide further discussion on the methods of both censoring contaminant groups and assessing false negative and false positive ELISA observations.

Immunologically Similar Contaminants

The ELISA method responds to immunologically similar (cross-reactive) contaminants, which provides an advantage of detecting target contaminants and associated degradates or metabolites (Eurofins-Abraxis Inc., 2019a, 2019b, 2019c, 2019d, 2019e, 2019f), and may provide an indication of the presence of contaminant mixtures. Therefore, the ELISA results were expected to be higher compared to the target contaminant results from NWQL and AXYS. The summation of the concentrations of target contaminants and immunologically similar contaminants reported by NWQL and AXYS were compared with ELISA results (table 1), and as previously mentioned, are referred to as contaminant groups. Not all immunologically similar contaminants with the potential to cross-react during the ELISA method analyses were analyzed by the other laboratories. The contaminants that make up each contaminant group are described in detail below.

Pesticides

The NWQL ATZ_{TOT} results are the summation of ATZ1 and five immunologically similar contaminant (ATZ2, ATZ3, ATZ4, ATZ5, and ATZ6; table 1) concentrations. Both ATZ3 and ATZ4 are metabolites and environmental degradation products of ATZ1, whereas ATZ2, ATZ5, and ATZ6 are herbicides belonging to the triazine class. The ELISA analytical method for ATZ_{TOT} also indicates the potential of cross-reactivity with terbuthylazine, an herbicide belonging to the triazine class, but this contaminant was not analyzed by the NWQL. The exclusion of terbuthylazine is considered trivial for this study because of its circumscribed use in industrial recirculating cooling water applications and ornamental fountains within the US (U.S. Environmental Protection Agency, 2010) and because it has a low cross-reactivity rate (0.33 percent) (Eurofins-Abraxis Inc., 2019a).

The NWQL IMD_{TOT} results used in this analysis represent only IMD1 because none of the immunologically similar contaminants indicated by the ELISA method were analyzed. The only immunologically similar contaminant that could be of importance is clothianidin because it has a high potential cross-reactivity rate (121 percent) with ELISA and has been detected in both surface water and groundwater in

Minnesota (Eurofins-Abraxis, 209d; Minnesota Department of Agriculture, 2020). Exclusion of the other immunologically similar contaminants from the comparison methods is expected to be trivial because of low cross-reactivity rates (less than 5 percent) or discontinued use in the State (Eurofins-Abraxis, 2019d, Minnesota Department of Agriculture, 2016b and 2020). The NWQL PYR_{TOT} results are the summation of *cis*-permethrin (PYR1) and bifenthrin (PYR2) concentrations. Both PYR1 and PYR2 are insecticides belonging to the pyrethroid family. The ELISA analysis method for PYR_{TOT} does not indicate the potential for cross-reactivity of any other contaminants outside the pyrethroid family (Eurofins-Abraxis Inc., 2019e), and no other contaminants within the pyrethroid family were analyzed at the NWQL.

Pharmaceuticals

The NWQL CAF_{TOT} results are the summation of CAF1, CAF2, and CAF3 (table 1) concentrations. CAF2 is a metabolite and environmental degradation product of CAF1. CAF3 is a bronchodilator used to treat breathing disorders in human and veterinary medical practices (Jilani and others, 2021). The ELISA analytical method for CAF_{TOT} indicates the potential of three other immunologically similar contaminants to exhibit cross-reactivity during analysis that were not analyzed by NWQL so were not included in the summation of CAF_{TOT}. The exclusion of the latter three potentially cross-reactive contaminants is considered trivial because the cross-reactivity rate for each contaminant is <3 percent (Eurofins Abraxis Inc., 2019b).

The AXYS CAF_{TOT} results are the summation of CAF1 and CAF2 (table 1) concentrations. The exclusion of CAF3 could be notable because it has been detected in Minnesota surface water (Minnesota Pollution Control Agency, 2017). Not including the other three potentially cross-reactive contaminants is considered trivial because the cross-reactivity rate for each contaminant is <3 percent (Eurofins Abraxis Inc., 2019b) and the presence of these contaminants in surface water and groundwater is unknown.

The NWQL CBZ_{TOT} results are the summation of CBZ1 and CBZ2 whereas the AXYS CBZ_{TOT} results represent CBZ1 only. The ELISA method indicates potential cross reactivity of six other immunologically similar contaminants that were not analyzed by NWQL. Only two of the immunologically similar contaminants are of potential importance (10,11-dihydro carbamazepine and 10,11-epoxy carbamazepine) because they have been detected in surface waters in Minnesota and worldwide (Miao and Metcalfe, 2003; Bahlmann and others, 2009; Writer and others, 2013) and have high cross-reactivity rates (97 and 78 percent, respectively).

The NWQL SMX_{TOT} results are the summation of SMX1–SMX3, whereas the AXYS SMX_{TOT} results are the summation of SMX1–SMX4. One sample was unquantifiable, resulting in one fewer sample result for SMX1, SMX2, and SMX4. The ELISA method indicates potential cross reactivity of 12 other immunologically similar contaminants (Eurofins-Abraxis, Inc., 2019f), none of which were analyzed by NWQL. The exclusion of 4 of the 12 immunologically similar contaminants is potentially important because these contaminants have been detected in Minnesota surface water (Minnesota Pollution Control Agency, 2017). The importance of excluding the other eight immunologically similar contaminants is unknown because their presence in the environment is unknown at the time of this report.

Analytical Method Comparisons

To evaluate the use of ELISA as a screening method for monitoring drinking water, we analyzed paired presence-absence agreement and concentration differences between ELISA and both the mass-spectrometry based analytical methods, as appropriate. The paired results have different reporting limits, and prior to comparison, each result of a pair was re-censored to the highest reporting limit of the two results. More detail is provided in appendix 1. Furthermore, because the data are highly censored (greater than 80 percent), the results of statistical analyses to discern agreement between paired concentrations by the Paired Prentice-Wilcoxon (PPW) test are tenuous. The PPW test and results are described in more detail in appendix 3.

The presence-absence test is a qualitative and presumptive test used to determine the presence of a contaminant in a sample rather than its concentration. The qualitative results (present or absent) for each result in a pair are then compared for agreement. For the presence-absence test, the contaminant was present in a sample if it was detected above the highest reporting limit; contrarily, the contaminant was determined absent in a sample if the contaminant was not detected above the highest reporting limit. If the contaminant was either present or absent in both results, the pair agreed. The result pair did not agree if the contaminant was present in one sample and absent from the other.

Results of Analyses

Sample data plus laboratory and field QC results for the three pesticides and three pharmaceuticals of interest determined by ELISA and NWQL methods, and for the three pharmaceuticals of interest determined by the AXYS method are available in Krall and Elliott (2022). As the results indicate, there were few detections of the contaminants. This made comparisons between detected concentrations seemingly insignificant. Appendix 4 provides detail on concentration differences between contaminant detections.

Table 2. Summary of pesticide and pharmaceutical detections analyzed in source and finish drinking-water samples collected from public water systems throughout Minnesota, 2019.

[See table 1 for definitions of immunologically similar contaminants. ELISA, enzyme-linked immunosorbent assay analytical method; CO, censored observation; UO, uncensored observation; *n*, number of ovservations; %, percent; NWQL, U.S. Geological Survey National Water Quality Laboratory; AXYS, SGS AXYS Services Ltd.; --, no data]

Immunologically similar contaminant	ELISA													
	Total *n*	False –	False +	Initial				Recensored (ELISA versus NWQL)				Recensored (ELISA versus AXYS)			
				CO		UO		CO		UO		CO		UO	
				n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
ATZTOT	122	27	0	117	95.9	5	4.1	117	95.9	5	4.1	--	--	--	--
ATZ1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
ATZ2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
ATZ3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
ATZ4	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
ATZ5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
ATZ6	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
IMDTOT	122	0	20	100	82.0	22	18.0	102	83.6	20	16.4	--	--	--	--
IMD1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
PYRTOT	122	0	7	115	94.3	7	5.7	115	94.3	7	5.7	--	--	--	--
PYR1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
PYR2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
CAFTOT	122	0	6	115	94.3	7	5.7	115	94.3	7	5.7	115	94.3	7	5.7
CAF1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
CAF2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
CAF3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
CBZTOT	122	2	0	121	99.2	1	0.8	121	99.2	1	0.8	121	99.2	1	0.8
CBZ1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
CBZ2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
SMXTOT	122	1	1	120	98.4	2	1.6	121	99.2	1	0.8	120	98.4	2	1.6
SMX1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
SMX2	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
SMX3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
SMX4	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
SMX5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Pesticides and Pharmaceuticals Determined by Enzyme-Linked Immunosorbent Assay (ELISA)

Overall, there were few detections by ELISA for all six unregulated contaminants (0.8 to 18 percent; table 2). ATZ_{TOT}, IMD_{TOT}, and PYR_{TOT} were detected by ELISA in 4.1 (5 of 122 samples), 18 (22 of 122 samples), and 5.7 percent (7 of 122 samples) of samples, respectively (table 2; fig. 2*A*). CAF_{TOT}, CBZ_{TOT}, and SMX_{TOT} were detected by ELISA in 5.7 (7 of 122 samples), 0.8 (1 of 122 samples), and 1.6 percent (2 of 122 samples) of samples, respectively (table 2; fig. 2*A*).

Pesticides and Pharmaceuticals Determined by the National Water Quality Laboratory (NWQL)

Overall, ATZ_{TOT} was detected in 77 percent (94 of 122 samples) of samples analyzed by the NWQL method (table 2; fig. 2*B*). A total of 71.3 percent (87 of 122 samples) of samples had ATZ1 detections, while 65.5 percent (80 of 122 samples) of samples had detections of at least one immunologically similar contaminant. The ATZ1 immunologically similar contaminants most frequently detected by the NWQL method were ATZ3 and ATZ4, with detections at 46.7 (57 of 122) and 33.6 percent (41 of 122 samples), respectively.

Table 2. Summary of pesticide and pharmaceutical detections analyzed in source and finish drinking-water samples collected from public water systems throughout Minnesota, 2019.—Continued

[ELISA, enzyme-linked immunosorbent assay analytical method; NWQL, U.S. Geological Survey National Water Quality Laboratory; AXYS, SGS AXYS Services Ltd.; *n*, number of ovservations; %, percent; --, no data]

NWQL									AXYS								
Toal	Initial				Recensored				Total	Initial				Recensored			
n	CO		UO		CO		UO		*n*	CO		UO		CO		UO	
	n	%	*n*	%	*n*	%	*n*	%		*n*	%	*n*	%	*n*	%	*n*	%
122	28	23.0	94	77.0	90	73.8	32	26.2	--	--	--	--	--	--	--	--	--
122	35	28.7	87	71.3	--	--	--	--	--	--	--	--	--	--	--	--	--
122	121	99.2	1	0.8	--	--	--	--	--	--	--	--	--	--	--	--	--
122	65	53.3	57	46.7	--	--	--	--	--	--	--	--	--	--	--	--	--
122	81	66.4	41	33.6	--	--	--	--	--	--	--	--	--	--	--	--	--
122	114	93.4	8	6.6	--	--	--	--	--	--	--	--	--	--	--	--	--
122	119	97.5	3	2.5	--	--	--	--	--	--	--	--	--	--	--	--	--
122	120	98.4	2	1.6	122	100.0	0	0.0	--	--	--	--	--	--	--	--	--
122	120	98.4	2	1.6	--	--	--	--	--	--	--	--	--	--	--	--	--
122	122	100.0	0	0.0	122	100.0	0	0.0	--	--	--	--	--	--	--	--	--
122	122	100.0	0	0.0	--	--	--	--	--	--	--	--	--	--	--	--	--
112	112	100.0	0	0.0	--	--	--	--	--	--	--	--	--	--	--	--	--
122	115	94.3	7	5.7	121	99.2	1	0.8	122	120	98.4	2	1.6	122	100.0	0	0.0
122	115	94.3	7	5.7	--	--	--	--	122	120	98.4	2	1.6	--	--	--	--
122	122	100.0	0	0.0	--	--	--	--	122	122	100.0	0	0.0	--	--	--	--
122	122	100.0	0	0.0	--	--	--	--	--	--	--	--	--	--	--	--	--
122	114	93.4	8	6.6	119	97.5	3	2.5	122	111	91.0	11	9.0	121	99.2	1	0.8
122	114	93.4	8	6.6	--	--	--	--	122	111	91.0	11	9.0	--	--	--	--
115	115	100.0	0	0.0	--	--	--	--	--	--	--	--	--	--	--	--	--
122	116	95.1	6	4.9	119	97.5	3	2.5	122	102	83.6	20	16.4	119	97.5	3	2.5
110	106	96.4	4	3.6	--	--	--	--	122	108	88.5	13	10.7	--	--	--	--
122	122	100.0	0	0.0	--	--	--	--	122	119	97.5	3	2.5	--	--	--	--
122	120	98.4	2	1.6	--	--	--	--	122	118	96.7	4	3.3	--	--	--	--
--	--	--	--	--	--	--	--	--	122	120	98.4	2	1.6	--	--	--	--
--	--	--	--	--	--	--	--	--	122	118	96.7	4	3.3	--	--	--	--

ATZ2, ATZ5, and ATZ6 were detected in ≤7 percent (≤8 of 122 samples) of samples. Generally, ATZ3 and ATZ4 made up a significant portion of the summed ATZ_{TOT} concentrations detected by the NWQL method, and ATZ4>ATZ3>ATZ1. IMD_{TOT} was detected in 1.6 percent (2 of 122 samples) of samples by the NWQL method, and PYR_{TOT} was not detected in any of the samples (fig. 2b).

Neither CAF2 nor CAF3 were detected in any of the samples by the NWQL method. CAF1 was detected in 5.7 percent (7 of 122 samples; table 2) of samples, thus CAF_{TOT} reflects CAF1 concentrations (table 2; fig. 2b). CBZ2 was not detected in any of the samples analyzed by the NWQL method. CBZ1 was detected in 6.6 percent (8 of 122 samples) of samples, thus CBZ_{TOT} reflects CBZ1 concentrations. Overall, 4.9 percent (6 of 122 samples) of samples had SMX_{TOT} detections by the NWQL method (table 2; fig. 2*B*). The target contaminant, SMX1, was detected in 3.3 percent (4 of 122 samples) of samples, while SMX3 was detected in <2 percent (2 of 122 samples) (table 2). SMX2 was not detected in any of the samples by the NWQL method (table 2).

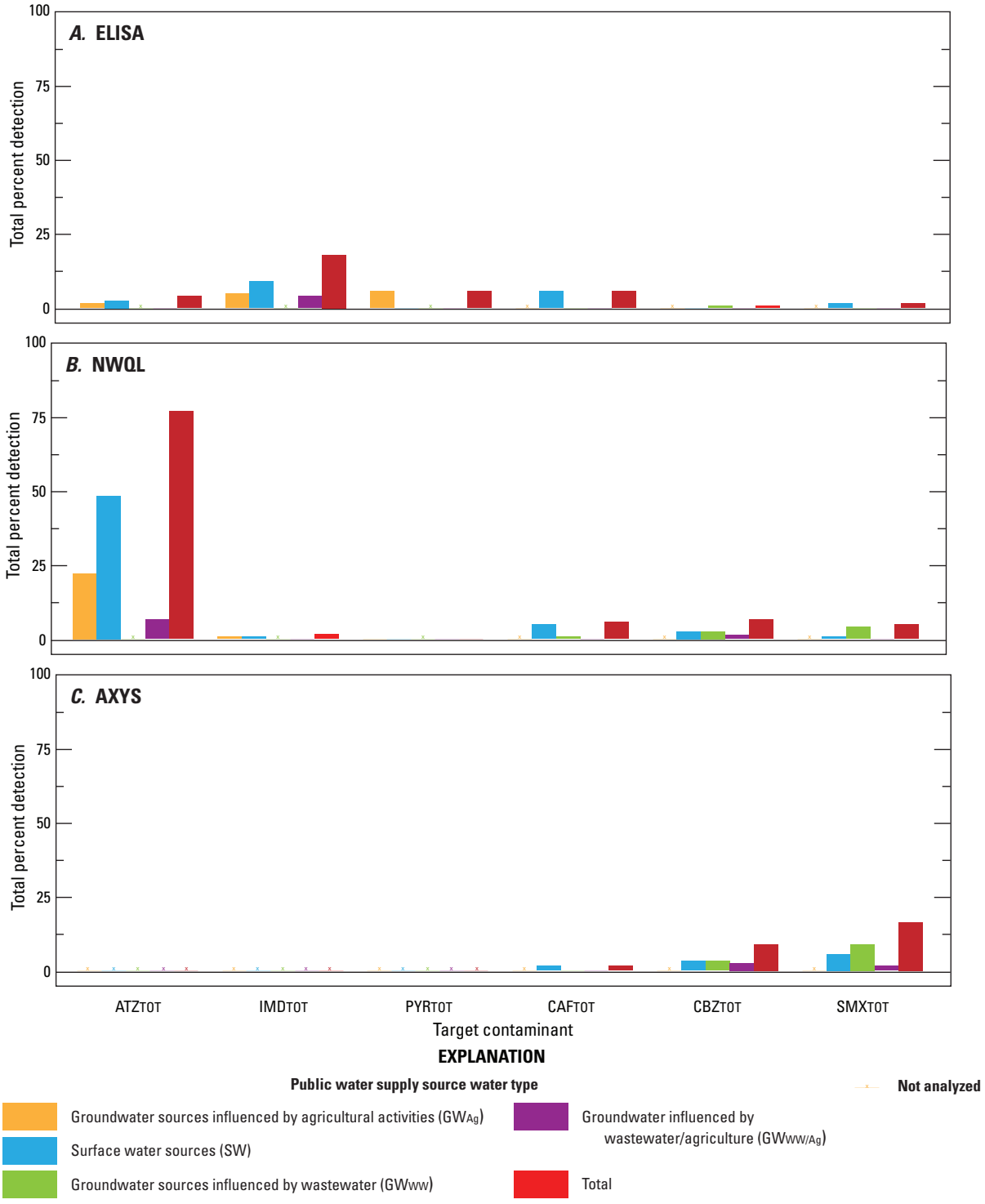

Figure 2. Percent of sample detections for six unregulated contaminant groups (atrazine group [ATZ_{TOT}], imidacloprid group [IMD_{TOT}], pyrethroids group [PYR_{TOT}], caffeine group [CAF_{TOT}], carbamazepine group [CBZ_{TOT}], and sulfamethoxazole group [SMX_{TOT}]) in source and finished drinking-water samples collected from public water systems throughout Minnesota, 2019. Samples were analyzed by *A*, enzyme-linked immunosorbent assay (ELISA) and by more advanced mass-spectrometry based analytical methods at: *B*, U.S. Geological Survey National Water Quality Laboratory (NWQL), and *C*, SGS AXYS Analytical Services Ltd. (AXYS).

Pharmaceuticals Determined by the SGS AXYS Analytical Services Ltd. (AXYS)

CAF2 was not detected in any of the samples by the AXYS method. However, CAF1 was detected in 1.6 percent (2 of 122 samples) of samples, thus CAF_{TOT} reflects CAF1 concentrations (table 2; fig. 2*C*). Overall, 9 percent (11 of 122 samples) of samples analyzed by the AXYS method had CBZ_{TOT} detections, whereas 16.4 percent (20 of 122 samples) had SMX_{TOT} detections (table 2; fig. 2*C*). SMX1 was detected in 10.7 percent (13 of 122) of samples. A total of 9 percent (11 of 122 samples) of samples had SMX1 was detected in ions but no immunologically similar contaminant detections.

Presence-Absence Agreement Among Analytical Methods

Figures 3 and 4 illustrate the differences in reporting limits for ELISA and the two comparison methods of analysis, where generally, reporting limits were ELISA>NWQL>AXYS. The higher reporting limits of the ELISA method between paired results (table 1), caused a decrease in the number of contaminant detections by NWQL and AXYS after re-censoring of the data (table 2).

The presence-absence agreement test between ELISA and the two comparison methods (NWQL and AXYS) ranged from 77.9 to 100 percent for all six contaminants (table 3). This indicates a generally good agreement between results of ELISA and those of the other two methods for detecting the presence of IMD_{TOT}, PYR_{TOT}, CAF_{TOT}, CBZ_{TOT}, and SMX_{TOT} but not for detecting the presence of ATZ_{TOT}, which will be discussed later in this report.

Of the 162 total samples collected from the 67 public water systems, up to five contaminant groups were detected in a single sample across all three methods (Krall and Elliott, 2022; fig. 5). Generally, the greatest number of contaminant group detections across all three methods were in the source water samples. However, differences between detections in source water and finished drinking-water samples were statistically insignificant because of the few detections within each dataset. No contaminant groups were detected in 29 percent (47 of 162) of all samples, across all methods (fig. 5*A*). Samples collected from GW_{WW} public water systems had the highest percentage (72.5 or 29 of 40) of samples with no contaminant group detections (fig. 5*A*). In contrast, samples collected from SW, GW_{Ag}, and $GW_{WW/Ag}$ public water systems had 7.6 (5 of 66), 22.5 (9 of 40), and 25 percent (4 of 16) of samples with no contaminant group detections. Two contaminant groups were detected in 21.6 (35 of 162) percent of all samples, across all methods. Samples with two contaminant groups detected were most commonly collected from $GW_{WW/Ag}$ public water systems (37.5 percent or 6 of 16 samples; fig. 5*A*). Less than 4 percent (1 to 6 of 162) of samples had more than two contaminant groups detected across all methods. Samples in which more than two contaminant groups were detected were most commonly collected from SW public water systems (fig. 5*A*).

Table 3. Paired result ties and presence-absence agreement between enzyme-linked immunosorbent assay (ELISA) and more advanced analytical methods by the U.S. Geological Survey National Water Quality Laboratory (NWQL) and SGS AXYS Analytical Services Ltd. (AXYS).

[ELISA, enzyme-linked immunosorbent assay analytical method; NWQL, National Water Quality Laboratory; AXYS, SGS AXYS Servies Ltd.; ATZTOT, atrazine plus immunologically similar contaminants; IMDTOT, imidacloprid plus immunologically similar contaminants; PYRTOT, pyrethroids plus immunologically similar contaminants; CAFTOT, caffeine plus immunologically similar contaminants; CBZ-TOT, carbamazepine plus immunologically similar contaminants; SMXTOT, sulfamethoxazole plus immunologically similar contaminants]

Comparisons	Target contaminant	Number of samples	Number of observation pairs (re-censored data)			Percent of observation pairs (re-censored data)			Percent presence/ absence agreement (re-censored)
			Tied	Untied		Tied	Untied		
			Nondetections in both samples of a matched pair (censored data)	Detections in both samples of a matched pair (uncensored data)	Detection in one sample and nondetection on one samples of a matched pair (censored and uncensored data)	Nondetections in both samples of a matched pair (censored data)	Detections in both samples of a matched pair (uncensored data)	Detection in one sample and nondetection on one samples of a matched pair (censored and uncensored data)	
ELISA versus NWQL	ATZTOT	122	90	5	27	74	4	22	77.9
	IMDTOT	122	102	0	20	84	0	16	83.6
	PYRTOT	122	115	0	7	94	0	6	94.3
	CAFTOT	122	115	1	6	94	1	5	95.1
	CBZTOT	122	119	1	2	98	1	2	98.4
	SMXTOT	122	118	0	4	97	0	3	96.7
ELISA versus AXYS	CAFTOT	122	115	0	7	94	0	6	94.3
	CBZTOT	122	121	1	0	99	1	0	100.0
	SMXTOT	122	118	1	3	97	1	2	97.5

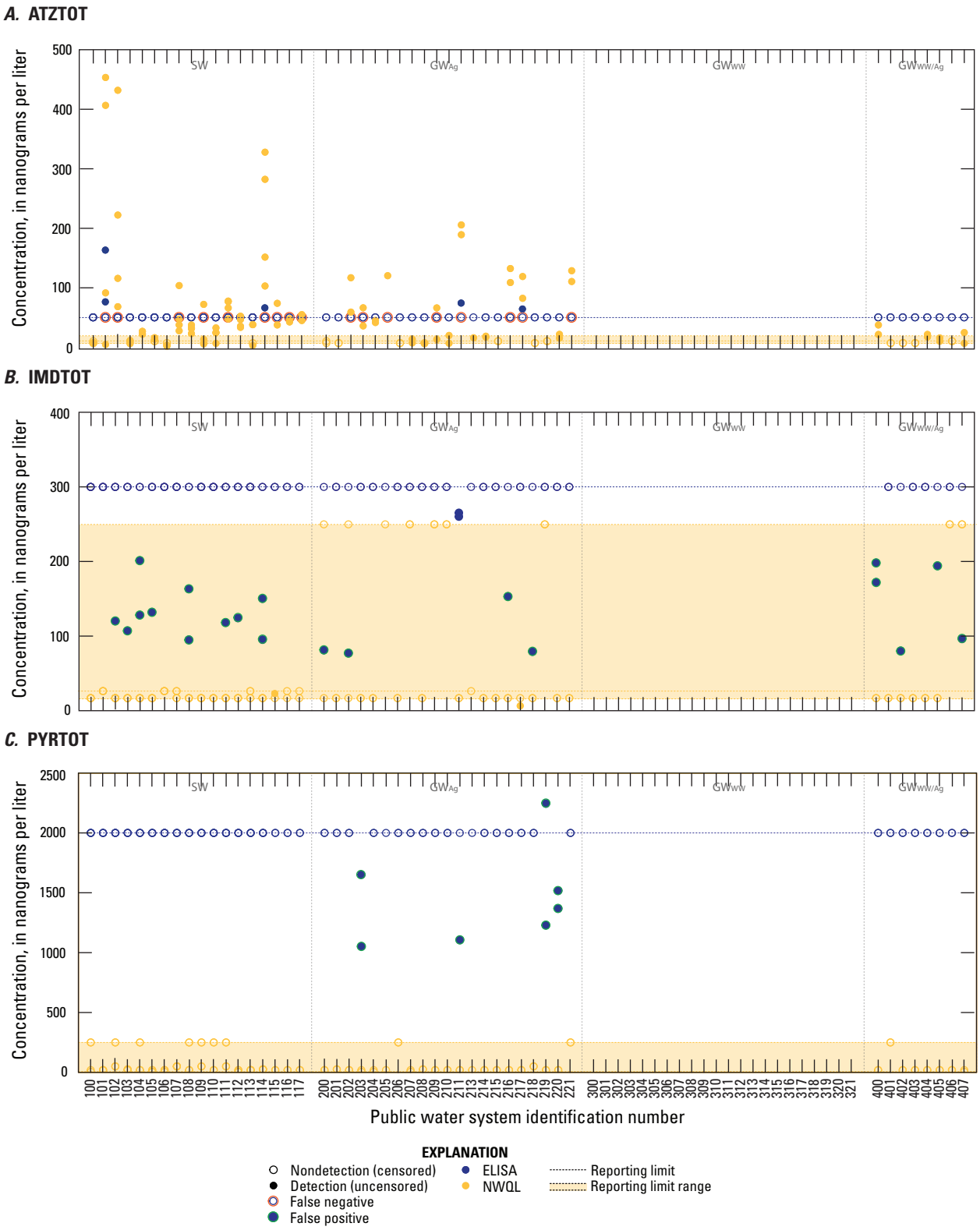

Figure 3. Concentrations of three unregulated pesticide contaminant groups: *A*, atrazine group (ATZ_{TOT}), *B*, imidacloprid group (IMD_{TOT}), and *C*, pyrethroids group (PYR_{TOT}) in source and finished drinking-water samples collected from public water systems throughout Minnesota, 2019. Samples were analyzed by enzyme-linked immunosorbent assay (ELISA) and by more advanced mass-spectrometry based analytical methods at the U.S. Geological Survey National Water Quality Laboratory (NWQL).

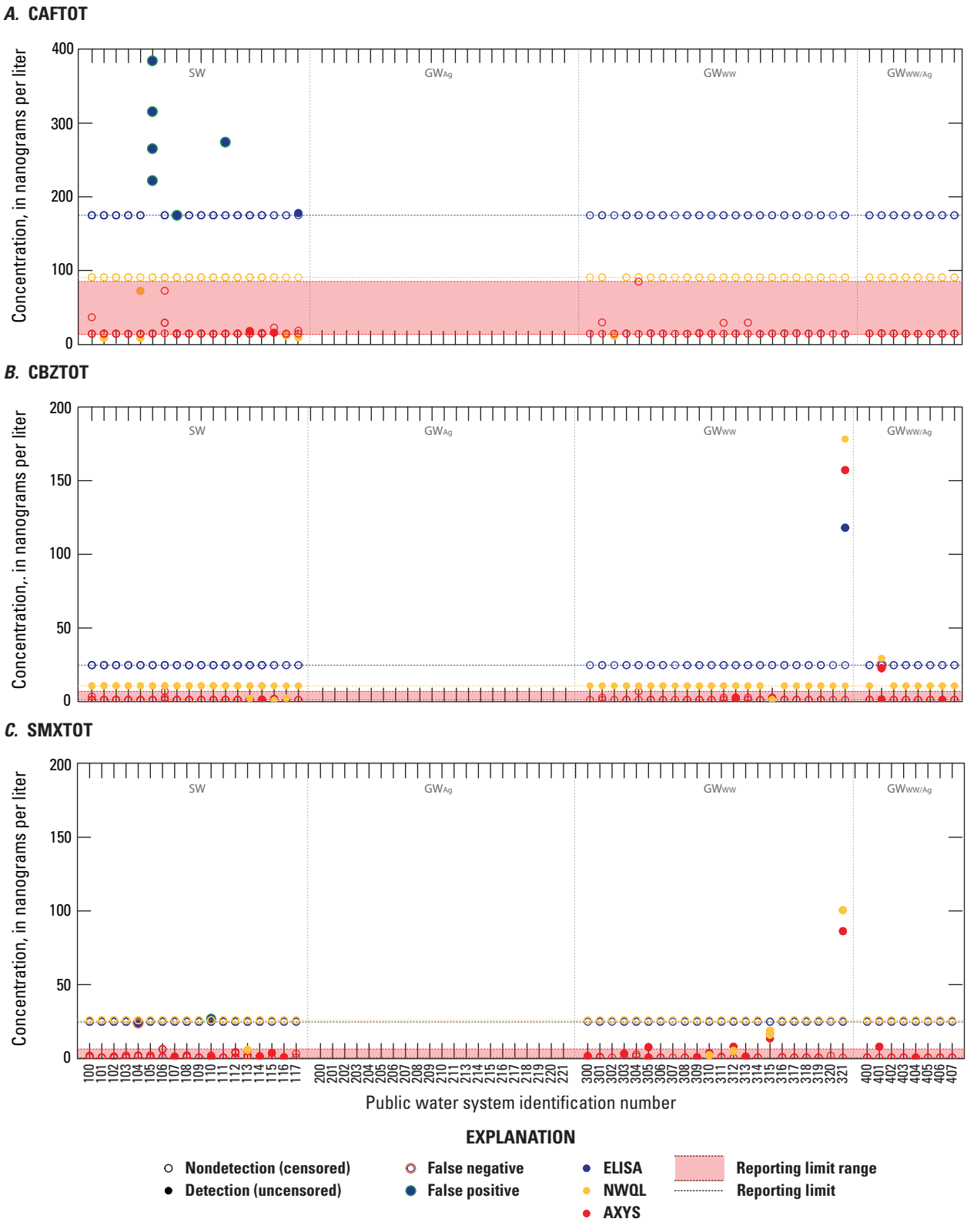

Figure 4. Concentrations of three unregulated pharmaceutical contaminant groups: *A*, caffeine group (CAF_{TOT}), *B*, carbamazepine group (CBZ_{TOT}), and *C*, sulfamethoxazole group (SMX_{TOT}) in source and finished drinking-water samples collected from public water systems throughout Minnesota, 2019. Samples were analyzed by enzyme-linked immunosorbent assay (ELISA) and more advanced mass-spectrometry based analytical methods at the U.S. Geological Survey National Water Quality Laboratory (NWQL) and SGS AXYS Analytical Services Ltd. (AXYS).

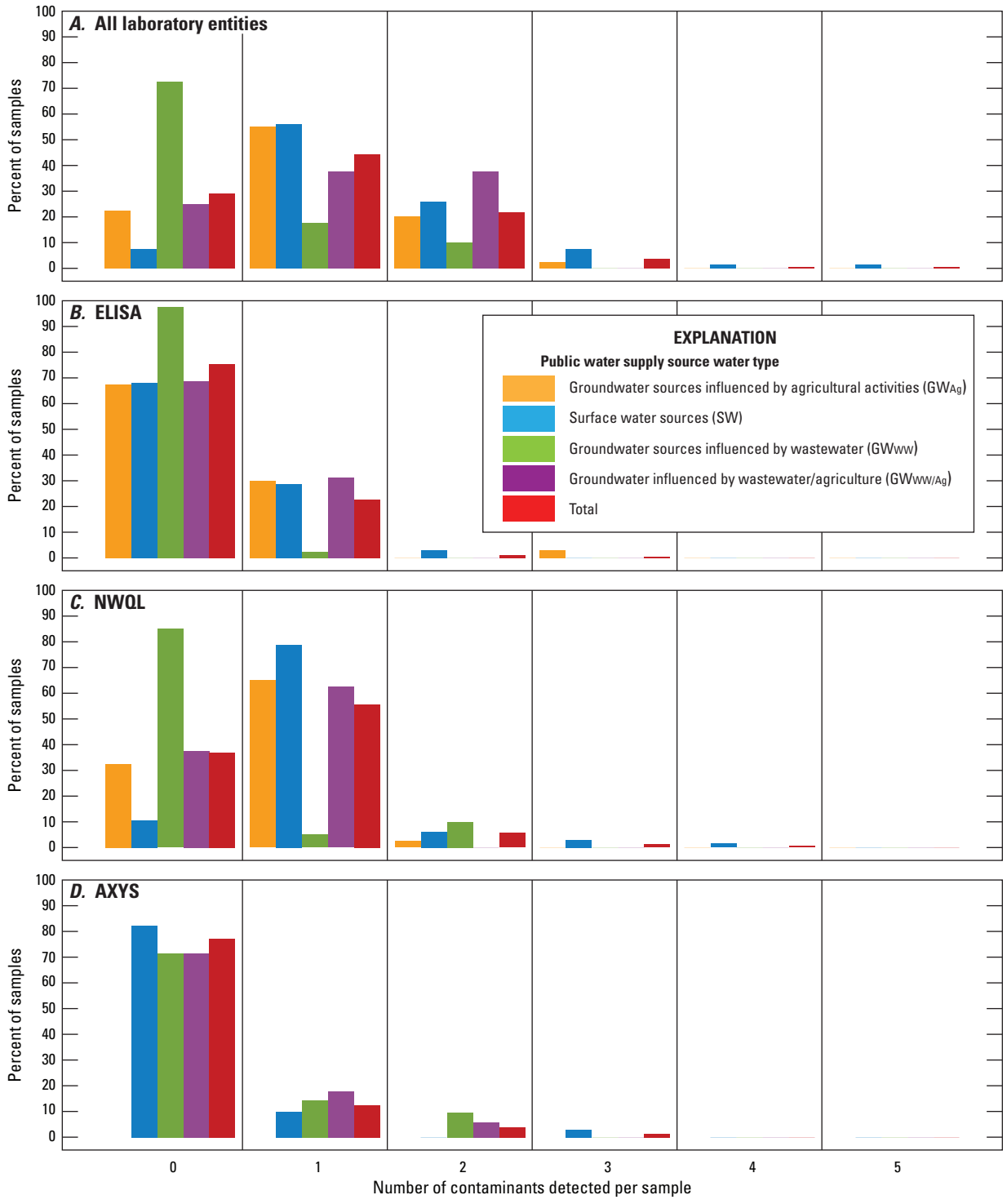

Figure 5. Percent of samples with zero to five contaminant group (atrazine group [ATZ_{TOT}], imidacloprid group [IMD_{TOT}], pyrethroids group [PYR_{TOT}], caffeine group [CAF_{TOT}], carbamazepine group [CBZ_{TOT}], and sulfamethoxazole group [SMX_{TOT}]) detections in a single source or finished drinking-water sample by public water system collected from public water systems throughout Minnesota, 2019, and by public water system source water type (surface water sourced [SW], groundwater sourced influenced by agricultural activities [GW_{Ag}], groundwater sourced influenced by wastewater [GW_{WW}], groundwater sourced influenced by both wastewater and agricultural activities [$GW_{WW/Ag}$]). Percent of samples with contaminant group detections are presented by *A*, all laboratory entities, *B*, enzyme-linked immunosorbent assay (ELISA), *C*, the U.S. Geological Survey National Water Quality Laboratory (NWQL), and *D*, SGS AXYS Analytical Services Ltd. (AXYS).

Implications of Using ELISA as a Screening Tool

Our ELISA ATZ_{TOT} analyses resulted in a high false negativity rate, which has also been documented in other studies (Graziano and others, 2006; Adams and others, 2004; Lydy and others, 1996). Generally, ELISA negatively biased results are assumed to be caused by matrix interference or mishandling of the test. Although some matrix samples had low recovery rates (<70 percent), most were within an ideal range (70 to 130 percent), indicating that matrix interference did not explain the high false negativity rate. One hypothesis for the negative bias is greater observed bias at higher concentrations. The consistent negative bias (underestimate) of ATZ_{TOT} results poses a monitoring risk if ELISA is used as a screening tool for drinking-water samples.

The false positivity rates of ELISA for analyses of IMD_{TOT}, PYR_{TOT}, and CAF_{TOT} (16, 6, and 5 percent, respectively) can be attributed to the sensitivity of ELISA to cross-react with immunologically similar contaminants. This is of importance because although not all target contaminants are detected in Minnesota surface water and groundwater, immunologically similar contaminants (that is, degradates) have been detected (Minnesota Department of Agriculture, 2020). Some data on the occurrence of immunologically similar contaminants are available, but not all laboratories may include those contaminants in analyses. Thus, the ELISA results may not be false positives, but simply reflect the presence of immunologically similar contaminants. Overall, these results indicate that using ELISA for detecting the presence of IMD_{TOT}, PYR_{TOT}, and CAF_{TOT} would be more overprotective than underprotective, which is considered beneficial for protecting human health.

Our analysis shows that results from ELISA generally are in good agreement (77.9 to 100 percent) with the results of advanced analytical methods for six unregulated contaminant groups: ATZ_{TOT}, IMD_{TOT}, PYR_{TOT}, CAF_{TOT}, CBZ_{TOT}, and SMX_{TOT}. Specifically, the false positivity and false negativity rates for ELISA CBZ_{TOT} and SMX_{TOT} are low, indicating that ELISA is not substantially underestimating or overestimating the presence of these two contaminant groups. The presence-absence agreement analysis, combined with analysis of false positive and false negative rates, indicates ELISA could provide a suitable screening method for analyzing CBZ_{TOT} and SMX_{TOT} in source and finished drinking-water samples collected from public water systems. However, the results also indicate that the use of ELISA as a screening tool for ATZ_{TOT} may not be a suitable method, and the use of ELISA for IMD_{TOT}, PYR_{TOT}, and CAF_{TOT} should be further evaluated.

Summary

We assessed the use of the enzyme-linked immunosorbent assay (ELISA), a rapid, inexpensive screening method for contaminants in water, as an alternative to more expensive and advanced mass-spectrometry analytical methods conducted at two laboratories, the U.S. Geological Survey National Water Quality Laboratory (NWQL) and SGS AXYS Analytical Services Ltd. (AXYS). Samples of source water and finished drinking-water collected from 67 public water systems throughout Minnesota were analyzed for three commonly detected pesticides (atrazine, imidacloprid, and pyrethroids) and three commonly detected pharmaceuticals (caffeine, carbamazepine, and sulfamethoxazole).

The ELISA method detects the target contaminant and immunologically similar contaminants, thereby providing an indication of contaminant mixtures. Other studies have reported positive bias in the results of ELISA compared to results of other analytical methods because of the potential for ELISA to respond to immunologically similar contaminants. Therefore, this study referred to the summation of the target contaminant and immunologically similar contaminants as contaminant groups. The potential cross-reactivity between the ELISA target contaminant and immunologically similar contaminants could not be fully assessed for all contaminants during this analysis because the number of immunologically similar contaminants varied by comparison method.

Overall, these datasets are highly censored (>80 percent) and contain multiple reporting limits within and between laboratories. The highly censored data caused tenuous statistical results when comparing paired concentrations between ELISA and the comparison methods (used at NWQL and AXYS). Thus, qualitative analysis of the data was deemed more appropriate. Generally, ELISA had higher reporting limits than the methods used by both the NWQL and AXYS, except for the analysis for SMX_{TOT} at NWQL. To discern agreement between paired results between ELISA and the comparison methods, the results of the presence-absence agreement analysis was coupled with false negative and false positive result analysis.

Both CBZ_{TOT} and SMX_{TOT} ELISA have very low false negative rates (1.6 and 0.8 percent, respectively) for samples with detections below the reporting limit (25 and 26 ng/L, respectively). These very low false negative results indicate that CBZ_{TOT} and SMX_{TOT} ELISA methods are not underestimating results. Conversely, SMX_{TOT} has a very low false positivity rate (0.8 percent) which indicates the SMX_{TOT} ELISA method is not overestimating results.

Although there is 78 percent agreement between ATZ_{TOT} ELISA and NWQL paired results, the false negativity rate of 84 percent indicates strong negative bias. This strong negative bias indicates that the ELISA method is underestimating ATZ_{TOT}, which is not an ideal result for monitoring drinking water.

The false positivity rates for IMD_{TOT}, PYR_{TOT}, and CAF_{TOT} (16, 6, and 5 percent) are higher than CBZ_{TOT} and SMX_{TOT} but are still relatively low. These results indicate the ELISA method could be overestimating the results in this

contaminant group. This positive bias could be attributed to the detection of other immunologically similar contaminants that have the potential to cross-react during ELISA analysis and are known to be present in Minnesota surface water and groundwater but were not analyzed by the comparison laboratories.

These qualitative results indicate the ELISA method could potentially provide an alternative screening method for the presence of CBZ_{TOT} and SMX_{TOT} in source and finished drinking-water samples collected from public water systems but did not prove to be a good alternative for ATZ_{TOT}. Efforts could be expanded to continue to study the use of ELISA as a reliable and cost-effective screening method to help drinking water monitoring entities prioritize sampling efforts for screening source and finished drinking-water samples for IMD_{TOT}, PYR_{TOT}, and CAF_{TOT}.

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Appendix 1. Censoring Analytical Result Data

The data in this study are left-censored, meaning the true values of the censored results are unknown and lie between zero and the reporting limit. The datasets in this study had different reporting limits for each laboratory and contaminant, and some had multiple reporting limits for each contaminant (table 1).

Contaminant Group Result Censoring

The ELISA method has the potential to detect the target contaminant and immunologically similar (cross-reactive) contaminants (Eurofins-Abraxis Inc., 2019a, 2019b, 2019c, 2019d, 2019e, 2019f). For this reason, the data in this study represent contaminant groups, or contaminant mixtures (the sum of the target contaminant and immunologically similar contaminants indicated by ELISA (Eurofins-Abraxis Inc., 2019a, 2019b, 2019c, 2019d, 2019e, 2019f) if analyzed and detected by the respective laboratory). In the case where a result was both a sum of the target contaminant and immunologically similar contaminants, and censored, the final result value used in analysis is the reporting limit of the target contaminant, except for PYR_{TOT}. PYR_{TOT} was censored to the higher reporting limit between PYR1 and PYR2, which most often was the reporting limit of PYR2 (table 1). A summary of censored and uncensored results for each contaminant group, target contaminant, and immunologically similar contaminants is provided in table 2.

Paired Result Re-censoring

Contaminant group results by ELISA were paired with results by the two comparison methods (NWQL and AXYS). These result pairs have different reporting limits, and for comparison, each result of a paired result was re-censored to the highest reporting limit of the two results. Generally, reporting limits were ELISA>NWQL>AXYS (table 1). The data were re-censored prior to analytical method comparisons under two scenarios: 1) if both results in a result pair were censored at different reporting limits, then the lower censored value was re-censored to the higher censored value where the result pair remains a tie; or 2) if one result in a result pair was censored at a higher limit than its uncensored result, then the uncensored result was re-censored to the censored result where the result pair then becomes a tie. No re-censoring occurred if: (1) both results in a result pair were uncensored (and therefore, untied) or (2) the censored result was less than the uncensored result (mixed and untied). Results of re-censoring are provided in table 3.

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Appendix 2. False Negative and False Positive Analysis

Determination of False Negative and False Positive Results

In the analysis of false negative and false positive ELISA results, the contaminant group results provided by the NWQL were considered accurate and, therefore, the ELISA results were compared to the NWQL group contaminant results. False negative results indicate the absence of a contaminant when it is present, whereas false positive results indicate the presence of a contaminant when it is either absent or present at a concentration below the detection limit. If the contaminant was not detected by ELISA but was detected by NWQL and above the higher reporting limit between ELISA and NWQL, then the ELISA result was determined to be a false negative. If the contaminant was detected by ELISA and above the NWQL reporting limit but was not detected by NWQL, then the ELISA result was determined to be a false positive.

Occurrence of False Negatives and False Positives

The ELISA analytical method produced 2 (of 122) and 1 (of 122) false negative results for CBZ_{TOT} and SMX_{TOT}, respectively (table 2 and fig. 4). No false negatives were produced for IMD_{TOT}, PYR_{TOT}, and CAF_{TOT} (table 2 and figs. 3 and 4). A total of 27 (of 122) false negatives were produced for ELISA ATZ_{TOT} (table 2; fig. 3*A*). CBZ_{TOT} and SMZ_{TOT} false negative results could be explained by the summation of the target contaminant and immunologically similar contaminants from NWQL causing a positive bias in the contaminant group concentrations when actual cross-reactivity is relatively low, whereas ATZTOT false negative results could be explained by an undetected interference during ELISA analysis, or by systematic bias at higher ATZ_{TOT} concentrations.

A total of 7 (of 122), 6 (of 122), and 1 (of 122) false positive results were produced for PYR_{TOT}, CAF_{TOT}, and SMX_{TOT}, respectively, with ELISA methods (table 2; figs. 3 and 4). No false positive results were produced for ATZ_{TOT} and CBZ_{TOT} (table 2; figs. 3*A* and 4*B*). A total of 20 (of 122) false positive results were produced for IMD_{TOT} (table 2; fig. 3*B*), which were detections below the reporting limit (300 ng/L) but above the detection limit (75 ng/L). False positive results could be explained by the potential of ELISA to detect immunologically similar contaminants that are not included in the NWQL contaminant group concentration (figs. 3 and 4).

Appendix 3. Paired Prentice-Wilcoxon Test

Paired Prentice-Wilcoxon Test Methods

ELISA ATZ_{TOT}, IMD_{TOT}, and PYR_{TOT} were compared to NWQL results, while ELISA CAF_{TOT}, CBZ_{TOT}, and SMX_{TOT} were compared to both NWQL and AXYS results. Paired results were compared using the Paired Prentice-Wilcoxon (PPW) test (O'Brien and Fleming, 1987), a nonparametric score test that compares paired results between two groups. The PPW test is well-suited for analysis of multiply censored data and is built to handle ties (both results in a paired result being censored) between results pairs (Helsel, 2012). Helsel indicates PPW test results can be tenuous when datasets are greater than 80 percent censored and have less than or equal to 2 uncensored result pairs. The PPW tests were computed in RStudio version 4.0.2 (R Studio Team, 2020) using the ppw. test function in the smwrQW package version 0.7.14 (Lorenz, 2017). Scores are computed for each result as a measure of position of the result in the datasets. The numeric differences between scores for the ELISA results and scores for the other laboratory results were computed to determine whether the sum of the differences for the entire dataset was significantly different from zero at the 95-percent confidence level. The computed PPW test provides a *Z*-score to describe the distribution of the data. The scores for ELISA results were less than scores for the other two laboratory results, if *Z* less and zero 0, equal if *Z* equals 0, and greater if *Z* greater than 0. Because the PPW test is not built to handle different reporting limits between results in a paired result (Helsel, 2012), result pairs are re-censored to the highest reporting limit of the two results (as described in app. 1) prior to computing the PPW test, as recommended by O'Brien and Fleming (1987) and Helsel (2012).

Paired Prentice-Wilcoxon Test Results

Differences between result pairs were evaluated to discern agreement between concentrations determined by the different methods. Result pairs for IMD_{TOT} could not be compared between ELISA and NWQL methods because of the lack of variance between the two datasets, as a result of re-censoring (described in app. 1), which changed all NWQL results to censored results. Paired results were significantly different for all possible comparisons (p less than 0.05), except for CBZ_{TOT} and SMX_{TOT} between ELISA and both comparison methods (table 3.1). Although ELISA CBZ_{TOT} and SMX_{TOT} concentrations deviated slightly from the comparison methods, the differences were not significantly different (p greater than 0.05, table 3.1). Plots of numeric differences between ELISA and NWQL and ELISA and AXYS paired results are depicted in figures 3.1 through 3.3. These plots suggest many of the method comparisons have outliers, but this could be explained by the high percent of censored results and little spread in the data.

Table 3.1. Paired Prentice-Wilcoxon test results for paired results for enzyme-linked immunosorbent assay (ELISA) and more advanced analytical methods for both the U.S. Geological Survey National Water Quality Laboratory (NWQL) and SGS AXYS Analytical Services Ltd. (AXYS).

[*Z*, *z*-score statistic; ELISA, enzyme-linked immunosorbent assay analytical method; NWQL, National Water Quality Laboratory; ATZTOT, atrazine plus immunologically similar contaminants; IMDTOT, imidacloprid plus immunologically similar contaminants; NA, not available; PYRTOT, pyrethroids plus immunologically similar contaminants; AXYS, SGS AXYS Servies Ltd.; CAFTOT, caffeine plus immunologically similar contaminants; CBZTOT, carbamazepine plus immunologically similar contaminants; SMXTOT, sulfamethoxazole plus immunologically similar contaminants]

Comparisons	Target contaminant	*p* value	*Z*
ELISA versus NWQL	ATZTOT	1.05×10^{-7}	−5.32
	IMDTOT	NA	NA
	PYRTOT	0.011	2.544
	CAFTOT	0.008	2.65
	CBZTOT	0.156	−1.42
	SMXTOT	0.782	−0.277
ELISA versus AXYS	CAFTOT	0.008	2.65
	CBZTOT	0.317	−1
	SMXTOT	0.576	0.56

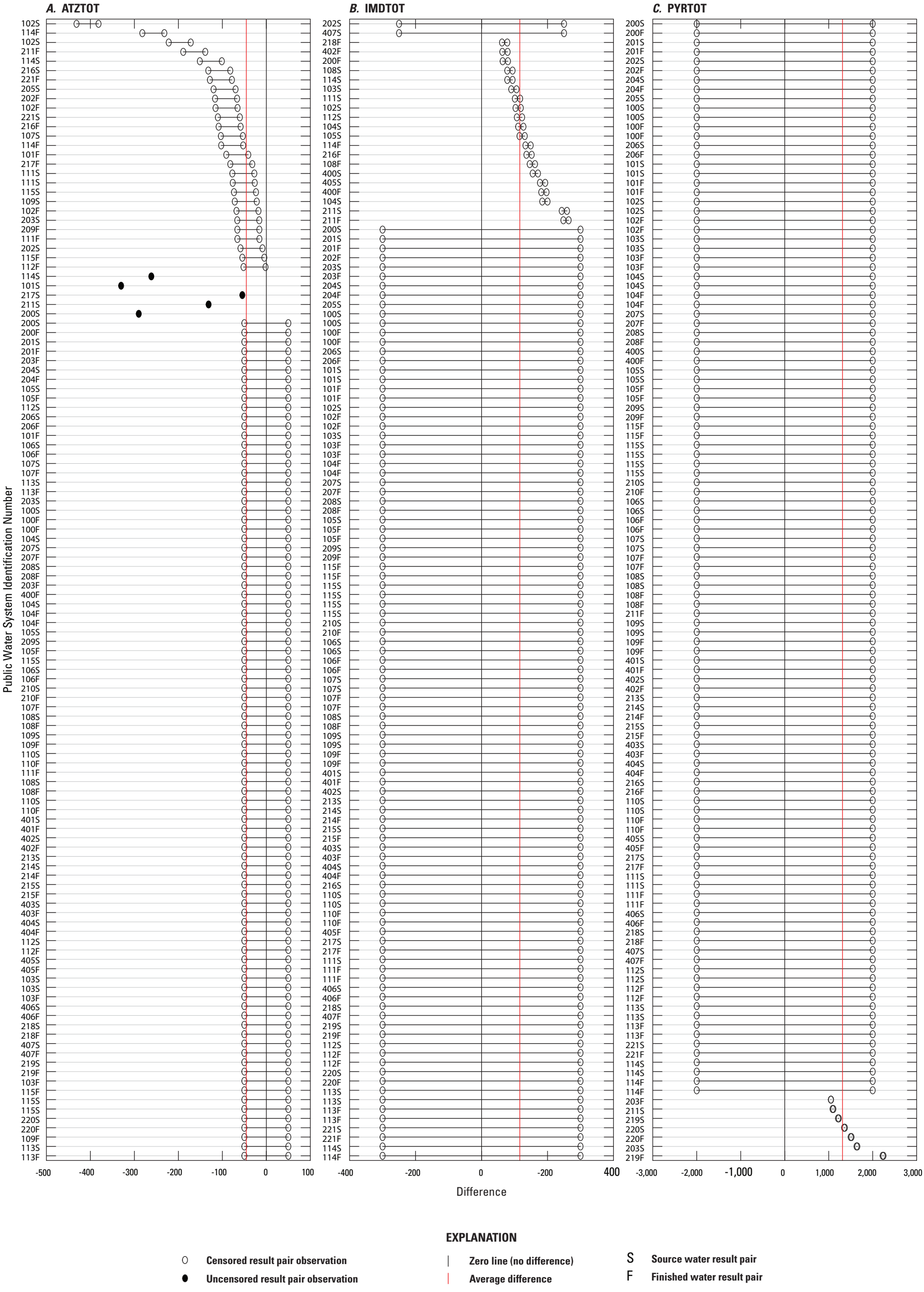

Figure 3.1. Plots of numeric differences between paired results from the enzyme-linked immunosorbent assay method (ELISA) and the U.S. Geological Survey National Water Quality Laboratory (NWQL) for three pesticide groups: *A*, atrazine (ATZTOT), *B*, imidacloprid (IMDTOT), and *C*, pyrethroids (PYRTOT).

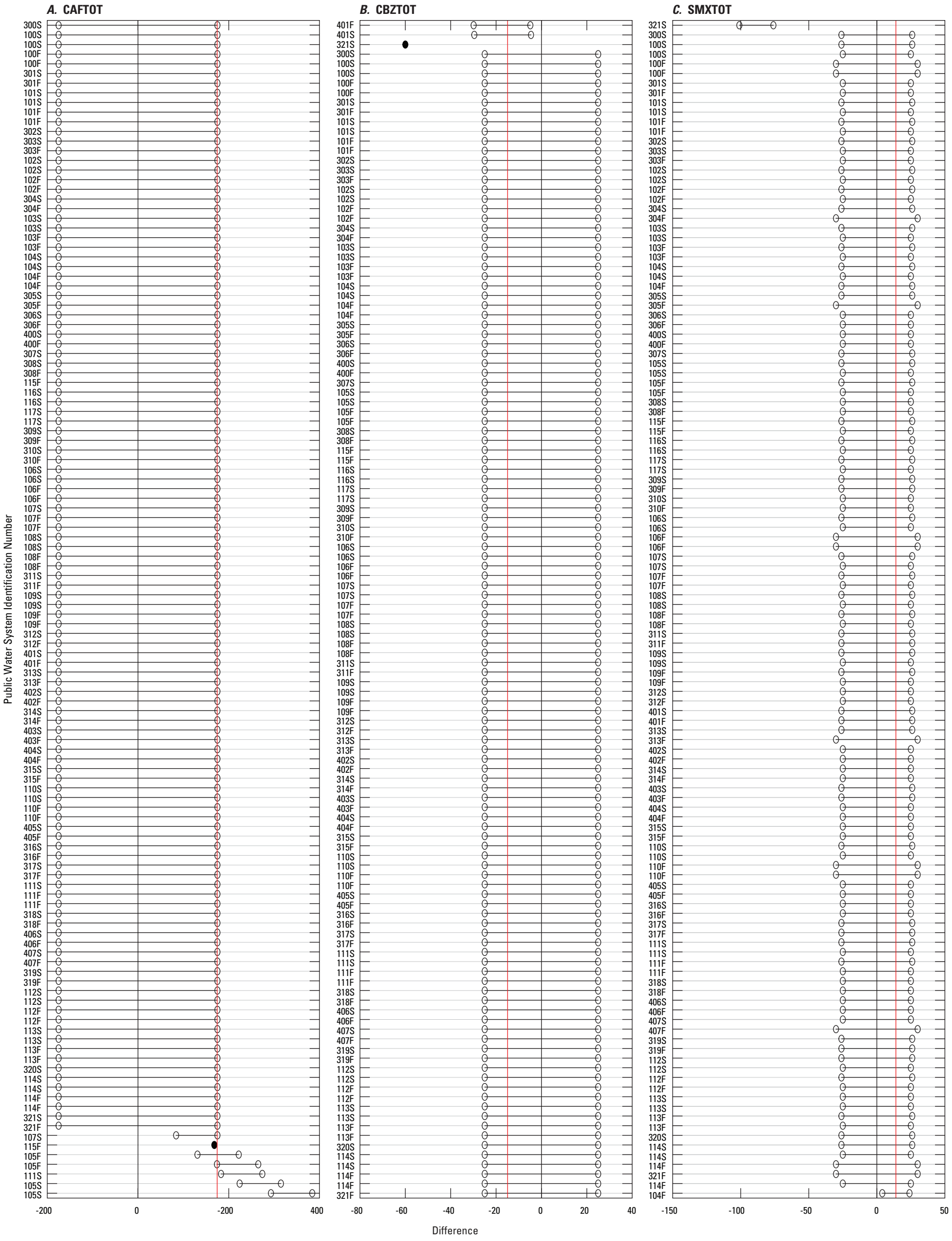

Figure 3.2. Plots of numeric differences between paired results from the enzyme-linked immunosorbent assay method (ELISA) and the U.S. Geological Survey National Water Quality Laboratory (NWQL) for three pharmaceutical groups: *A*, caffeine (CAFTOT), *B*, carbamazepine (CBZTOT), and *C*, sulfamethoxazole (SMXTOT).

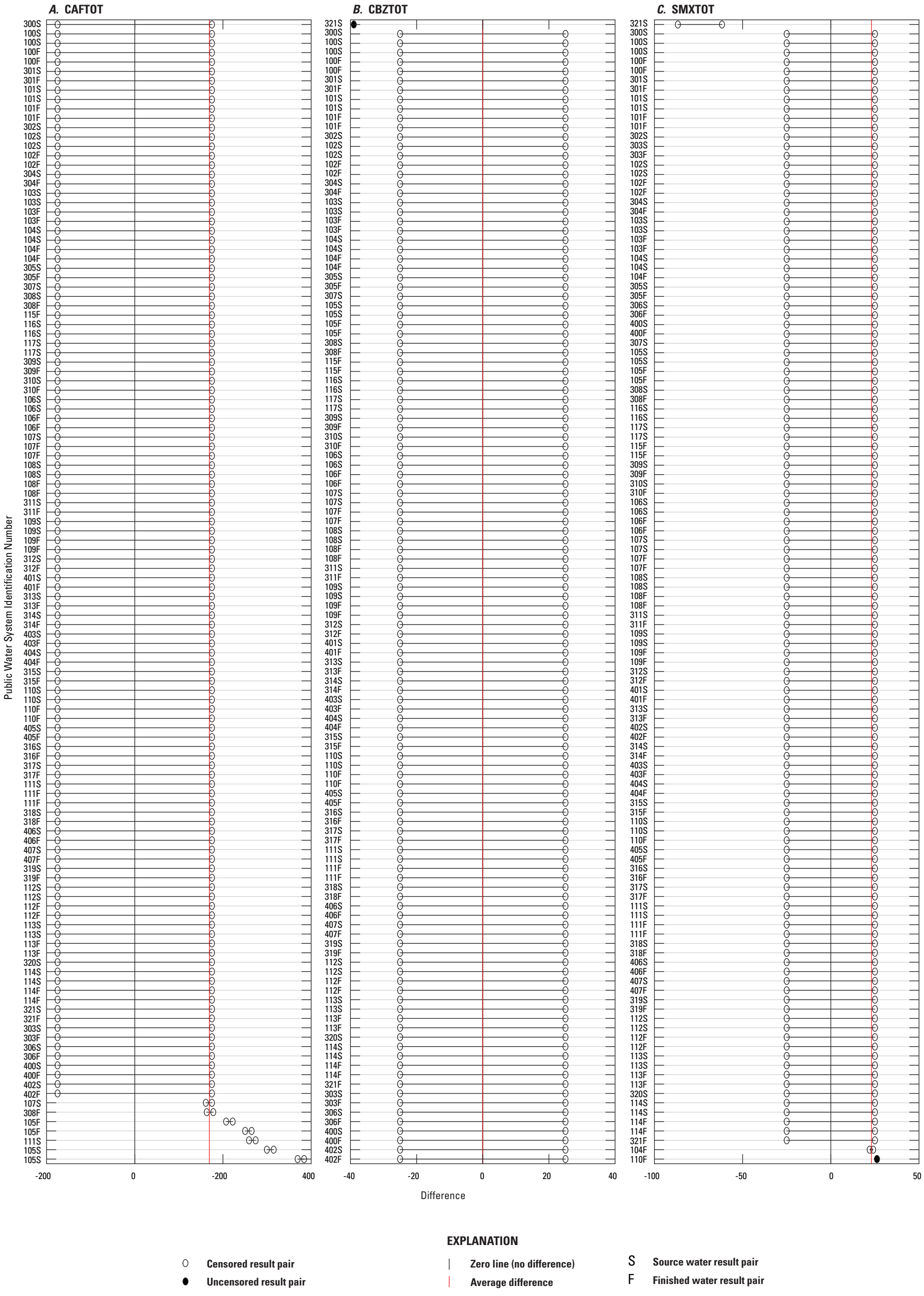

Figure 3.3. Plots of numeric differences between paired results from the enzyme-linked immunosorbent assay method (ELISA) and the SGS AXYS Analytical Services Ltd. (AXYS) for three pharmaceutical groups: *A*, caffeine (CAFTOT), *B*, carbamazepine (CBZTOT), and *C*, sulfamethoxazole (SMXTOT).

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Appendix 4. Comparison of Sample Concentration Ranking Among Analytical Methods

The contaminant data reflected multiple reporting limits within and between laboratories for each of the contaminant groups. Generally, reporting limits were ELISA>NWQL>AXYS (table 1; figs. 3 and 4). ATZ_{TOT} concentrations were highest at public water system 101 (SW) by both ELISA and NWQL methods (fig. 3*A*). Most commonly, higher concentrations of ATZ_{TOT} were analyzed in samples collected from SW-sourced public water systems. Public water system 321 (GW_{WW}) had the greatest CBZ_{TOT} concentrations, as determined by all methods, and the greatest SMX_{TOT} concentrations, as determined by NWQL and AXYS methods (fig. 4*B* and *C*). CBZ_{TOT} and SMX_{TOT} concentrations by NWQL and AXYS methods were below or just above the ELISA reporting limit, except at public water system 321 (fig. 4*B* and *C*). Generally, the greatest CBZ_{TOT} concentrations by NWQL and AXYS methods were in samples collected from public water system 321, with progressively lower concentrations at public water systems 401, 315, and 117, while the greatest SMX_{TOT} concentrations determined by NWQL and AXYS methods were in samples collected from public water system 321, with progressively lower concentrations at public water systems 315, 401, and 310 (fig. 4*B* and *C*).

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