# GCxGC-TOFMS Utilized as a Broad-Spectrum Analysis for Endocrine Disruptor Compounds in Urban and Rural Watersheds

Allen Zhang, John Heim, and Joe Binkley | LECO Corporation, St. Joseph, Michigan USA

*Industrial plant next to a river*

## Introduction

Endocrine-disrupting compounds (EDCs) encompass a variety of chemical classes, including drugs, pesticides, polymer additives, coatings materials, personal consumer products, industrial by-products and pollutants. There is worldwide concern over long-term environmental exposure to EDCs leading to serious health effects including a range of reproductive problems such as reduced fertility, male and female reproductive abnormalities, skewed male/female sex ratios, brain and behavior problems, impaired immune functions, and various cancers. This research presents a robust, broad range analysis for the detection of EDCs in impacted natural waters using Comprehensive Two-Dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry (GCxGC-TOFMS). GCxGC facilitates enhanced detection, chromatographic resolution, and peak capacity while TOFMS allows the fast acquisition (up to 500 spectra per second) necessary to successfully acquire the data density needed to fully characterize low levels of targeted and untargeted compounds in complex samples. A reference standard of 108 known endocrine-disrupting compounds was prepared and analyzed. Methods for solid-phase extraction and GCxGC-TOFMS analysis were developed. GCxGC-TOFMS analysis was conducted on multiple water samples from a rural and urban Midwestern U.S. watershed. Extraction of 1.0 liter water samples was conducted using Supel-Select HLB SPE cartridges (Supelco Analytical, Sigma-Aldrich) designed to recover a wide range of analytes. Subsequent analysis was conducted by GCxGC-TOFMS and the data was processed using a 152 component reference standard. The results are reported as targeted and untargeted analytes found. This research study presents a practical, robust, sensitive, and reliable method for the detection of EDCs in urban and rural watersheds.

## Experimental Methods

**Reference Standard Preparation**

- An endocrine disruptor reference stock standard at 1ng/µL was prepared in acetone with EPA Method standards purchased from Restek Corp.
- The commercial standards purchased were Method 8270 Megamix, Method 527 pesticide mix # 1, Method 551.1 pesticide/herbicide mix, plus Bisphenol A.

**Solid-Phase Extraction Procedure**

- Adjust 1 liter water sample to pH 2 with 37% HCl.
- Condition SPE Supel™ Select HLB, 500 mg cartridge with 5 mL HPLC Water/5% Methanol, then 5 mL Acetone, followed by 5 mL HPLC water.
- Load 1 Liter of water using the Supelco Visiprep Vacuum Manifold (Supelco Analytical, Sigma –Aldrich) slowly into the SPE cartridge.
- Dry SPE tube with vacuum for approximately 15 minutes.
- Elute slowly, 3 mL of acetone/5% methanol into a 20 mL clean glass test tube.
- Elute slowly, 3 mL of dichloromethane into the same 20 mL glass test tube.
- Speedvac to dryness for approximately 2 hours.
- Reconstitute dried residue in 500 µL of acetone, vortex, and pipet into autosampler vial.
- Inject 1 µL sample for GCxGC-TOFMS analysis.

**Method Development**

- The solid-phase extraction (SPE) procedure was developed using 1 liter of HPLC water spiked with the EDC reference standard at 50ppb.
- The GCxGC-TOFMS method was developed using the EDC reference standard injected at 5 ng on-column.

## Methods

### GCxGC-TOFMS Analysis Parameters

- Gas Chromatograph: Agilent 7890 equipped with a LECO dual stage, quad jet thermal modulator, and a GERSTEL MPS2 autosampler
- GC Primary Column: 30 m x 0.25 mm id. x 0.25 µm film thickness Rxi-5SilMS (Restek Corp.)
- GC Secondary Column: 1.0 m x 0.18 mm id. x 0.18 µm film thickness Rxi-17Sil-MS (Restek Corp.)
- Carrier Gas: Helium set @ 1.5 mL/min
- Injection Mode: Splitless
- Injection Volume: 1 µL
- Inlet Temperature: 250°C
- Primary Column Temperature Program: Initial temperature 75°C for 1.0 min ramped @ 6.0°C/min to 300°C held for 10 min
- Secondary Column Temperature Program: Initial temperature 80°C for 1.0 min ramped @ 6.0°C/min to 305°C held for 10 min
- GCxGC Modulator Temperature Offset: 20°C
- Transfer Line Temperature: 280°C
- Total Run Time: 48.50 min

### GCxGC Variable Modulation

Variable modulation shown in Table 1 below allows the user to change and modify the modulation period, hot pulse, and cool time throughout the GCxGC method to optimize the separation, thereby maximizing the peak capacity in the chromatographic plane.

**Table 1.**

Modulation Timing: For 1D GC set second dimension time to 0

| # | Start | End | Modulation Period (s) | Hot Pulse Time | Cool Time Between Stages |
|---|---|---|---|---|---|
| 1 | Start of Run | 614 s | 2.00 | 0.50 | 0.50 |
| 2 | 614 s | 1838 s | 3.00 | 0.60 | 0.90 |
| 3 | 1838 s | End of Run | 4.00 | 0.70 | 1.30 |

### Mass Spectrometer: Pegasus® 4D GCxGC-TOFMS Analysis Parameters

- Mass Range: 35 – 800 u
- Acquisition Rate: 200 spectra/s
- Ion Source Temperature: 230°C
- Detector Voltage: 1700 V
- Acquisition Delay: 150 sec.

## Using The Reference Standard Feature

A Reference Method is built in ChromaTOF® software from a user-created standard that is applied and compared to a sample. The purpose of a Reference Method is to determine the component differences between a sample and a reference standard within user-defined limits of retention time, peak area, and spectral match. In this study, untargeted components found in the water extracts were added to the original 108-component reference standard. The final Reference used in data analysis contained 152 compounds. The Reference was applied as part of the data processing method. The processed sample peak table displays each compound from the reference in the Type column as either a "Match", found but "Out of Tolerance" by percent, or "Not Found".

**Table 2.** An example of a peak table is shown below for the EDC Reference used for this analysis. The columns show the Compound Name, Type of Match, the Match similarity score, retention time, peak area, unique mass, signal to noise, and the library used to identify the mass spectral peak.

| Name | Type | Match | R.T. (s) | Area | UniqueMass | S/N | Library |
|---|---|---|---|---|---|---|---|
| Bisphenol A | Match | 895 | 1724 , 2.600 | 740588 | 213 | 2979.2 | mainlib |
| Isonol 2 * (ANTIOXIDANT- TO PREVENT GUMMING IN FUELS) | Out of Tolerance | 918 | 1085 , 1.450 | 733755 | 219 | 5824.1 | mainlib |
| Lindane/ EDC STD 8S rt dev 600 SIM | Not Found | 600 | | | | | mainlib |

## GCxGC-TOFMS Results

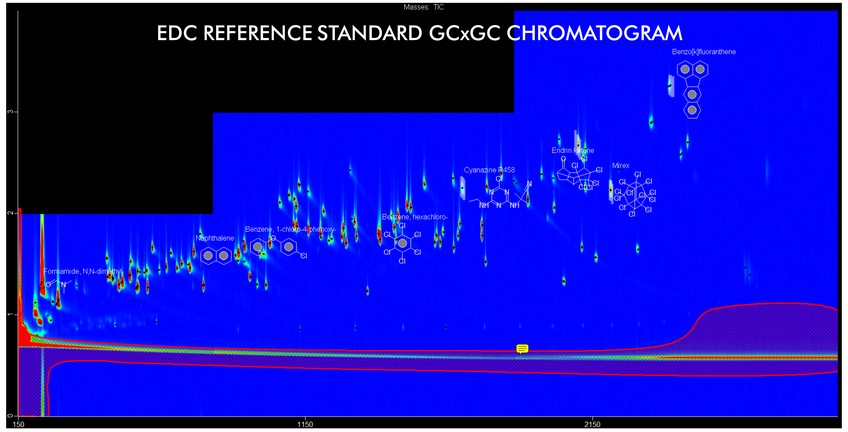
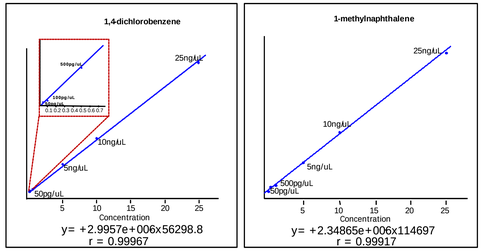



**Figure 1.** The two-dimensional contour plot chromatogram above shows the 108-component endocrine disruptor Reference standard used to develop the SPE extraction procedure and GCxGC-TOFMS method. The on-column concentration for each component is 5 nanograms. The red hatched unprocessed classification region represents the unwanted solvent and erroneous background excluded from the processed data.

## Calibration Linearity & Targeted GCXGC-TOFMS Quantitation







| Name | Concentration | Conc. Units | R.T. (s) | Similarity | Area | Height | UniqueMass | S/N | Library |
|---|---|---|---|---|---|---|---|---|---|
| 1,4-Dichlorobenzene | 0.08 | ng | 396 , 1.345 | 941 | 169194 | 12113 | 146 | 364.46 | Wiley9 |
| Naphthalene, 1-methyl- (CAS) | 0.06 | ng | 791 , 1.830 | 732 | 7533.1 | 605.24 | 141 | 70.566 | Wiley9 |

**Figure 2.** The six-point calibration curves for 1,4-dichlorobenzene and 1-methylnaphthalene are shown above in Figure 2. Calibration linearity for both components above show calculated Pearson's r values greater than 0.999. Targeted analysis was utilized in the data processing method with calibration curves for components contained within the endocrine disruptor standard. The peak table above shows the quantitative results calculated for two selected analytes detected in a 1 liter water sample.

## GCxGC Increased Peak Capacity & NIST Searchable TOFMS

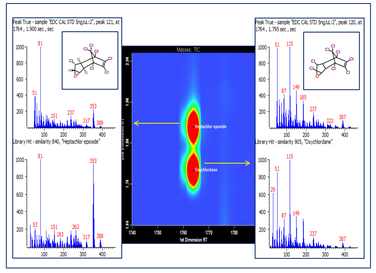



**Figure 3.** The example above shows the two-dimensional Contour Plot for two peaks that are resolved in the second dimension by 105 milliseconds yet coeluted completely in the 1st dimension separation at 1764 s. The expanded peak capacity and advantage of a two-dimensional separation is illustrated by this example of two chemically similar pesticides that would be coeluted and difficult to characterize by one-dimensional chromatography. Notice the high mass spectral match scores by the NIST 08 library searches of 84% and 90.5% for both compounds.

## EDCs & Pollutants Detected

**Table 3.** The results for thirteen 1 liter water samples prepared with solid-phase extraction and analyzed by GCxGC-TOFMS are shown below. A total of 102 chemical compounds were detected from six point sources in a Midwestern watershed. The compounds detected matched the Reference standard with at least a 60% library match similarity. Endocrine-disrupting compounds (EDCs) encompass a variety of chemical classes, including pharmaceuticals, pesticides, polymer additives, coatings materials, personal consumer products, flame retardants, plasticizers, industrial by-products and miscellaneous pollutants. This data shows that out of 26 GCxGC-TOFMS analyses, 81% of the 102 chemicals detected were found at least 5 times.

| | UNTARGETED AND TARGETED ANALYTES DETECTED | 1ST DIMENSION RETENTION TIME (s) | COUNT # OF TIMES DETECTED | CHEMICAL TYPE |
|---|---|---|---|---|
| 1 | Phenol (CAS) | 354 | 23 | EDC |
| 2 | Bis(2-chloroethyl) ether | 364 | 1 | EDC |
| 3 | 1,4-Dichlorobenzene | 392 | 21 | EDC |
| 4 | 1,3-Dichlorobenzene | 394 | 9 | EDC |
| 5 | 1,2-DICHLOROBENZENE | 418 | 3 | EDC |
| 6 | Benzylalcohol P194 | 420 | 19 | EDC |
| 7 | 2-Methylphenol | 440 | 22 | EDC |
| 8 | 3-Methylphenol | 458 | 24 | EDC |
| 9 | Nitrobenzene | 480 | 7 | EDC |
| 10 | Isophorone * Solvent | 532 | 26 | EDC, INDUSTRIAL CHEMICAL |
| 11 | 2-Nitrophenol | 548 | 11 | EDC |
| 12 | 2,4-Dimethylphenol | 566 | 11 | EDC |
| 13 | 2,4 Dichlorophenol | 600 | 7 | EDC |
| 14 | Benzene, 1,2,4-trichloro- (CAS) | 608 | 9 | EDC |
| 15 | Naphthalene | 623 | 25 | EDC, PAH |
| 16 | D-Glucitol, 1,4-3,6-dianhydro-2,5-di-O-methyl- * used in Personal Care Products; cosmetics | 731 | 7 | PCP |
| 17 | Naphthalene, 2-methyl- | 770 | 20 | EDC, PAH |
| 18 | Naphthalene, 1-methyl- | 791 | 22 | EDC, PAH |
| 19 | 1,3-Isobenzofurandione (CAS) * RUBBER RETARDER, CURING AGENT | 794 | 14 | EDC, INDUSTRIAL CHEMICAL |
| 20 | Propofol | 842 | 6 | PCP, PHARMACEUTICAL |
| 21 | 2,4,6-Trichlorophenol | 842 | 6 | EDC |
| 22 | 2,4,5-Trichlorophenol | 845 | 6 | EDC |
| 23 | á DAMASCONE | 863 | 12 | FOOD, FLAVOR, FRAGRANCE |
| 24 | 2-Chloronaphthalene | 878 | 7 | EDC, PAH |
| 25 | Phenol, 4-chloro-3,5-dimethyl- (CAS) | 884 | 6 | EDC |
| 26 | á-Patchoulene | 887 | 7 | FOOD, FLAVOR, FRAGRANCE |
| 27 | Naphthalene, 1,7-dimethyl- | 911 | 7 | EDC, PAH |
| 28 | 1H-3a,7-Methanoazulene, 2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3á,3aâ,7á,8aâ)]- | 929 | 5 | EDC |
| 29 | Acenaphthylene (CAS) | 971 | 14 | EDC, PAH |
| 30 | Acenaphthene | 1010 | 21 | EDC, PAH |
| 31 | BUTYL HYDROXY TOLUENE * BHT ANTIOXIDANT | 1025 | 23 | EDC, FOOD, FLAVOR, FRAGRANCE |
| 32 | Tributyl phosphate * SOLVENT & PLASTICIZER | 1031 | 19 | EDC, INDUSTRIAL CHEMICAL |
| 33 | Benzeneacetic acid, ethyl ester * flavor fragrance | 1037 | 25 | EDC, FOOD, FLAVOR, FRAGRANCE |
| 34 | á-N-METHYL IONONE | 1043 | 6 | EDC, FOOD, FLAVOR, FRAGRANCE |
| 35 | TRIPROPYLENE GLYCOL S | 1046 | 21 | EDC, INDUSTRIAL CHEMICAL |
| 36 | Dibenzofuran | 1052 | 14 | EDC |
| 37 | Lilial | 1055 | 12 | PCP, FOOD, FLAVOR, FRAGRANCE |
| 38 | 1H-Benzotriazole, 4-methyl- * CORROSION INHIBITOR | 1070 | 4 | EDC, INDUSTRIAL CHEMICAL |
| 39 | 1H-Benzotriazole, 5-methyl- *RETROCURE G USED IN PREVULCANIZATION IN RUBBER MANUFATURE | 1076 | 4 | EDC, INDUSTRIAL CHEMICAL |
| 40 | Ionol 2 * (ANTIOXIDANT- TO PREVENT GUMMING IN FUELS) | 1085 | 11 | EDC, INDUSTRIAL CHEMICAL |
| 41 | 2-tert-Butylhydroquinone * TBHQ, FOOD PRESERVATIVE ANTIOXIDANT | 1088 | 2 | FOOD, FLAVOR, FRAGRANCE |
| 42 | Dodecanamide, N,N-bis(2-hydroxyethyl)- *FOAM STABILIZER IN HOUSEHOLD DETERGENTS AND SHAMPOOS | 1091 | 22 | PCP |
| 43 | á-N METHYL IONONE | 1109 | 6 | PCP, FOOD, FLAVOR, FRAGRANCE |
| 44 | DEET * INSECTICIDE | 1112 | 26 | PCP, EDC, INSECTICIDE |
| 45 | Gabapentin | 1127 | 13 | PCP, PHARMACEUTICAL |
| 46 | Fluorene | 1130 | 16 | EDC, PAH |
| 47 | 4-Chlorophenyl phenyl ether | 1139 | 5 | EDC, FLAME RETARDANT |
| 48 | Benzothiazole, 2-(methylthio)- (CAS) | 1151 | 14 | EDC |
| 49 | Ibuprofen | 1160 | 5 | PCP, PHARMACEUTICAL |
| 50 | 2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol | 1166 | 23 | EDC, UV LIGHT STABILIZER |
| 51 | Diphenylamine * ANTIOXIDANT, SCALD INHIBITOR USED ON APPLES | 1169 | 2 | EDC, FOOD, FLAVOR, FRAGRANCE |
| 52 | Azobenzene | 1175 | 9 | EDC |
| 53 | Kaysacure bp * used in the manufacturing of antihistamines, hypnotics, insecticides. | 1178 | 11 | EDC, PHARMACEUTICAL, INSECTICIDE |
| 54 | N,N,N',N'-Tetraacetylethylenediamine *LIGAND FOR METAL IONS, ACRYLAMIDE POLYMERIZATION | 1178 | 10 | EDC, INDUSTRIAL CHEMICAL |
| 55 | Clovene | 1181 | 18 | FOOD, FLAVOR, FRAGRANCE |
| 56 | TRANS-METHYL DIHYDROJASMONATE | 1196 | 21 | EDC, FOOD, FLAVOR, FRAGRANCE |
| 57 | Trifluralin | 1214 | 6 | EDC, HERBICIDE |
| 58 | 4-Bromophenyl phenyl ether *USED AS A (PAST) FLAME RETARDANT | 1250 | 4 | EDC, BFR, INDUSTRIAL CHEMICAL |
| 59 | Ibuprofen-M (HO-)- HIZO P329 | 1253 | 6 | PCP, PHARMACEUTICAL |
| 60 | Hexachlorobenzene | 1259 | 18 | EDC, FUNGICIDE, PCP, banned globally |
| 61 | Simazine | 1298 | 14 | EDC, HERBICIDE, banned by EU |
| 62 | Atrazine P363 | 1307 | 22 | EDC, HERBICIDE, banned EU, US still uses |
| 63 | Myristic acid P412 | 1313 | 24 | FOOD, FLAVOR, FRAGRANCE |
| 64 | Cedryl acetate | 1331 | 10 | PCP, FOOD, FLAVOR, FRAGRANCE |
| 65 | Ibuprofen-M (HO-) isomer-1 ME P444 * Pharmaceutical | 1334 | 6 | PCP, PHARMACEUTICAL |
| 66 | Tris(1-chloro-2-propyl)phosphate *TCPP Flame retardant | 1349 | 26 | EDC, INDUSTRIAL CHEMICAL |
| 67 | Fenanthrene | 1352 | 24 | EDC, PAH |
| 68 | Anthracene | 1358 | 11 | EDC, PAH |
| 69 | Naphthalene, 6,7-diethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl- (CAS) | 1364 | 13 | EDC |
| 70 | 3,5-DITERT-BUTYLBENZALDEHYDE | 1373 | 7 | EDC |
| 71 | Caffeine (CAS) | 1406 | 10 | PCP, FOOD, FLAVOR, PHARMA |
| 72 | Carbazole | 1415 | 12 | EDC, INDUSTRIAL CHEMICAL |
| 73 | 7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin * synthetic musk | 1415 | 24 | PCP, FOOD, FLAVOR, FRAGRANCE |
| 74 | Gemfibrozil * Medication to lower lipid levels | 1490 | 11 | PCP, PHARMACEUTICAL |
| 75 | metolachlor | 1529 | 24 | EDC, HERBICIDE |
| 76 | 5H-dibenz[b,f]azepine (CAS) * Pharmacutical - intermediate used in drug manufacturing | 1550 | 9 | PCP, PHARMACEUTICAL |
| 77 | Benzenesulfonanilide * production of synthetic dyes, photochemicals, disinfectants, electroplating | 1589 | 8 | EDC, INDUSTRIAL CHEMICAL |
| 78 | Sunscreen uv-15 | 1592 | 6 | PCP, PHARMACEUTICAL |
| 79 | Heptachlor epoxide | 1612 | 2 | EDC, POP, INSECTICIDE, US limits use |
| 80 | Oxychlordane | 1613 | 2 | EDC, POP, INSECTICIDE, banned US |
| 81 | Bioallethrin | 1625 | 4 | 1988 EDC, PESTICIDE |
| 82 | FLUORANTHENE | 1628 | 23 | EDC, PAH |
| 83 | Lauryl acrylate * for manufacturing polymers used in hairstyling | 1640 | 6 | PCP |
| 84 | Naproxen * Pharmaceutical | 1655 | 1 | PCP, PHARMACEUTICAL |
| 85 | Triclosan * antibacterial, antifungal agent used in toothpaste | 1661 | 7 | PCP, PHARMACEUTICAL |
| 86 | Pyrene | 1679 | 23 | EDC, PAH |
| 87 | Butyl citrate * used as a plastiizer, antifoam agent | 1721 | 5 | EDC, INDUSTRIAL CHEMICAL |
| 88 | Bisphenol A * Used in making plastics | 1721 | 13 | EDC, INDUSTRIAL CHEMICAL |
| 89 | Nitrofen | 1769 | 2 | EDC, PESTICIDE, banned in EU and US |
| 90 | Endrin P1112 | 1772 | 2 | EDC, INSECTICIDE, banned 2004 |
| 91 | TCPP , Tris(1,3-dichloroisopropyl)phosphate * (PBDE) flame retardant REPLACEMENT | 1838 | 16 | EDC, INDUSTRIAL CHEMICAL |
| 92 | 2H-1-Benzopyran-2-one, 7-(diethylamino)-4-methyl- * (Optical bleach in textile industry) | 1854 | 4 | EDC, INDUSTRIAL CHEMICAL |
| 93 | Hexazinone P513 | 1882 | 1 | EDC, HERBICIDE |
| 94 | Bifenthrin | 1958 | 5 | EDC, INSECTICIDE, class C carcinogen |
| 95 | Benzo[a]anthracene | 1962 | 4 | EDC, PAH |
| 96 | Chrysene (CAS) | 1970 | 8 | EDC, PAH |
| 97 | Methoxychlor | 1974 | 2 | EDC, INSECTICIDE U.S. BANNED 2003 |
| 98 | Mirex | 2062 | 2 | EDC, INSECTICIDE U.S. BANNED 1976 |
| 99 | Benzo[b or k]fluoranthene | 2198 | 15 | EDC, PAH |
| 100 | Benzo[a]pyrene (CAS) | 2262 | 3 | EDC, PAH |
| 101 | Fenvalerate isomer-1 P1241 | 2302 | 1 | EDC, INSECTICIDE |
| 102 | Fenvalerate isomer-2 P1242 | 2322 | 2 | EDC, INSECTICIDE |

## Conclusions

In conclusion, comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry was utilized in this research to detect endocrine disruptors as well as other pollutants in samples obtained from urban and rural point sources along a Midwestern watershed. A solid-phase extraction method was developed using a hydrophilic modified styrene-based polymer for a broad range of compounds from aqueous samples. An optimized GCxGC method was developed using a conventional non-polar and mid-polarity column set. The GCxGC method utilized variable modulation which aids in optimization of the available peak capacity and chromatographic resolution of the first- and second-dimension separation. A TOFMS method was created which offers continuous full-range non-skewed mass spectral information, True Signal Deconvolution®, and fast acquisition rates ideal for the characterization of EDCs and other contaminants in water. TOFMS generates NIST library searchable mass spectral data as illustrated in Figure 3.

Thirteen solid-phase extractions were conducted on 1 liter water samples obtained from 6 different rural and urban point sources along a Midwestern watershed. GCxGC-TOFMS analysis was followed by data processing that utilized the "Reference" feature in ChromaTOF software. The final Reference developed to detect EDCs contained 152 compounds which was applied as part of the data processing method. Targeted analysis was conducted using a six point calibration developed in the 50 ppb to 25 ppm range for selected EDC standard compounds illustrated in Figure 2. Calibration linearity of greater than 99.9% and quantitative examples are shown for selected analytes. Results of this research detected 102 chemicals in aqueous extractions from 6 different sampling sites. The detected compounds matched the reference standard with at least a 60% library match similarity. Furthermore, results show that 81% of the 102 detected chemicals were found at least 5 times.

The research presented in this study emphasizes the need for instrumentation that will detect, quantify, and characterize sources of long-term environmental exposure to EDCs that can lead to ecological destruction and serious health effects. The application of GCxGC-TOFMS for this work presents an excellent instrumental option for the detection of targeted and untargeted pollutants in impacted natural waterways. The data presented illustrates the advantages and benefits of GCxGC-TOFMS to provide a robust analysis as well as a data mining strategy using the "Reference" feature and quantitative calibration available in ChromaTOF software to detect a broad range of chemical contaminants in impacted natural waterways.

For further information regarding this study, contact the authors at john_heim@leco.com.

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