Application Note

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Comprehensive Profiling of Environmental Contaminants in Surface Water Using High-Resolution GC/Q-TOF

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Abstract

Monitoring of environmental pollutants in surface water is a challenging task due to large number of contaminants, continuous change of their relevance in the environment, and toxicity at low concentration (for example, for pyrethroids and some organophosphate pesticides) requiring methods with low detection limits.[1] The use of accurate mass high-resolution MS (HRMS) techniques to characterize known and unknown pollutants in a sample is gaining in popularity. However, several environmental contaminants are low molecular weight, volatile, or nonpolar, making them much more amenable to analysis by GC rather than LC.

Therefore, to achieve high sensitivity together with an expanded analysis scope, a comprehensive workflow including targeted quantitation, suspect screening, and a nontargeted approach with a high-resolution accurate mass GC/Q-TOF was applied to screen for environmental pollutants in water samples.

Introduction

The investigation of organic micropollutants is an important aspect of assessing environmental quality. The conventional approach to this monitoring involves analyzing a defined number of target compounds by mass spectrometry with the instrument operated in a selected data acquisition mode for targeted analytes. However, there is evidence that such an approach may significantly underestimate the exposure and risk of pollutants, compared to a more comprehensive untargeted screen.

Recent advances in mass spectrometry allow an increased scope of analysis, no longer sensitivity or selectivity limited when using high-resolution accurate mass instruments operated in full spectrum acquisition mode. Accurate mass information enhances the amount of detail and allows for the determination of both targeted and nontargeted components.

One of the challenges that this information rich data presents is determining what samples warrant a more detailed investigation. This Application Note offers a workflow using an accurate mass high-resolution GC/Q-TOF for profiling environmental contaminants in samples of interest. This work also provides guidance for identification of unknown compounds.

Experimental

Sample preparation

Sampling was carried out at several sites throughout the Cache Slough Complex, located in the Sacramento – San Joaquin River Delta in Northern California. The main input of point-source micropollutants as well as diffuse pollutants is expected to be through Ulatis Creek. All samples were cooled during transport and stored in the dark at 4 °C until extraction.

Extraction was performed by passing 1 L of surface waters through a GF/F filter. The filtrates were passed through a polymeric solid phase extraction (SPE) cartridge. After drying for one hour, the cartridges were eluted with 10 mL of ethyl acetate. The filters were extracted with hexane/acetone, and followed by a partial solvent exchange into ethyl acetate.

Data acquisition and preprocessing

The data were acquired using a high-resolution Agilent 7250 GC/Q-TOF system following sample separation on an Agilent 7890B GC with midcolumn backflush configuration (Figure 1) used to reduce source contamination, run time, and carryover. A 20 minute method was retention time locked (RTL) to chlorpyrifos-methyl (at an RT of 9.143 minutes) to ensure RT consistency with the GC/Q-TOF accurate mass library of pesticides and environmental pollutants. Table 1 describes the GC/Q-TOF parameters.

The acquired data files were converted to the SureMass format[2] for all the downstream data processing.

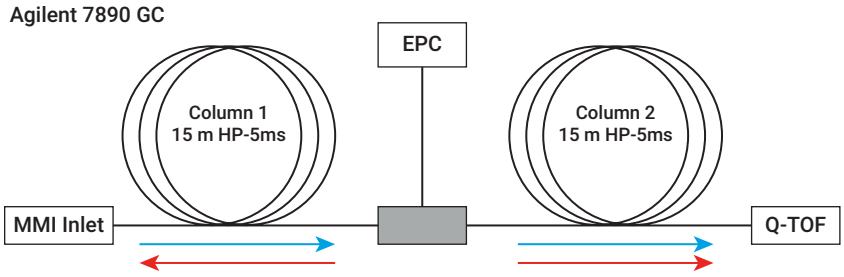


Figure 1. Midcolumn backflush configuration. Helium flow path during the backflushing at the end of the run is depicted by red arrows. The pressure at the purged union is increased while the pressure at the inlet drops. This results in reversing the flow on the first column, and allows high-boiling compounds to be removed through the split vent.

Suspect screening and nontargeted screening workflow

A workflow combining target quantitation and suspect screening was used to identify pollutants in water and filter extracts (Figure 2). This unified workflow used a GC/Q-TOF Screening feature available in Agilent MassHunter Quantitative Analysis software 10.1. The suspect screening was based on the Agilent GC/Q-TOF accurate mass personal compound database and library (PCDL) of pesticides and environmental contaminants, containing RTs and spectra for over 1,000 compounds. The screening method used the most specific accurate mass ions automatically selected from each PCDL spectrum. The screening method parameters such as library match score, RT window, coelution score, and mass error among others were selected in accordance with SANTE and FDA guidelines.[3,4]

To identify contaminants beyond the PCDL scope, Agilent MassHunter Unknowns Analysis from MassHunter Quantitative Analysis 10.1 was used to perform chromatographic deconvolution and NIST17.L library search. The ExactMass tool of Unknowns Analysis was then used to annotate deconvoluted accurate mass GC/Q-TOF spectra with fragment formulae based on the unit mass NIST17 library hit. This step was included to help remove false positive hits based on the accurate mass discrepancy.

Table 1. GC/Q-TOF acquisition parameters.

GC	Agilent 7890B GC
Inert Flow Path Configuration	Midcolumn backflush
Columns	2 × Agilent J&W HP-5ms Ultra Inert, 15 m, 0.25 mm id, 0.25 µm film
Inlet	MMI, 4 mm UI liner single taper with wool
Injection Volume	1 µL
Injection Mode	Cold splitless
Injection Temperature Program	60 °C for 0.2 minutes
	600 °C/min to 300 °C, hold
	330 °C, postrun
Inlet Flow (Column 1)	1.0 mL/min (chlorpyrifos-methyl locked at 9.143 minutes)
PUU Flow (Column 2)	Column 1 flow + 0.2 mL/min
Oven Temperature Program	60 °C (hold 1 minutes)
	then 40 °C/min to 170 °C,
	then 10 °C/min to 310 °C (hold 3 minutes)
	Run time 20.75 minutes
Transfer Line	280 °C
Midcolumn Backflush	
Timing	5 minutes duration during post run
Oven Temperature	310 °C
AUX EPC Pressure	~50 psi
Inlet Pressure	~2 psi
MS	**Agilent 7250 Q-TOF**
Source Temperature	280 °C
Quad Temperature	150 °C
Collision Cell Gas Flows	1 mL/min N_2
	4 mL/min He
Electron Energy	70 eV (standard EI)
	15 eV (low energy EI)
Acquisition Mass Range	*m/z* 45–550
Spectral Acquisition Rate	5 spectra/sec

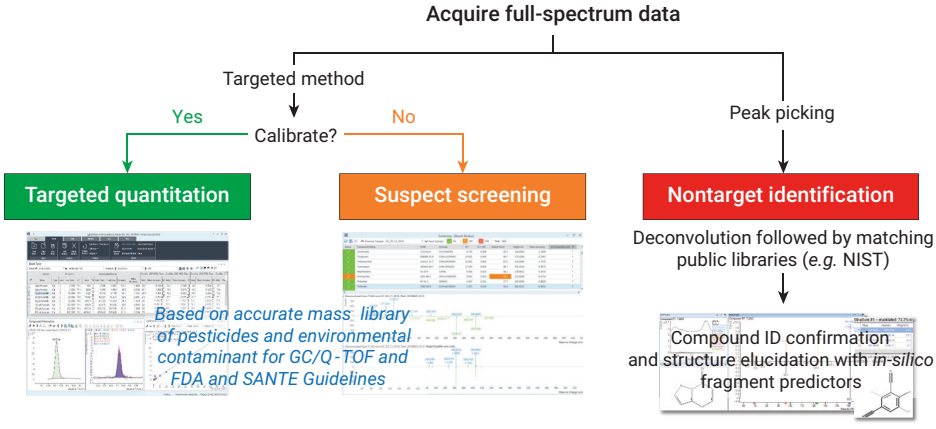


Figure 2. Combined contaminants screening workflow based on targeted and suspect screening approach using GC/Q-TOF PCDL and nontargeted screening using NIST library followed by structure elucidation of the unknowns.

Unknowns identification

The first step in unknowns identification is finding the *m/z* of a molecular ion. To identify the molecular ion of an unknown, soft ionization, the low electron energy mode of the GC/Q-TOF, was used. After molecular ion confirmation, accurate mass product ion spectra of unknowns were generated in target MS/MS mode using the tentatively identified molecular ion *m/z* as a precursor. The MS/MS data were then imported into Molecular Structure Correlator (MSC) software to assist structure elucidation.

Results and discussion

Suspect screening results

Large number of pesticides and environmental contaminants (>100) were identified and confirmed in each water extract (collected at day three after rain) using the GC/Q-TOF Screening workflow with the GC/Q-TOF PCDL. Some of the criteria used for compound verification included accurate mass of < 5 ppm, a library match and coelution score both of >70, and S/N >3.

Figure 3 shows an example of the GC/Q-TOF Screening (top) and quantitation results (bottom) windows.

The screening results table is set up such that only verified (labeled in green) and tentatively identified (labeled in orange) compounds are shown. Tentatively identified compounds (not visible in the current view) are those that failed one or more criteria (for example, RT difference) but may still be real hits. These tentative hits need manual review for their verification. The two mirror plots below the screening summary table show deconvoluted (blue, top) versus PCDL (green, bottom) compound spectra for either full spectrum or only specific ions selected by the screener displayed in the upper and lower panels, respectively.

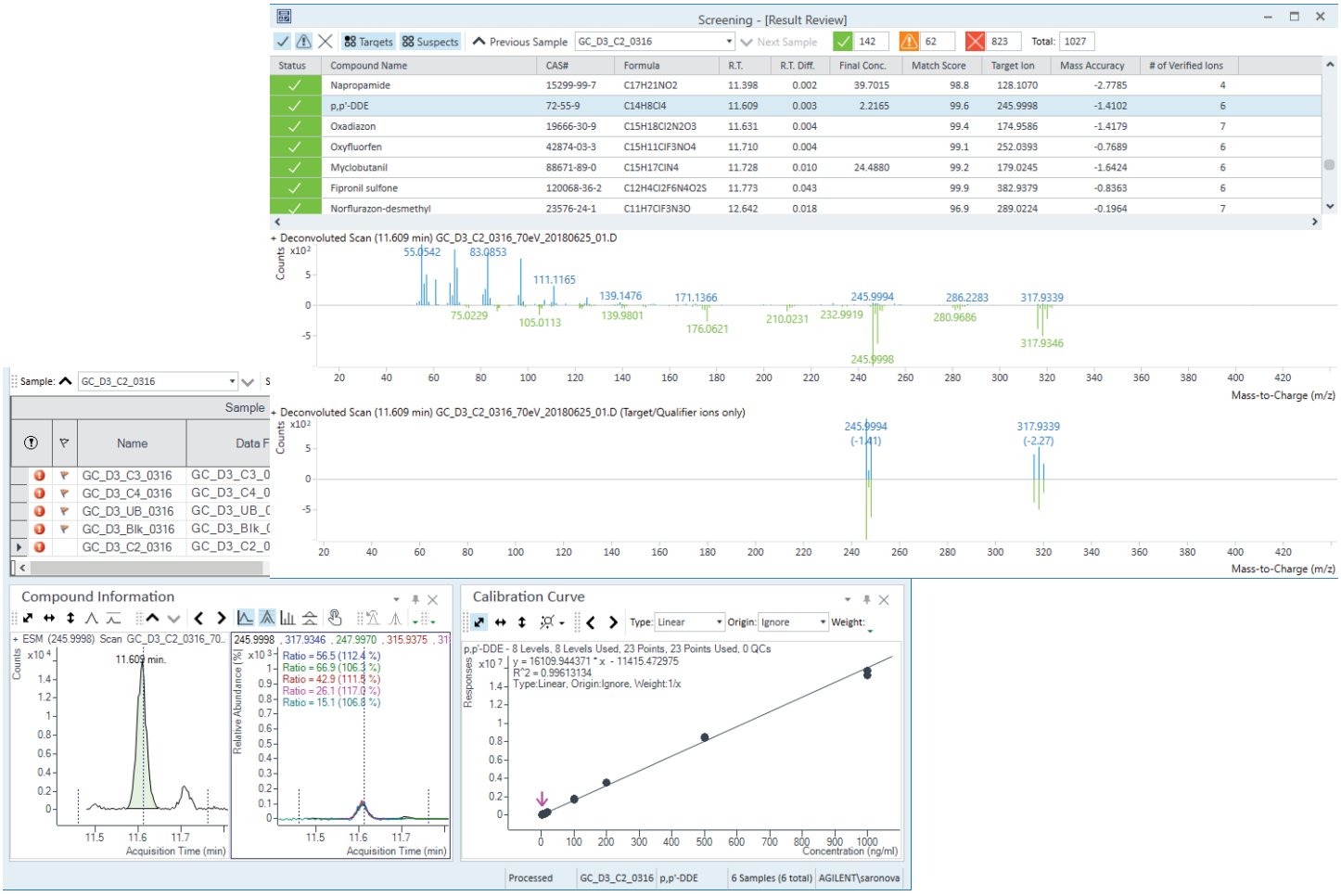


Figure 3. Suspect screening in Agilent MassHunter Quantitative Analysis software 10.1. Both Screening and Quantitation Results windows are linked to simplify the review process.

Table 2 shows a summary of targeted quantitation and suspect screening results for the most upstream (UB) sampling site included in this study. This list excluded pollutants that were also identified in blank extracts. The identified contaminants were mainly herbicides (36%) followed by fungicides (25%) and insecticides (21%), which was not surprising given the proximity of the sampling site to an agricultural development (Figure 4).

Table 2. Target and suspect screening results summary from UB sampling site. Reported amounts are concentrations in the injected solution.

Compound Name	Fragment Ratio Score	Mass Difference (ppm)	Amount or Suspect ID Only (ng/mL)
2,4,6-Tribromoanisole	99.6	1.68	ID only
2-Phenylphenol	86.2	0.59	ID only
Anthraquinone	93.7	2.35	ID only
Atrazine	98.5	0.77	6.5
Atrazine-Desethyl	90.1	3.41	ID only
Atrazine-Desisopropyl	94.4	2.42	ID only
Azoxystrobin	99.9	0.89	95.1
BAM/Dichlorbenzamide	84.3	0.57	ID only
Boscalid (Nicobifen)	99.8	0.03	ID only
Bromacil	99.4	0.53	116.5
Carvone	86.6	3.5	ID only
Chlorantraniliprole	96.1	0.59	304.6
Chloroneb	96.1	0.57	ID only
Chlorothalonil	99.9	0.83	7.3
Coumaphos	88.4	0.47	ID only
Cyprodinil	99.7	1.53	ID only
DCPA/Chlorthal-Dimethyl	99.4	2.06	ID only
Diethyltoluamide (DEET)	99.7	1.47	ID only
Diazinon (Dimpylate)	86.5	0.86	265
Diazoxon	99.5	0.21	ID only
Dichlobenil	98.1	1.24	ID only
Difenoconazole(I)	95.7	1.32	26.1
Dimethenamid-P	99	1.11	ID only
Dimethoate	98.6	2.03	1048.1
Disugran	67.9	2.44	ID only
Dithiopyr	99.8	1.38	ID only
Diuron Metabolite [3,4-Dichlorophenylisocyanate]	100	0.64	ID only
Fenbuconazole	92.8	0.64	ID only
Fipronil	91.9	1.26	ID only
Fipronil sulfide	99.6	0.27	ID only
Fipronil sulfone	99.9	0.06	ID only
Flonicamid	89.1	0.73	ID only
Flumioxazin	96.6	0.26	ID only
Fluopyram	99.1	1.11	ID only
Fluridone	96.1	1.43	ID only
Flurprimidol	92.6	2.3	ID only
Flutolanil	78.5	0.34	ID only
Fluxapyroxad	99.3	0.9	ID only
Fthalide	84.9	1.22	ID only
Hexazinone	84.4	1.89	ID only
Indoxacarb	71.6	1.5	37.9
Iprodione (Glycophen)	99.4	0.78	ID only

Compound Name	Fragment Ratio Score	Mass Difference (ppm)	Amount or Suspect ID Only (ng/mL)
Isoxaben	88.1	1.46	ID only
Malathion	94.5	0.98	7.9
Metalaxyl	90.4	0.59	11.6
Metolachlor	99.1	0.21	178
Metribuzin	97.4	2.98	ID only
Myclobutanil	99.5	1.22	10
N-(2,4-Dimethylphenyl)Formamide	80.9	3.27	ID only
Napropamide	90.7	0.47	11.5
Nitrapyrin	72.2	2.84	ID only
Norflurazon	96.3	0.98	ID only
Norflurazon-Desmethyl	94.7	0.75	ID only
Octhilinone	94.3	1.06	ID only
Omethoate	98.5	0.19	31.8
Oryzalin	99.8	0.35	ID only
Oxadiazon	99.9	0.78	ID only
Oxyfluorfen	99.2	0.27	ID only
p,p'-DDE	99.8	1.41	1.9
PCP/pentachlorophenol	72.8	1.35	3.1
Pendimethalin (Penoxalin)	99.8	0.54	ID only
Pentachloroanisole	89.8	0.09	ID only
Phenanthrene	99.5	1.76	ID only
Phenothiazine	87.5	1.43	ID only
Phosmet (Imidan)	80.6	1.79	ID only
Phthalide	94.5	2.81	ID only
Prodiamine	99.9	0.31	ID only
Prometon	90.1	1.04	ID only
Propiconazole(I)	99.3	1.13	ID only
Propiconazole(II)	99.4	0.42	ID only
Propyzamide (Pronamide)	80.1	1.07	2.2
Pyraclostrobin	93.8	0.71	ID only
Pyrimethanil	88.6	2.26	ID only
Simazine	99.8	0.27	ID only
Sulfentrazone	99.9	0.32	ID only
Tebuconazole(I)	91.4	1.03	ID only
Tebuthiuron	90.4	0.89	ID only
Tetraconazole	84.3	1.74	ID only
Thanite	86.5	3.98	ID only
Thiamethoxam	97.1	1.24	34.1
Triclosan	95.7	1.15	ID only
Trifloxystrobin	87	1.27	ID only
Trifluralin	95.8	2.22	ID only
Tris(2-Butoxyethyl)Phosphate	96	2.02	ID only
Tris(3-Chloropropyl)Phosphate	98.6	2.63	ID only
Tris(b-Chloropropyl)Phosphate	99.1	0.9	ID only

In addition to water samples, extractions were also performed from filter particles. Most contaminants were present in water extracts, but a few pollutants were also identified in filter extracts (Figure 5). Interestingly, some pyrethroids and PAHs were identified uniquely in the filter extracts. Their strong affinity to filter particles can be explained by their high hydrophobicity.

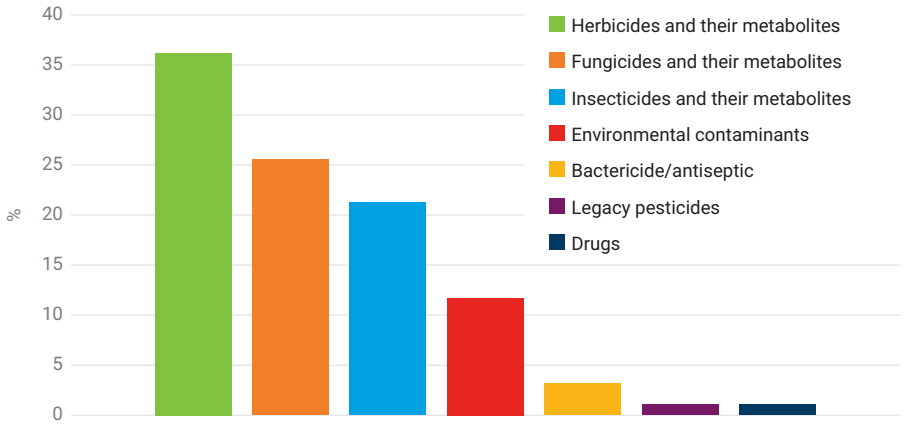
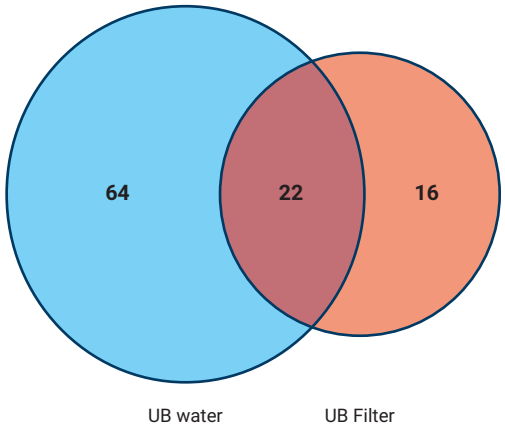


Figure 4. Distribution of pollutants identified at UB sampling site by the GC/Q-TOF Screening workflow and PCDL.

A



B

Compounds uniquely identified in the UB filter extract:

Diphenylamine (DFA)
Hexachlorobenzene
Pentachloroaniline
Fluoranthene
Pyrene
Nonachlor-trans
p,p'-DDD
Dihexylphthalate
Bifenthrin
Chrysene
cis-Permethrin
trans-Permethrin
Benzo[b]fluoranthene
Benzo[a]pyrene
Dinonylphthalate
Indeno[1,2,3-cd]pyrene

Figure 5. Distribution of the contaminants between water and filter extracts from the UB site.

Pollutants identified by a targeted and suspect screening approach in water extracts were compared across different sampling sites. The highest number of pollutants was identified using the PCDL screening approach in a water extract from the C2 sampling site. Approximately half of all identified pollutants were in common between UB, C2, and C4 sampling sites (Figures 6A and 6B). Relative amounts of contaminants identified across all sampling sites were also plotted on the 3D-area graphs to better visualize the spatial trends (Figure 7).

Nontargeted screening results and verification of tentative hits

Using a nontargeted analysis, a complimentary approach to target and suspect screening, a few additional compounds were identified in water extracts. Figure 8 shows examples of compounds tentatively identified by Unknowns Analysis with NIST17.L library in the extract from the UB site. Since NIST17.L is a unit mass library, accurate mass data are not automatically considered for library matching. However, Unknowns Analysis can help confirm the identity of tentative hits or invalidate false positives based on the accurate mass information when correlated with the molecular formula of the NIST hit. This is performed using the ExactMass feature of Unknowns Analysis. As shown in Figure 8, most ions for each of these tentative hits can be explained by a subset of the molecular formula of the hit within 5 ppm mass error.

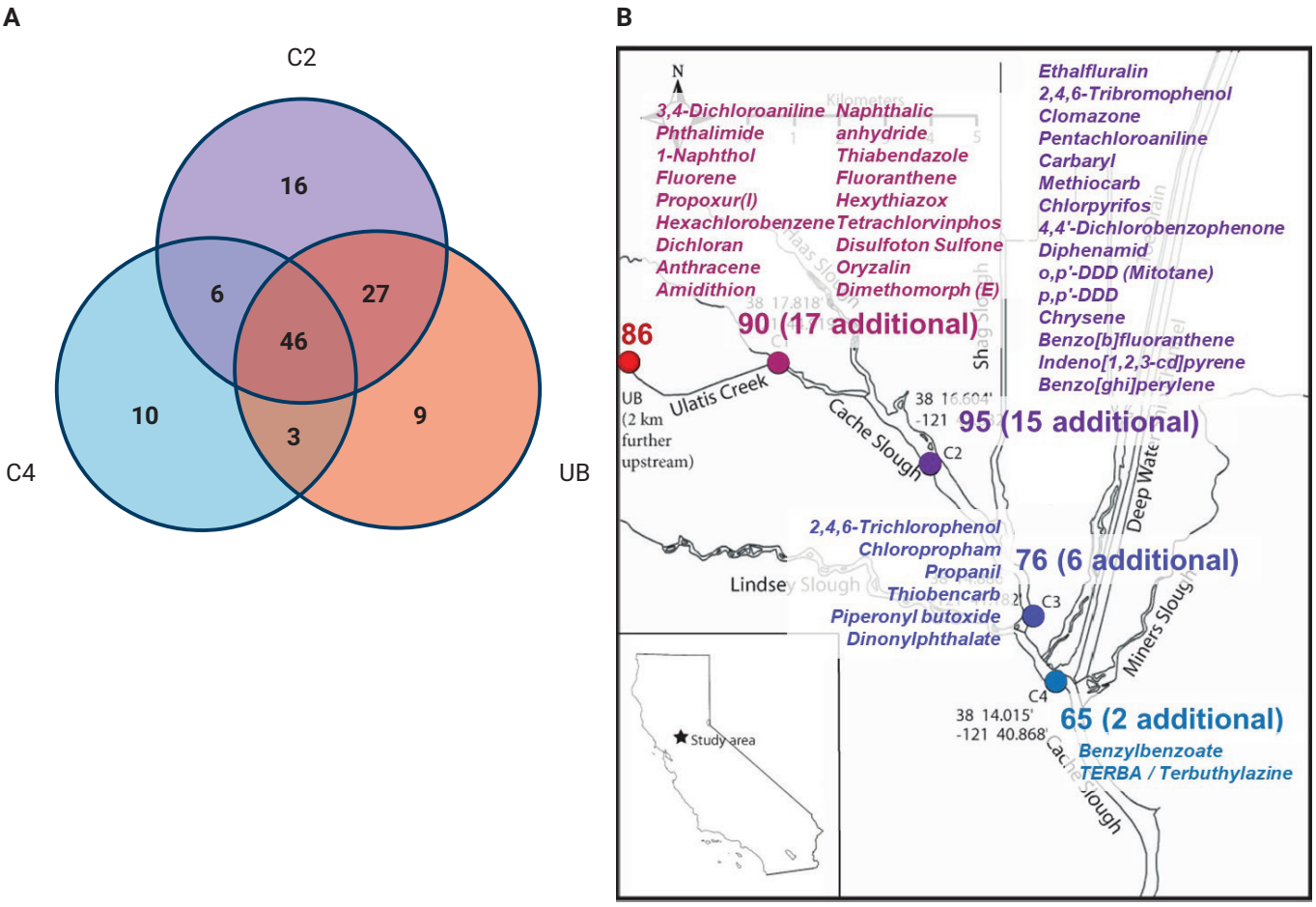


Figure 6. Geographic distribution of pollutants. Comparison of the identified contaminants between UB, C2, and C4 sites (A). Sampling map showing the number of identified pollutants as well as the new contaminants added to the flow stream from each site (B).

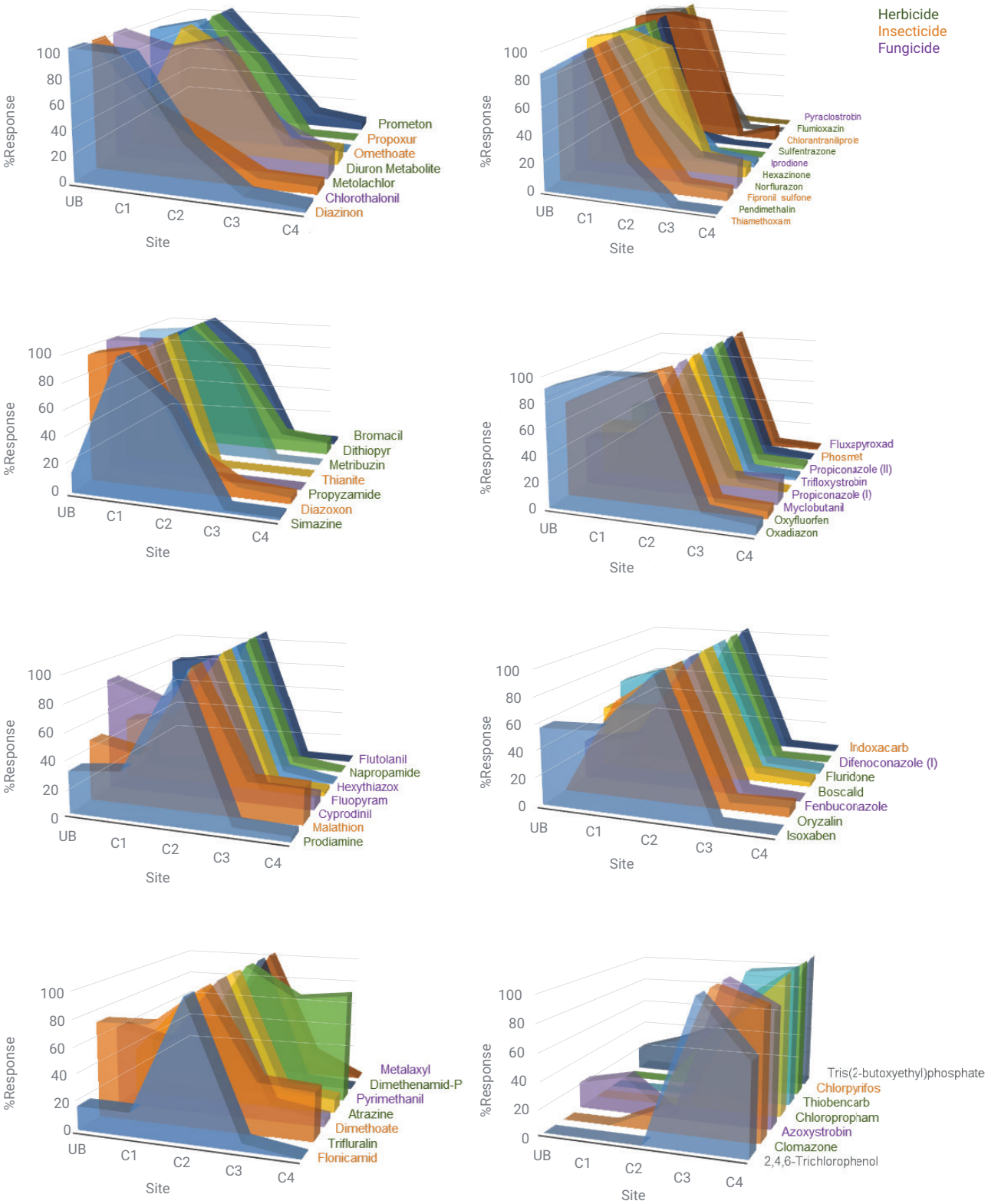
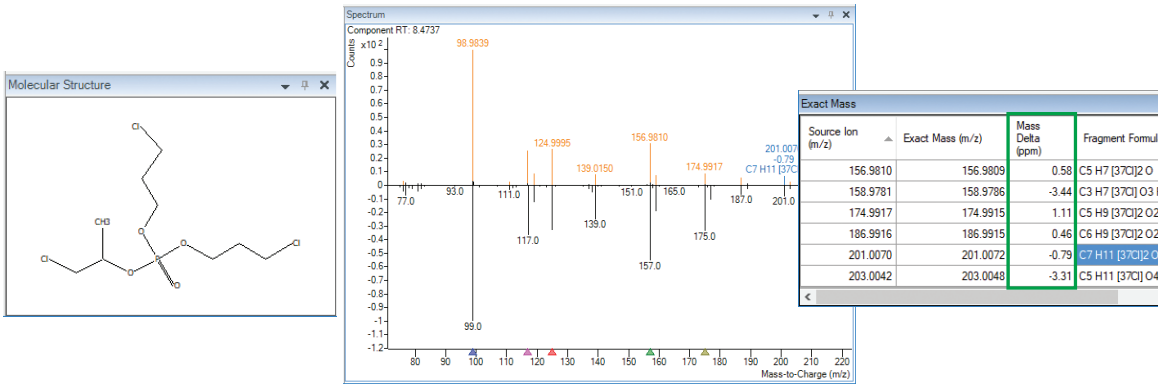
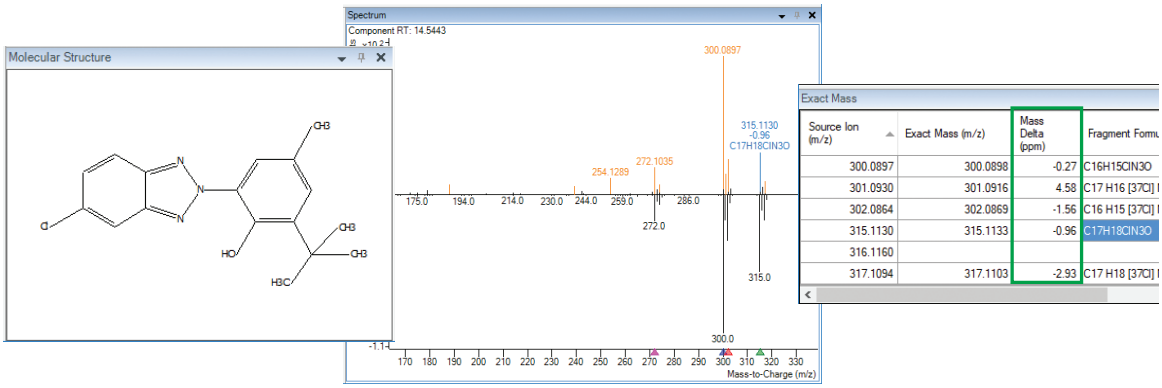


Figure 7. Relative amount of pollutants identified across all sampling sites. The compounds are grouped based on their geographic distribution profile.

Tentative hit: Bis(3-chloro-2-propyl)(1-chloro-2-propyl)phosphate ($C_9H_{18}Cl_3O_4P$)



Tentative hit: Bumetrizole ($C_{27}H_{18}ClN_3O$)



Tentative hit: Methoxsalen ($C_{12}H_8O_4$)

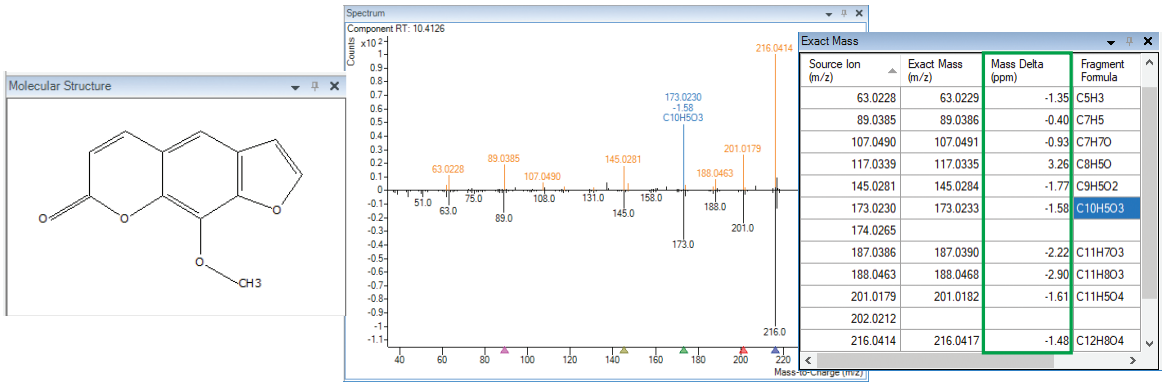


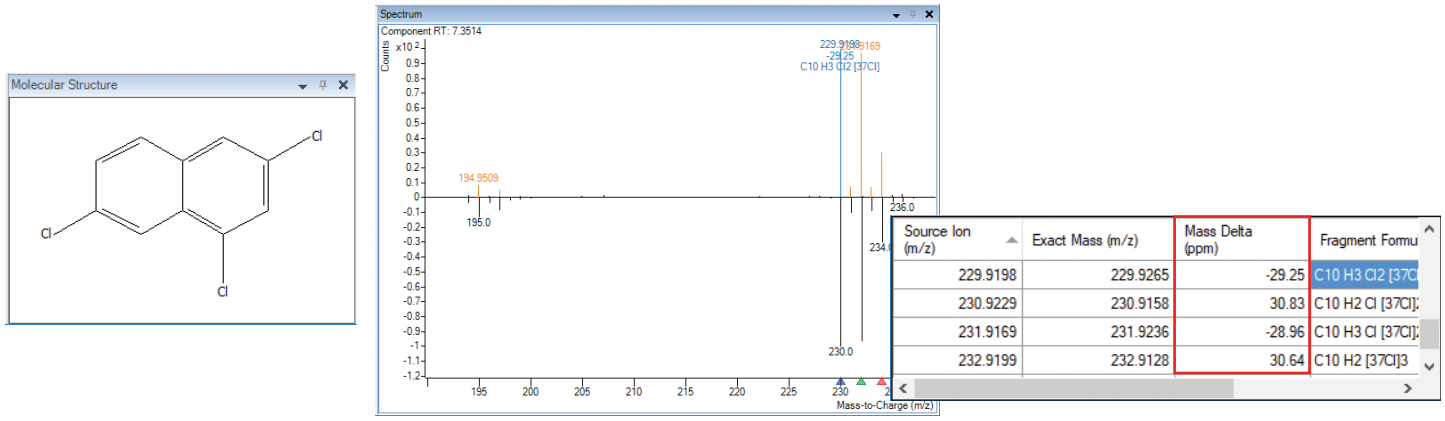
Figure 8. Examples of tentatively identified contaminants from UB site, using Agilent MassHunter Unknowns Analysis software and NIST17.L library. Low mass error for the fragments in the deconvoluted spectrum provides additional point for confirmation of the molecular formula of the hit.

Identification of unknowns

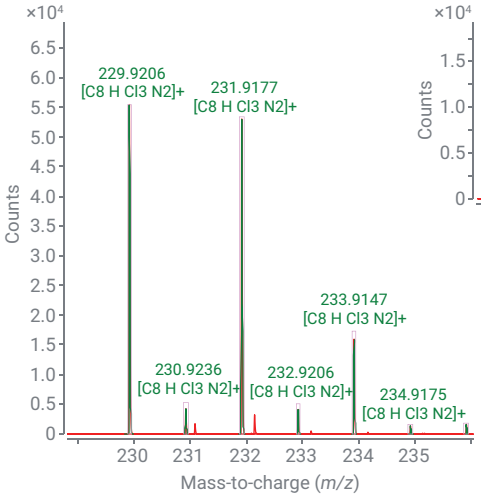
Some of the tentative hits were not confirmed due to large mass error, and were subjects for further investigation. Figure 9 shows an example of such a case. A hit, tentatively identified as 1,3,7-trichloronaphthalene by NIST library with a library match score of 73.9, was rejected after evaluation with the ExactMass feature due to a large mass difference (around 30 ppm) between the compound spectrum ions and theoretical ions corresponding to the molecular ion isotope cluster of the hit.

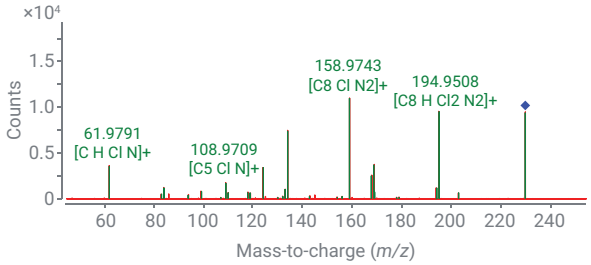
To identify the compound, the molecular ion was confirmed using a low electron energy setting (15 eV), and an isotopic cluster of the tentative molecular ion was annotated using the Molecular Formula Generation (MFG) feature of MassHunter Qualitative Analysis software

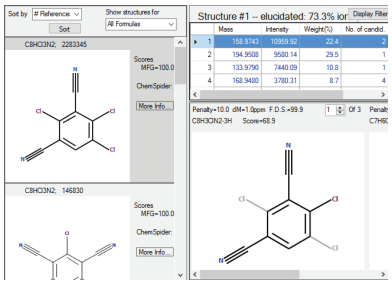
A **Tentative NIST17 hit:** 1,3,7-trichloronaphthalene ($C_{10}H_5Cl_3$)



B **Step 1:** Confirm M+ → **Step 2:** Confirm fragment ions → **Step 3:** Structure elucidation on candidate







Most likely: 2,4,5-trichloroisophthalonitrile, a degradation product of chlorothalonil

C

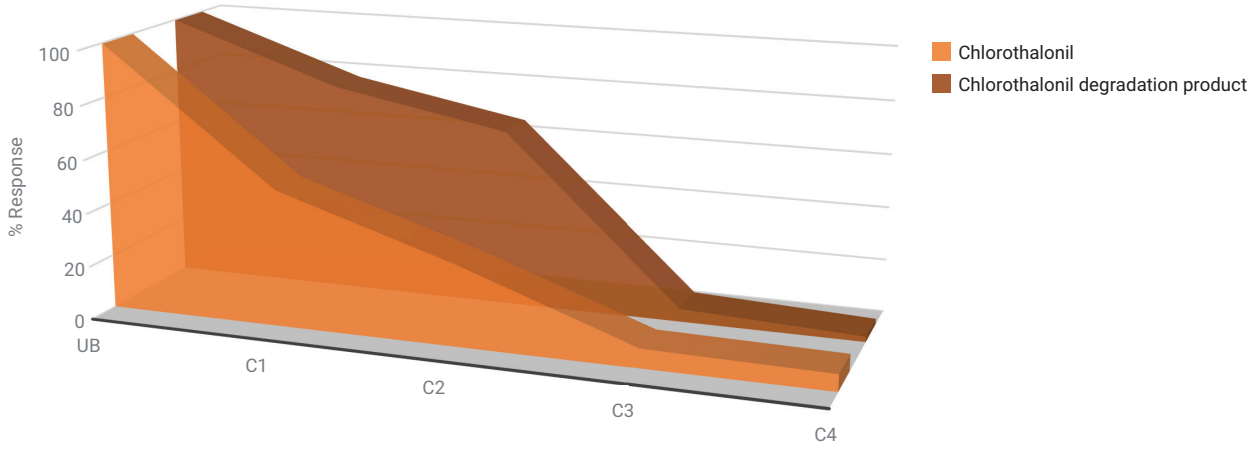


Figure 9. Identity confirmation and structure elucidation of one of the tentative hits. Significant mass error suggested incorrect identity of the compound (A). The compound was identified using the Molecular Structure Correlator tool with accurate mass product ion spectrum as an input (B). Distribution of chlorothalonil and its degradation product 2,4,5-trichloroisophthalonitrile across sampling sites (C).

(Figure 9B, Step 1). Next, MS/MS was performed using a tentative molecular ion as a precursor (Figure 9B, Step 2). MS/MS data were then processed using Molecular Structure Correlator to propose a structure for this unknown compound (Figure 9B, Step 3). The most likely structure based on the number of references is 2,4,5-trichloroisophthalonitrile, a degradation product of chlorothalonil. Remarkably, the profile of chlorothalonil closely follows its degradation product (Figure 9C).

Conclusion

A large number of pesticides and other environmental pollutants have been identified in surface water samples using a comprehensive workflow that included targeted quantitation, suspect screening based on GC/Q-TOF accurate mass PCDL, and a nontargeted approach.

Low energy EI and accurate mass MS/MS facilitated compound identification in a nontargeted screening and structure elucidation of unknowns. One of the unknowns has been tentatively identified as 2,4,5-trichloroisophthalonitrile, a degradation product of chlorothalonil.

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