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Analysis of the Wastewater Effluent Samples to Identify Toxic Chemicals Using High-Resolution GC/Q-TOF

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Introduction

Identification of toxic chemicals in wastewater effluents is a key step towards improving environmental water quality of downstream ecosystems. Conventional targeted analysis approaches are often not sufficient to identify the source of toxicity due to their limited scope. Comprehensive screening, often with non-targeted analysis can help to address this issue and lead to a deeper understanding of the possible cause of toxicity. The current study combined both non-targeted and broad scope suspect screening for pesticides and environmental pollutants using high-resolution GC/Q-TOF to identify toxic chemicals in wastewater effluent samples.

Experimental

Samples were filtered through a GF/F filter (0.45 µm) and passed over an Oasis HLB cartridge. Dried cartridges were eluted with ethyl acetate and methanol. Dried filters were extracted in sonicating bath with hexane/acetone 1:1. Both extracts were combined and spiked with Dibromooctafluorobisphenol (DBOFB, 10 ng) as an internal standard. The samples were analyzed using

Figure 1. Agilent 7250 GC/Q-TOF.

Experimental

high-resolution 7250 Q-TOF coupled to a new 8890 GC (Figure 1) using GC mid-column blackflush configuration and a retention time locked (RTL) method to ensure RT consistency with the GC/Q-TOF accurate mass library of pesticides and environmental pollutants. The samples were screened for contaminants in both EI as well as Negative CI (NCI) modes to ensure best sensitivity for pyrethroids and other halogenated compounds. Positive CI (PCI) was later used to assist compound identification. The conditions are described in detail in Table 2.

GC and MS Conditions:	EI	Negative CI	Positive CI
GC	8890		
Column	2 x HP-5MS UI, 15 m, 0.25 mm, 0.25 µm		
Inlet	MMI, 4-mm UI liner single taper w wool		
Injection volume	1 µL		
Injection mode	Cold splitless		
Inlet temperature	60°C for 0.2 min; 600°C/min to 320°C		
Oven temperature program	60°C for 1 min; 40°C/min to 170°C, 10°C/min to 310°C, 3 min hold		
Carrier gas	Helium		
Column 1 flow	~1.2 mL/min		
Column 2 flow	~ 1.4 mL/min		
Backflushing conditions	5 min (Post-run), 310 °C (Oven), 50 psi (Aux EPC pressure), 2 psi (Inlet pressure)		
Transfer line temperature	280°C		
Mass range	50 to 650 m/z		
Spectral acquisition rate	5 Hz		
Quadrupole temperature	150°C		
Source temperature	280°C	150°C	280°C
Electron energy	70 eV	250 eV	100 eV
Emission current	5 µA	10 µA	15 µA

Table 1. GC/Q-TOF acquisition parameters.

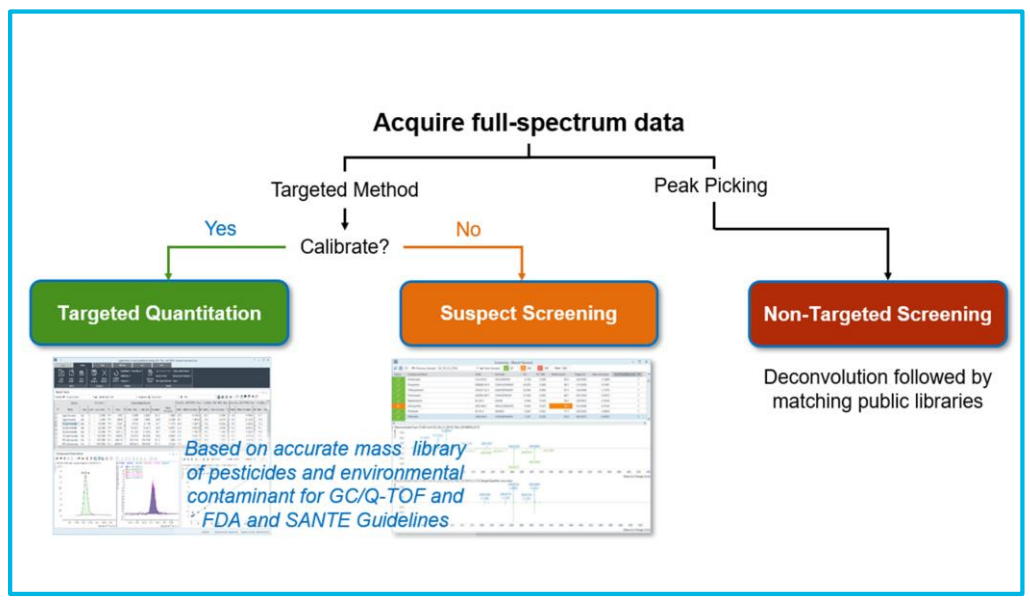

Figure 2. Combined contaminants screening workflow based on PCDL and non-targeted screening using NIST library, all performed using MassHunter Quantitative Analysis 10 software.

EI Broad Scope Targeted Screening

The wastewater effluent samples were collected on days 1, 2, 4 and 5 of a five-day series in duplicates and displayed varying degrees of acute toxicity (0-80%) toward Ceriodaphnia dubia. The extracted samples were analyzed using high-resolution GC/Q-TOF. A new screening workflow available in MassHunter Quantitative Analysis Software 10 (Figure 2) was used to surveil a large number of important pesticides and environmental pollutants contained in the GC/Q-TOF accurate mass library containing over 1000 compounds. In addition, a smaller PCDL containing NCI spectra for 118 compounds was created to be able to utilize the same approach for contaminants screening in NCI.

An example of the screener window as well as the report (for EI) are shown in Figure 3. The identified compounds that demonstrated some difference in the response levels between the samples grouped by mortality % towards *C. dubia* are shown in Table 2.

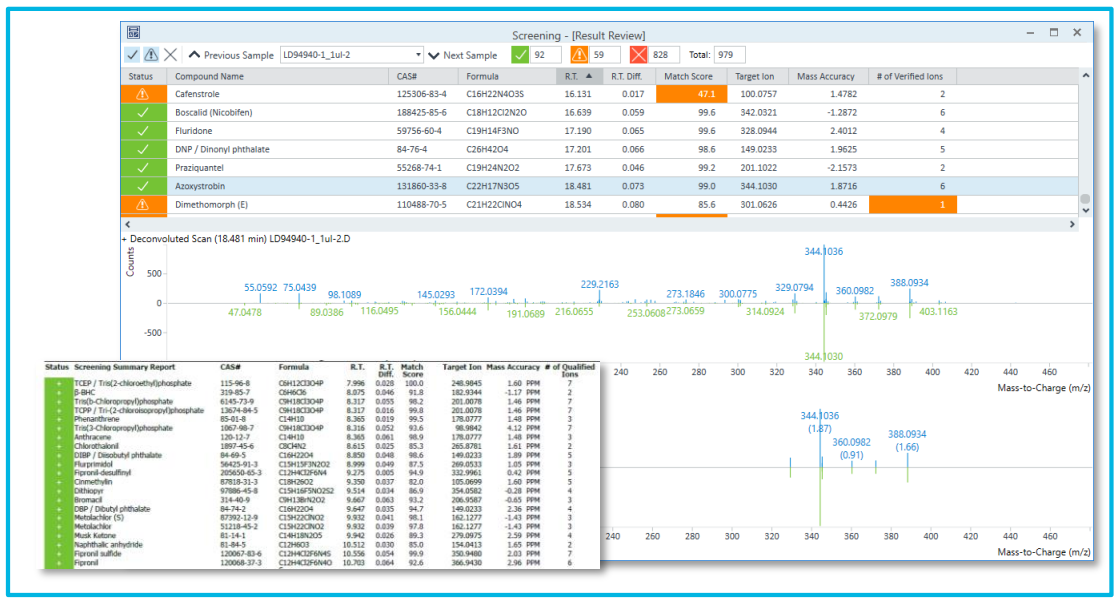

Figure 3. EI PCDL-based screening in MassHunter Quantitative Analysis Software 10. Automatically verified compounds are labeled in green. Those compounds that need additional review are in orange. Partial report is shown in the lower corner.

NCI Analysis of Wastewater Effluent Samples

NCI screener results are shown in the Figure 4 and Table 3. Although some compounds have only one fragment and may require additional data for confirmation (Figure 4A), many others produce enough fragments with methane NCI to be used in the PCDL screening workflow (Figure 4B).

Using the EI and NCI screening approaches, numerous environmental pollutants were identified in wastewater samples, including many pesticides. However, most of them, e.g. fipronil and fipronil degradation products, were also identified in wastewater samples that exhibited no toxicity (Table 3), and thus are unlikely to be the source of the observed toxicity.

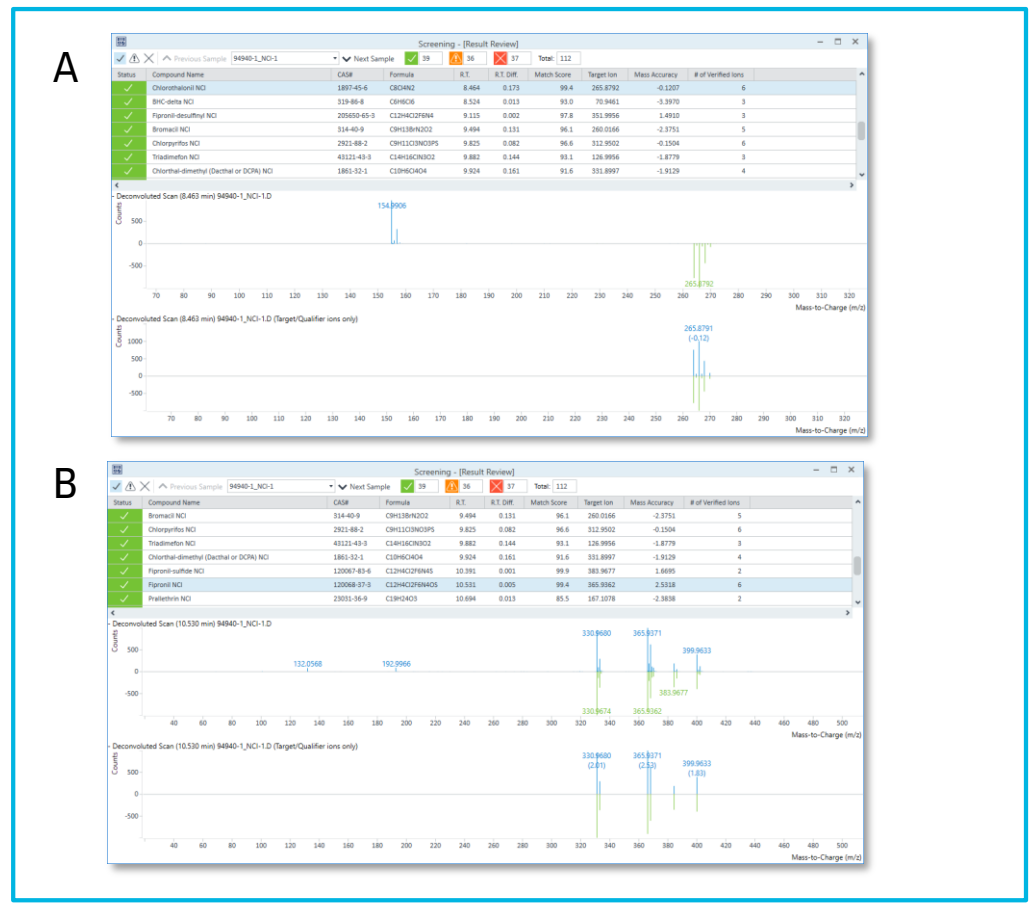

Figure 4. Examples of compounds identified using PCDL-based screening in NCI.

Sample	80 % mortality						20 % mortality						0 % mortality					
	LD94940-1			LD94940-2			LD94941-1			LD94941-2			LD94943-1			LD94943-2		
Compound Name	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score
TBEP / Tris(2-butoxyethyl) phosphate	2013504	2.8	99.9	1502528	3.9	99.9	1289372	2.5	99.9	1559301	3.8	99.9	787113	3.1	99.9	784473	3.8	99.9
tert-Butylphenyldiphenylphosphate	16799	2.1	92.9	4948	3.2	74.6	2828	1.1	82.5	10468	0.8	91.9	2950	1.3	70.6	2766	0.8	91.9
Chlorantraniliprole	6298	0.2	76.8	5330	2.0	79.4	3572	1.7	63.2	3494	1.8	66.4	3458	1.1	52.4	2710	1.8	66.4
Flurprimidol	16518	1.3	80.4	15240	0.5	76.4	10698	2.6	73.7	12065	2.1	80.2	6038	2.0	74.2	4976	2.1	80.2
Paclobutrazol	16985	0.9	96.8	15763	1.6	98.7	10725	0.9	92.4	12090	2.1	94.9	9106	1.8	79.1	8448	2.1	94.9
TBZ / Thiabendazole	1570235	1.4	99.7	1536170	2.4	99.7	1282402	0.6	99.7	1368732	2.2	99.8	774093	0.6	99.7	675439	2.2	99.8
Azoxystrobin	134463	1.8	99.1	139960	3.0	98.9	109579	1.4	98.9	119004	1.7	98.8	104804	1.7	89.9	94511	1.7	98.8

Table 2. EI screening results. The response value is color-coded based on the relative level across the samples. Mass error for the quant ion as well as library match score for the ions selected within the screener are also shown.

Results and Discussion

Untargeted Analysis of Wastewater Effluent Samples

In addition, the data were processed using an untargeted workflow that involves feature finding in Unknowns Analysis and NIST 17 library search, followed by the differential analysis in Mass Profiler Professional (MPP) to identify compounds with more significant presence in the samples that displayed higher toxicity.

Sample	94940-1		94940-2		94941-1		94941-2		94943-1		94943-2	
Compound Name	Response	Library Match Score	Response	Library Match Score	Response	Library Match Score	Response	Library Match Score	Response	Library Match Score	Response	Library Match Score
Deltamethrin	16837	71.4	14202	42.7	6474	83.7	4138	36.3	3253	56.5	4294	36.3
Endosulfan sulfate	3372	98.8	3013	91.9	12182	98.9	11865	99.1	18103	98.8	15859	99.1
Fipronil sulfone	1184481	99.4	989538	99.3	1058932	99.4	898204	99.3	1218463	99.4	1089462	99.3
Chlorfenvinphos	22450	94.7	13196	86.3	16668	94.4	14469	94.7	14757	95.7	12834	94.7
Fipronil	1312800	98.7	1269915	97.7	1255881	97.1	1307988	96.8	1519654	95.8	1350814	96.8
Fipronil-sulfide	201344	99.9	192041	100.0	224062	100.0	218654	100.0	241255	100.0	274001	100.0
Chlorthal-dimethyl	1730		1409	94.1	1468	94.3	1489	96.2	2204	80.0	1807	96.2
Triadimefon	22376	94.1	16547	94.2	19705	96.0	17006	96.4	18710	97.2	16675	96.4
Malathion	474	86.6	249	86.6	0	-	0	-	0	-	0	-
Fipronil-desulfinyl	128886	97.8	111722	97.8	122423	97.8	119001	97.9	164450	97.8	135773	97.9
Chlorothalonil	23789	99.4	12226	99.2	14367	99.0	15765	99.1	14714	99.2	12680	99.1
BHC-beta	36573	88.4	19696	91.7	25594	81.4	19439	84.7	23983	69.4	13527	84.7
Dicloran	30089	92.3	33303	93.2	34005	92.6	39632	93.8	44118	95.1	35911	93.8
Hexachlorobenzene	13573	99.3	10353	99.6	11863	99.3	9934	99.1	14371	98.7	12048	99.1
Trifluralin	10334	86.6	11119	94.1	12089	94.2	11454	92.8	13550	94.5	9293	92.8
2,4-Dinitrotoluene	81406	90.1	91627	89.0	75770	84.4	67256	83.3	43423	91.1	41979	83.3
2,4,6-Trichlorophenol	2551498	92.2	2250861	91.6	2525758	91.5	2544336	91.4	2707308	91.2	2736603	91.4

Table 3. NCI Screening results.

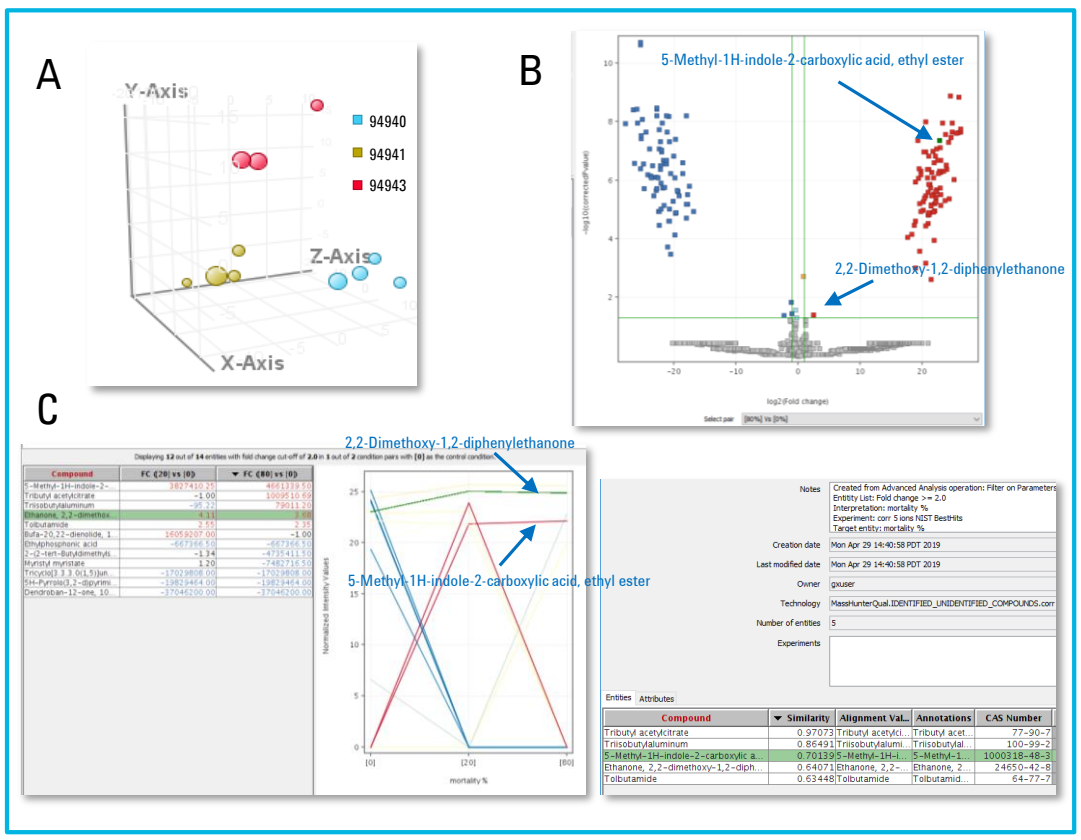

Figure 5. MPP Results: PCA plot (A), Volcano plot comparing 80 % mortality group against 0% (B), and correlation analysis (C). Note the same compounds showed up in both B and C.

Unknowns ID and Verification of NIST Library Hits

To confirm identity of the compounds identified using NIST 17 library in the Unknowns Analysis, "ExactMass" - fragment formula annotation feature was used (Figure 6A). Using the same tool, it become obvious that the second compound of interest was not identified correctly (Figure 6B). PCI was used in this case to identify the molecular ion and propose molecular formula of the compound (Figure 6C).

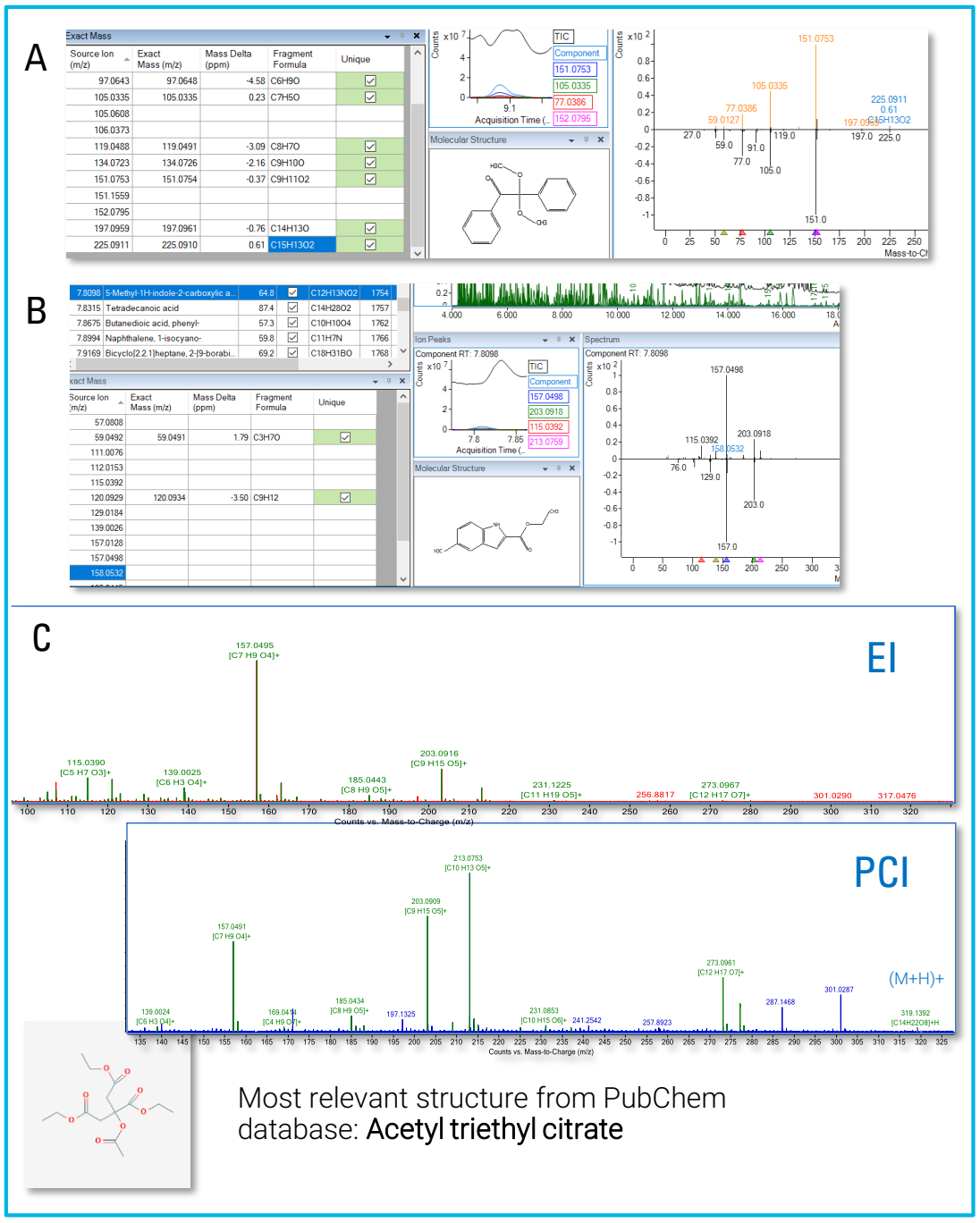

Figure 6. Compound ID and verification using Unknowns Analysis and PCI

Conclusions

- EI and NCI broad scope environmental contaminant target screening approach combined with non-targeted screening was performed using 7250 GC/Q-TOF system.
- Few compounds of interest including pesticides, such as flurprimidol, paclobutrazol, azoxystrobin and chlorantraniliprole, were identified predominantly in the samples associated with some degree of toxicity.
- Non-targeted approach helped to identify additional compounds that might be associated with mortality of *C. dubia*. While non-targeted approach is unlikely to detect minor differences in the levels of trace compounds, unlike targeted approach, it is able to find potential contaminants outside of the accurate mass PCDL.

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