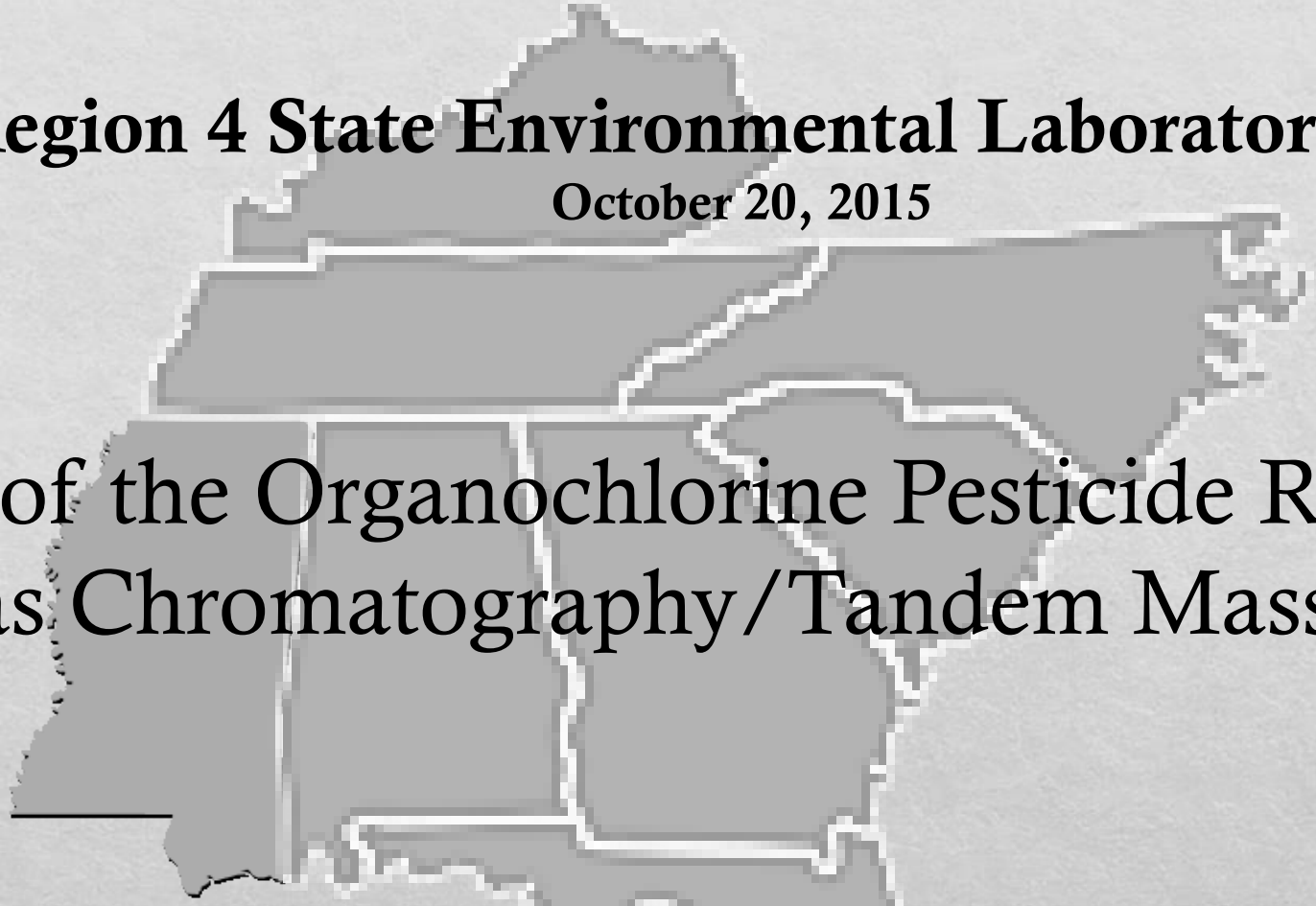
EPA Region 4 State Environmental Laboratories Meeting

October 20, 2015



Analysis of the Organochlorine Pesticide Routine Target List by Gas Chromatography/Tandem Mass Spectrometry

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US EPA Region 4

Pesticide Analysis Overview

Currently, all analyses for our routine organochlorine (OC) pesticides target list are run by Method 8081 on GC/ECD.

- ECD is a highly sensitive detector for compounds containing electronegative atoms or functional groups (halogens, organometallics, nitrites, nitro groups), and is capable of achieving (and exceeding) the low reporting limits required for target list OC pesticides.

ECD response- 1 pg on column

4,4'-DDT	6.983	7.505	14497272	13386839	0.000940	0.001090
End Aldehyde	7.193	7.610	15290925	13791739	0.000886	0.000616
Methoxychlor	7.329	7.965	8398409	7442961	0.000682	0.000865
Endo SO4	7.493	7.797	14236360	13406688	0.000832	0.001046
End Ketone	7.686	8.167	19244305	16260744	0.000812	0.000997

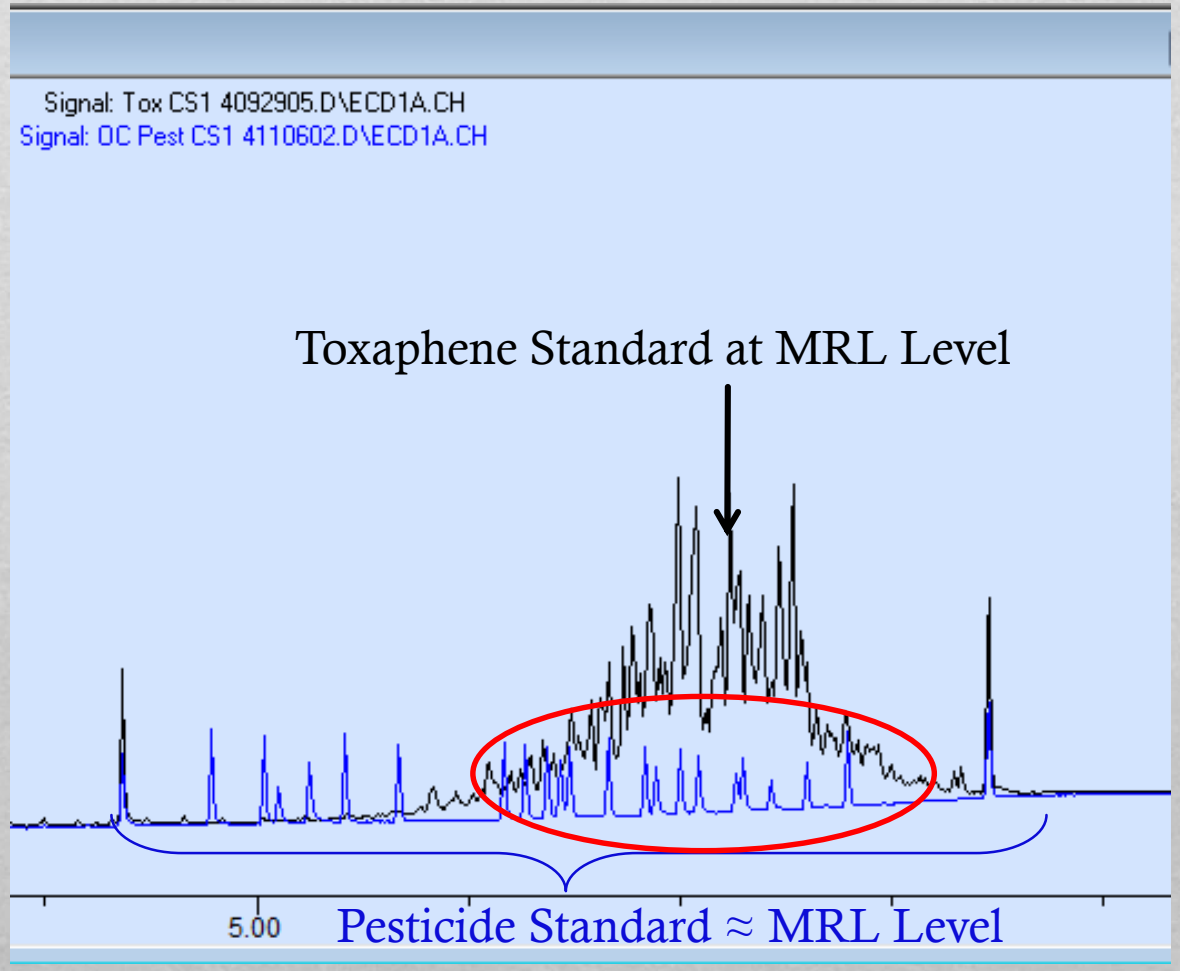
- As a non-specific detector, target compound identification is achieved via agreement between sample chromatographic peak retention time (RT) and the its expected retention time as determined during calibration. This must be confirmed by a second dissimilar stationary phase column or other qualitative technique (e.g. GC/MS).

This method performs very well if sample extracts are relatively free of interferences. Unfortunately, environmental sample extracts rarely meet this criterion.

Limitations of GC/ECD Analysis

Detector selectivity

The presence of reportable levels of technical toxaphene- a target pesticide on our routine list- precludes reporting of a large subset of single-component pesticides down to their MRLs.



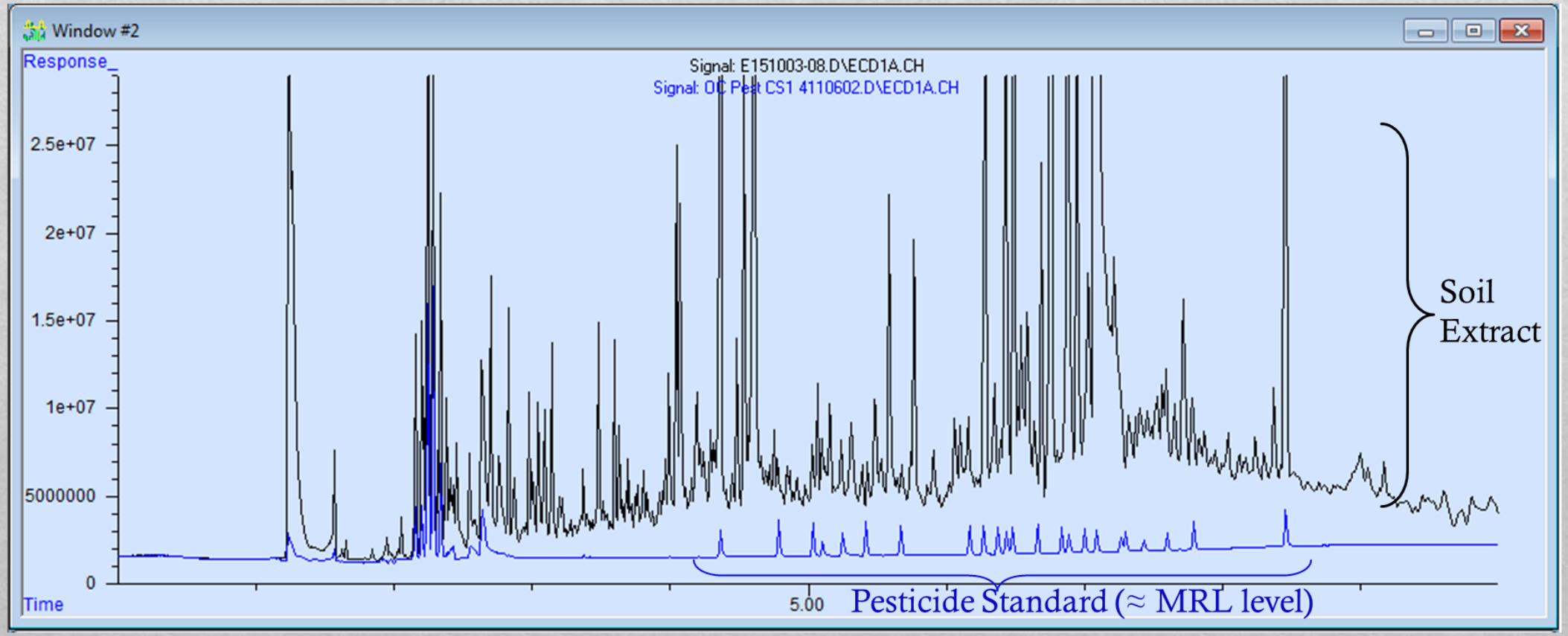
8081 SOP Target List

Aldrin
Heptachlor
Heptachlor Epoxide
alpha-BHC
beta-BHC
gamma-BHC (Lindane)
delta-BHC
Endosulfan-I
Dieldrin
4,4'-DDT
4,4'-DDE
4,4'-DDD
Endrin
Endosulfan-II
Endosulfan-SO4
Endrin Aldehyde
Endrin Ketone
Methoxychlor
gamma-Chlordane
alpha-Chlordane
Toxaphene

Limitations of GC/ECD Analysis

Chemical interferences

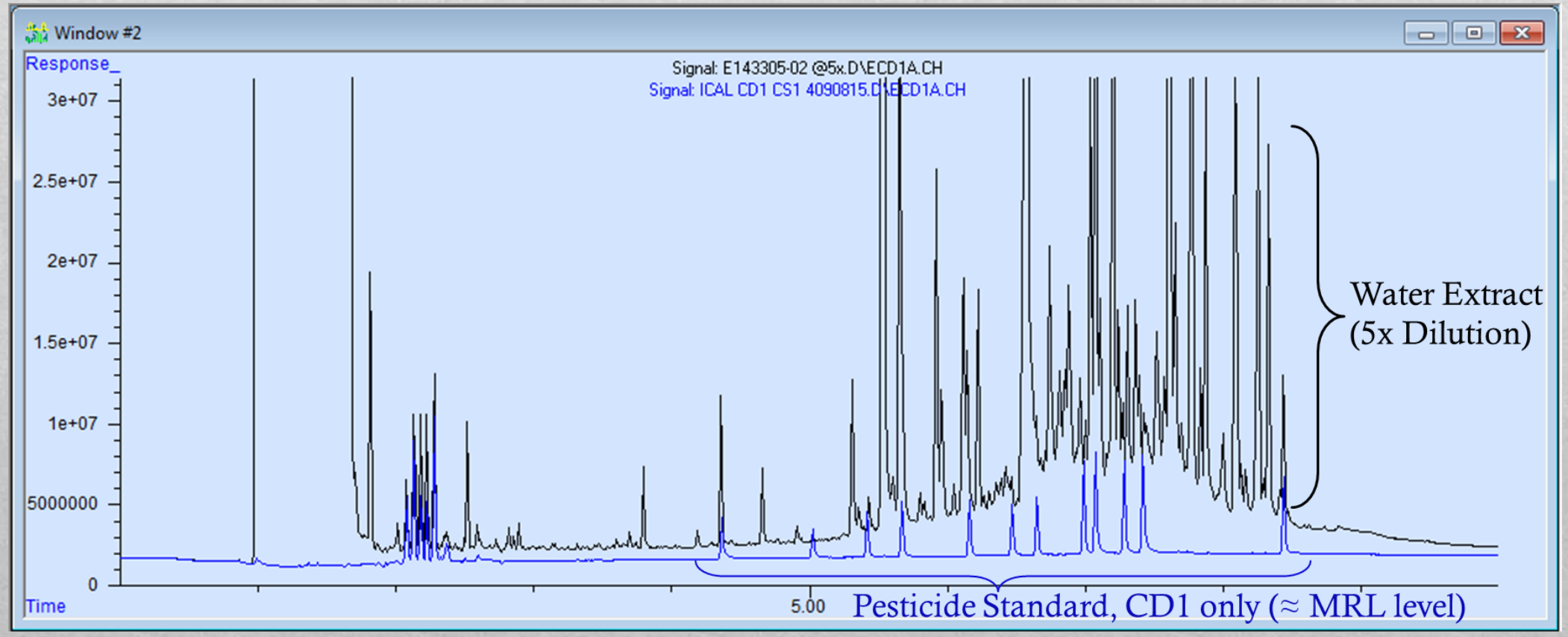
Environmental analysis normally involves samples with very complex matrices (soil, tissue, waste), and these extracts often contain high levels of co-extracted interferences.



Limitations of GC/ECD Analysis

Chemical interferences

Aqueous sample extracts from heavily contaminated sites can also have similar issues as a result of high levels of co-extracted interferents.



Limitations of GC/ECD Analysis

Chromatographic noise that decreases data quality is not an uncommon occurrence, and often results in more data qualification than would otherwise be required.

Clearly, detector selectivity is the limiting factor of GC/ECD. Improving the data quality depends most on improving detector specificity in order to reduce the levels of interferences that reach the detector, and/or reducing the level of these interferences in the sample extracts themselves.

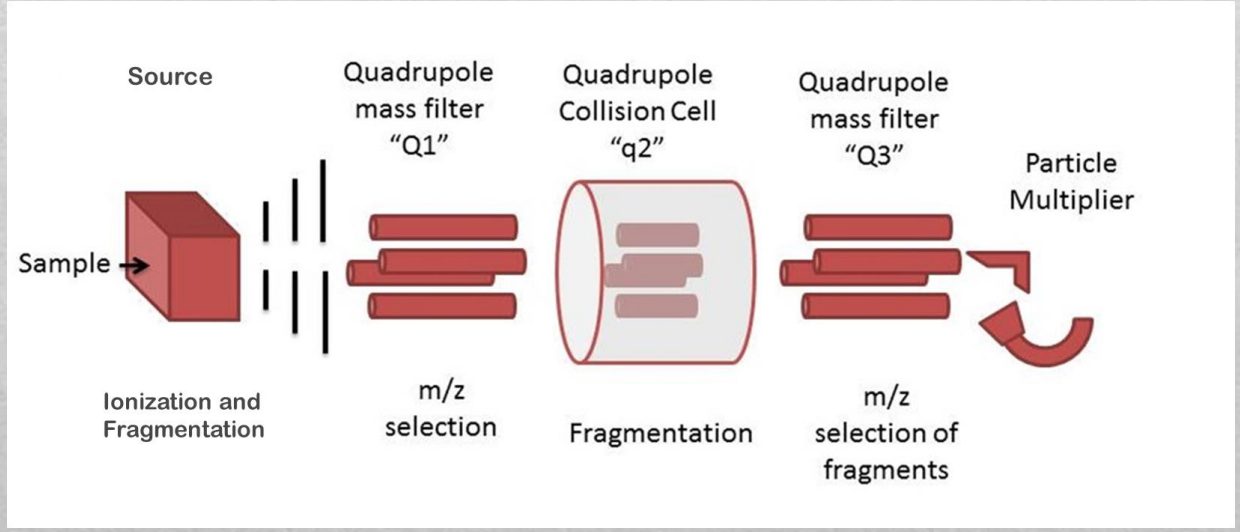
CAS Number	Analyte	MDL Draft Only	Results Qualifiers
72-54-8	4,4'-DDD (p,p'-DDD)	0.54	11 U, D-4
72-55-9	4,4'-DDE (p,p'-DDE)	1.4	70
50-29-3	4,4'-DDT (p,p'-DDT)	3.1	90
309-00-2	Aldrin	0.89	11 U, D-4
319-84-6	alpha-BHC	0.86	11 U, D-4
5103-71-9	alpha-Chlordane	1.4	11 U, D-4
319-85-7	beta-BHC	0.81	11 U, D-4
319-86-8	delta-BHC	0.76	11 U, D-4
60-57-1	Dieldrin	0.70	11 U, D-4
959-98-8	Endosulfan I (alpha)	0.89	11 U, D-4
33213-65-9	Endosulfan II (beta)	0.90	11 U, D-4
1031-07-8	Endosulfan Sulfate	0.95	11 U, D-4
72-20-8	Endrin	0.79	11 U, D-4
7421-93-4	Endrin aldehyde	0.84	15 U, D-4
53494-70-5	Endrin ketone	0.69	11 U, D-4
58-89-9	gamma-BHC (Lindane)	0.84	11 U, D-4
5566-34-7	gamma-Chlordane	0.72	11 U, D-4
76-44-8	Heptachlor	1.1	11 U, D-4
1024-57-3	Heptachlor epoxide	0.70	11 U, D-4
72-43-5	Methoxychlor	0.90	11 U, D-4
8001-35-2	Toxaphene		410 U, D-4

U	The analyte was not detected at or above the reporting limit.
D-4	MRL elevated due to interferences.

Benefits of MS/MS Detection

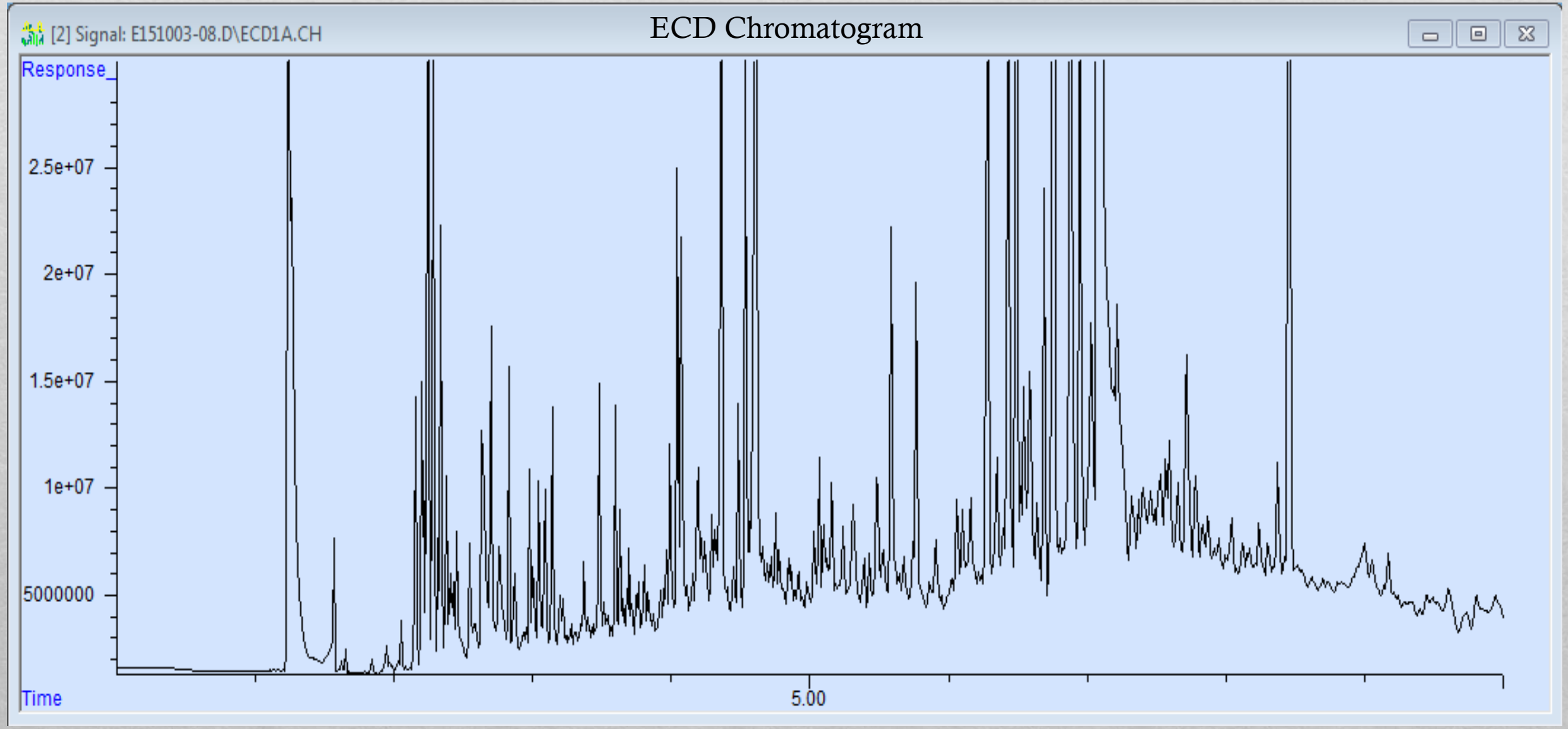
MSMS is an obvious choice for targeted environmental analyses, due to the high level of selectivity that the detector configuration is able to achieve.

- RT selectivity (e.g. ECD)
- m/z selectivity (e.g. MS-SIM)
- **structure related selectivity** (e.g. …MSMS)

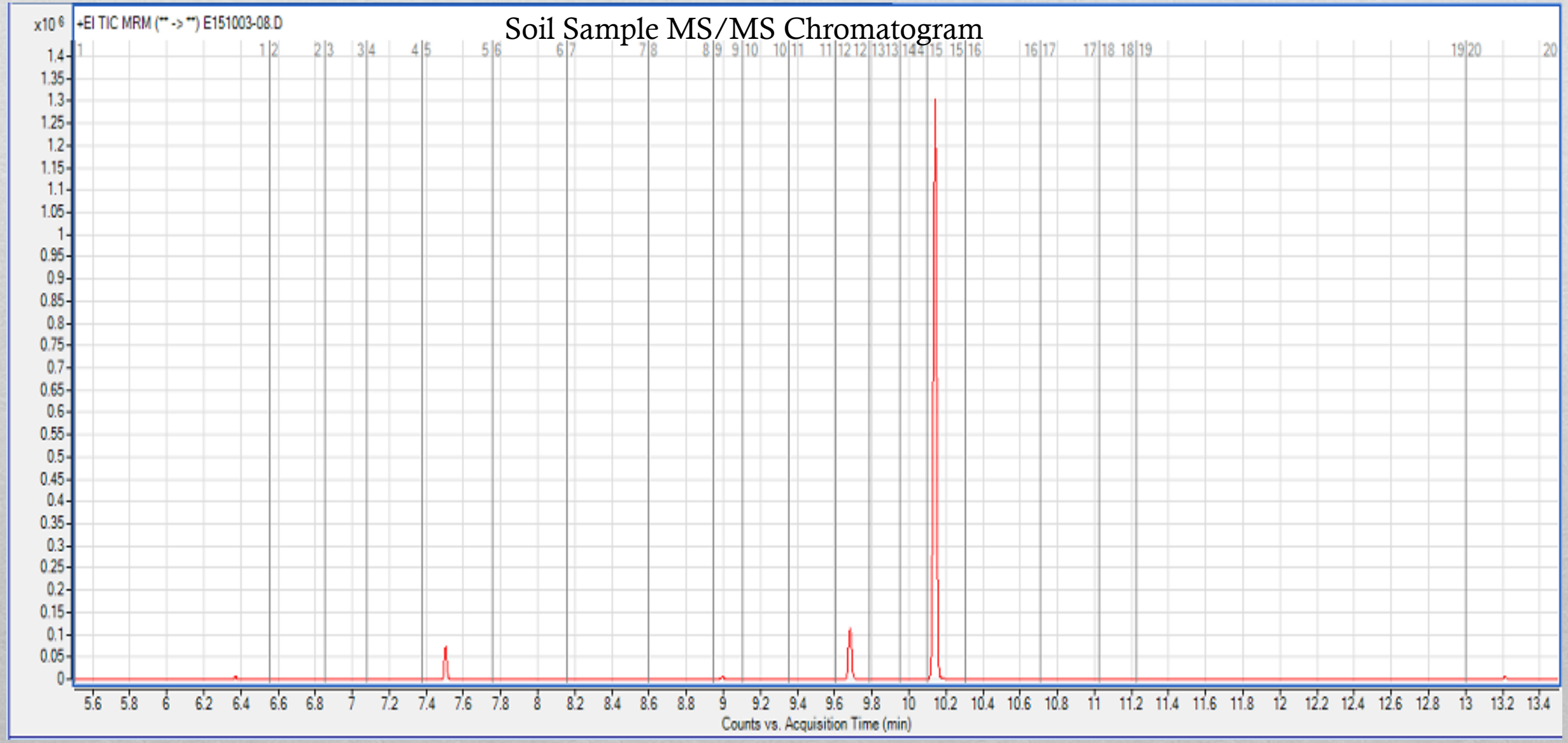


Chemical noise in the chromatography is virtually eliminated, greatly improving S/N and detector sensitivity

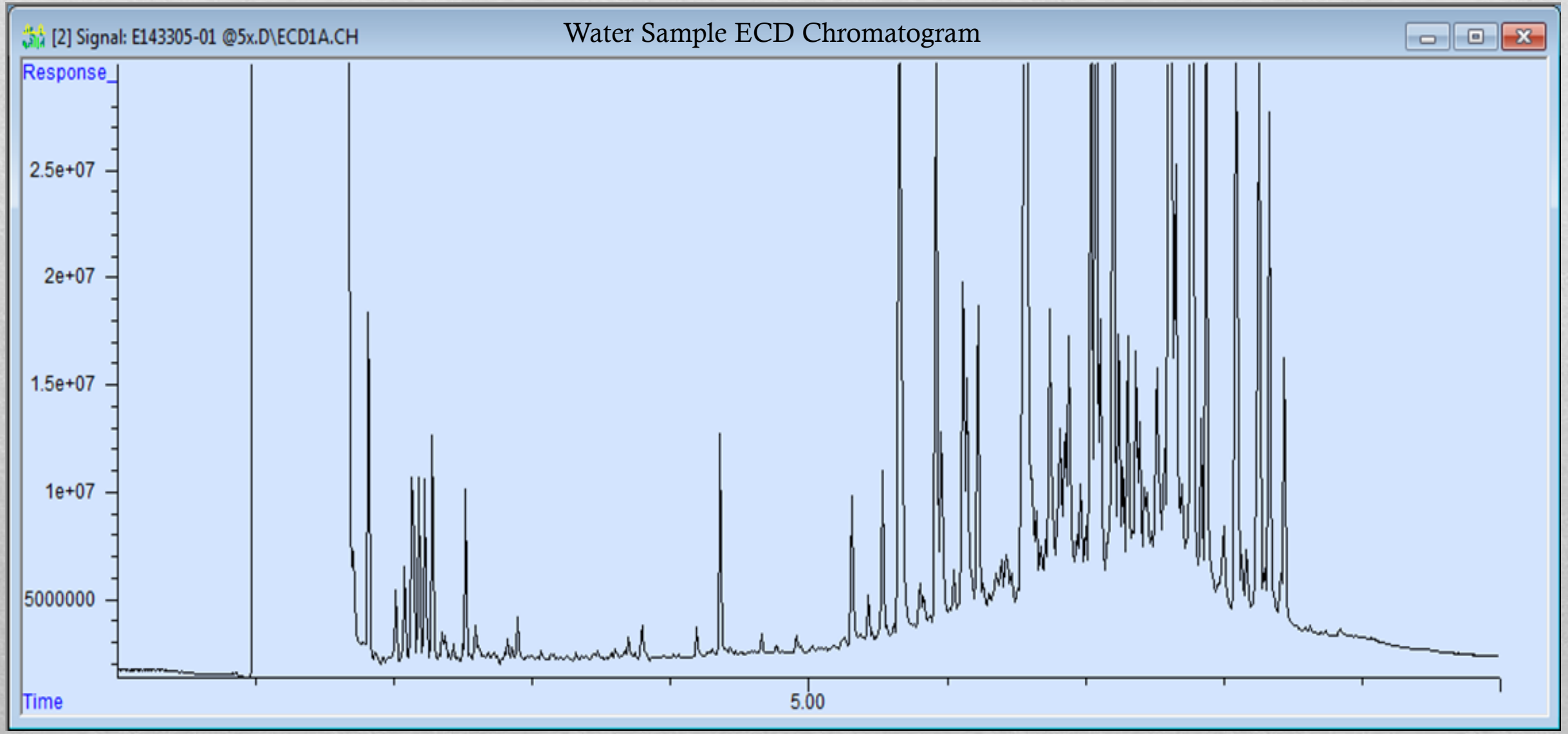
GC/ECD and MSMS Comparison – Soil Sample Extract



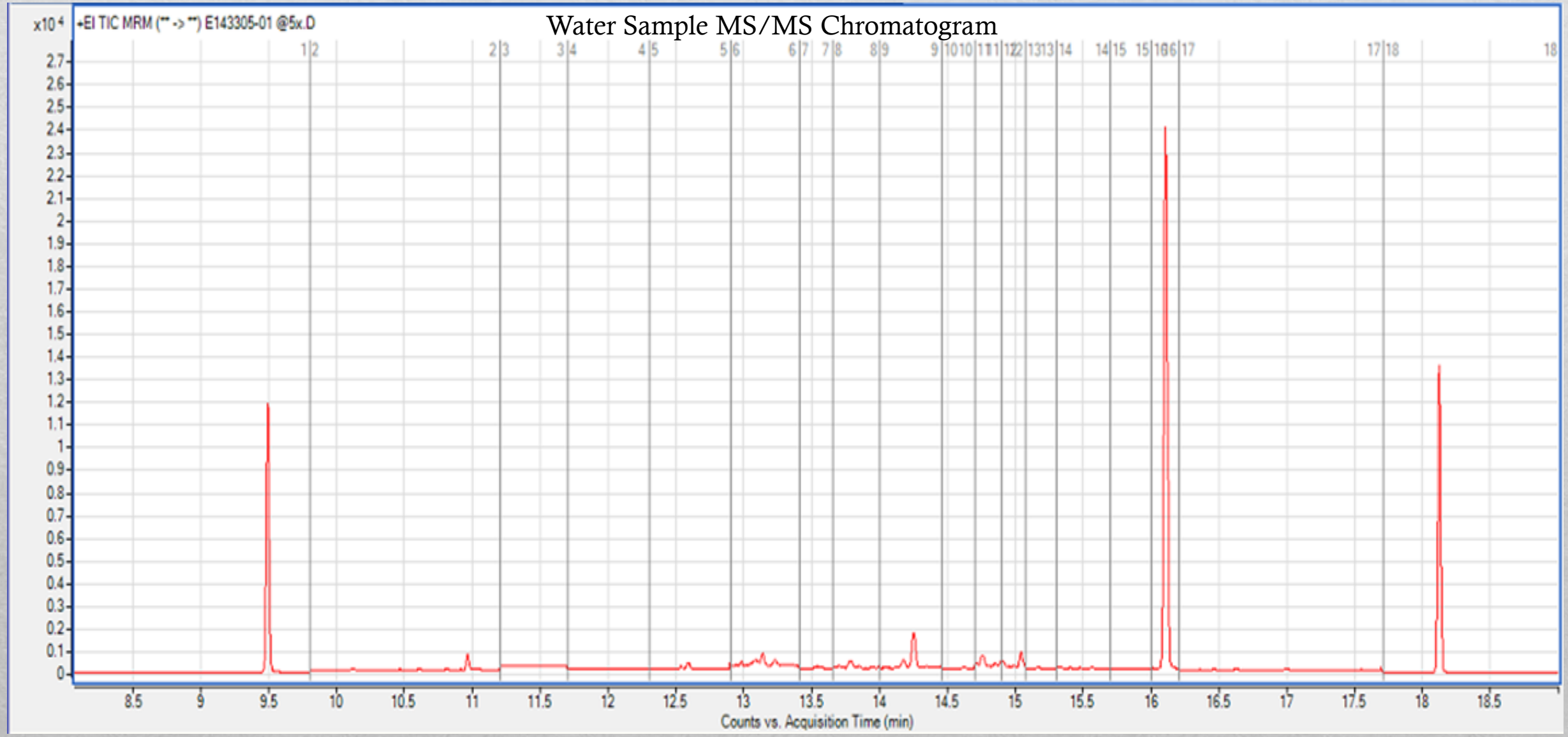
GC/ECD and MSMS Comparison – Soil Sample Extract



GC/ECD and MSMS Comparison – Water Sample Extract



GC/ECD and MSMS Comparison – Water Sample Extract

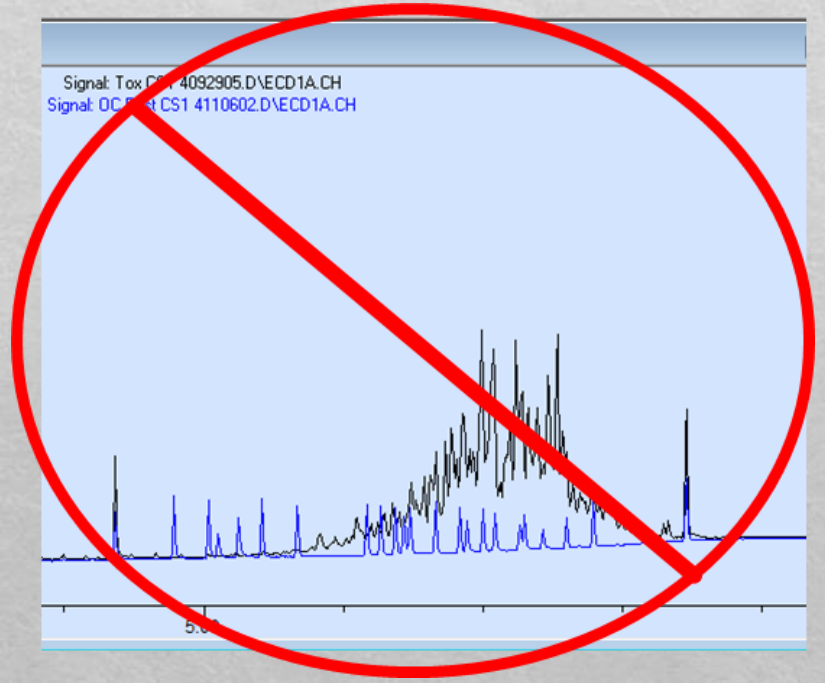


What toxaphene interference?

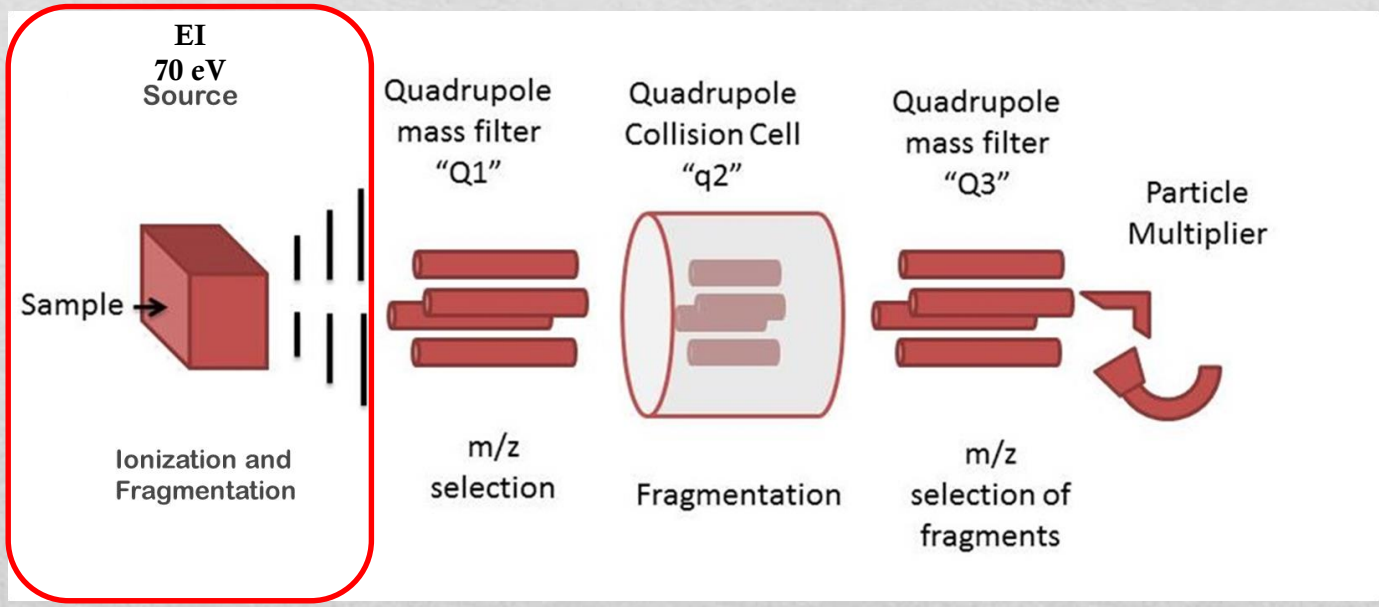
GC/MSMS is able to analyze for toxaphene and all of the single component pesticides… at one time.

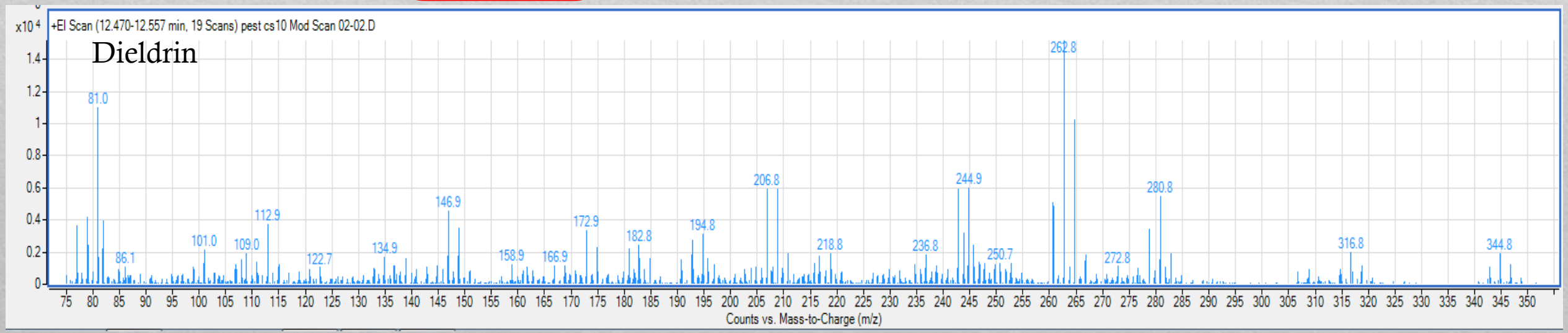
- Through careful selection of precursor and product ions in the MSMS method, interference of toxaphene with the other target pesticides has been eliminated.

- As a result, instrument calibration requires only one set of calibration standards containing all the target compounds, and demonstrates the capability to truly analyze for the entire target list down to the reporting limits in a single analysis.



MSMS Overview

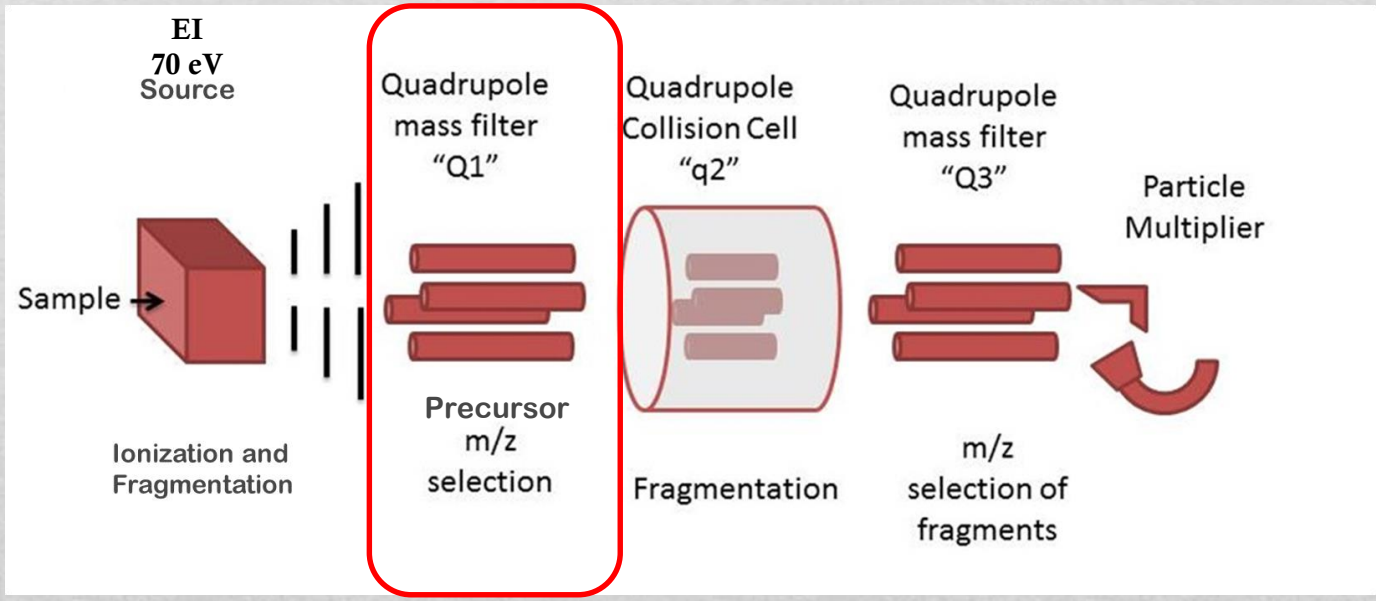


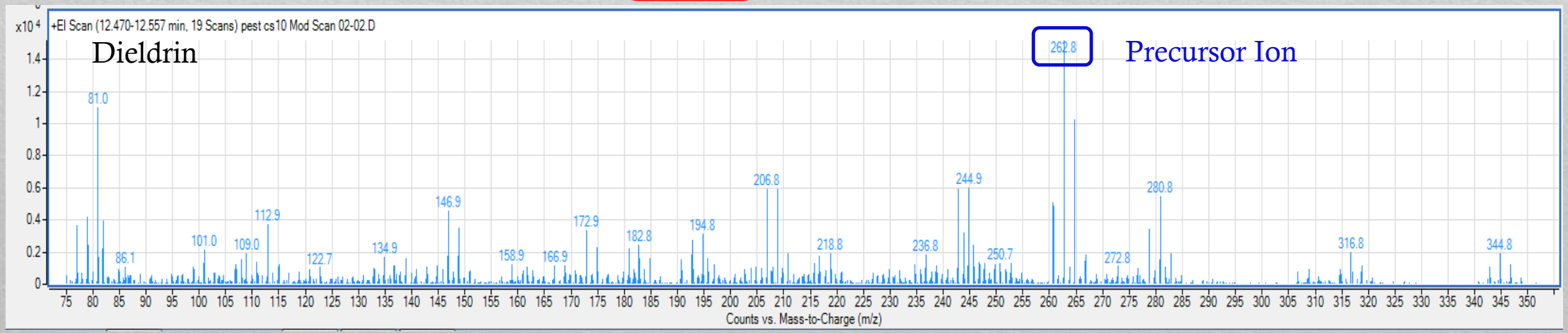


MSMS Overview

Structure Related Selectivity

Q1- Every sample component within the RT window that produces ions of the same m/z as precursors chosen for the target will pass through the mass filter.

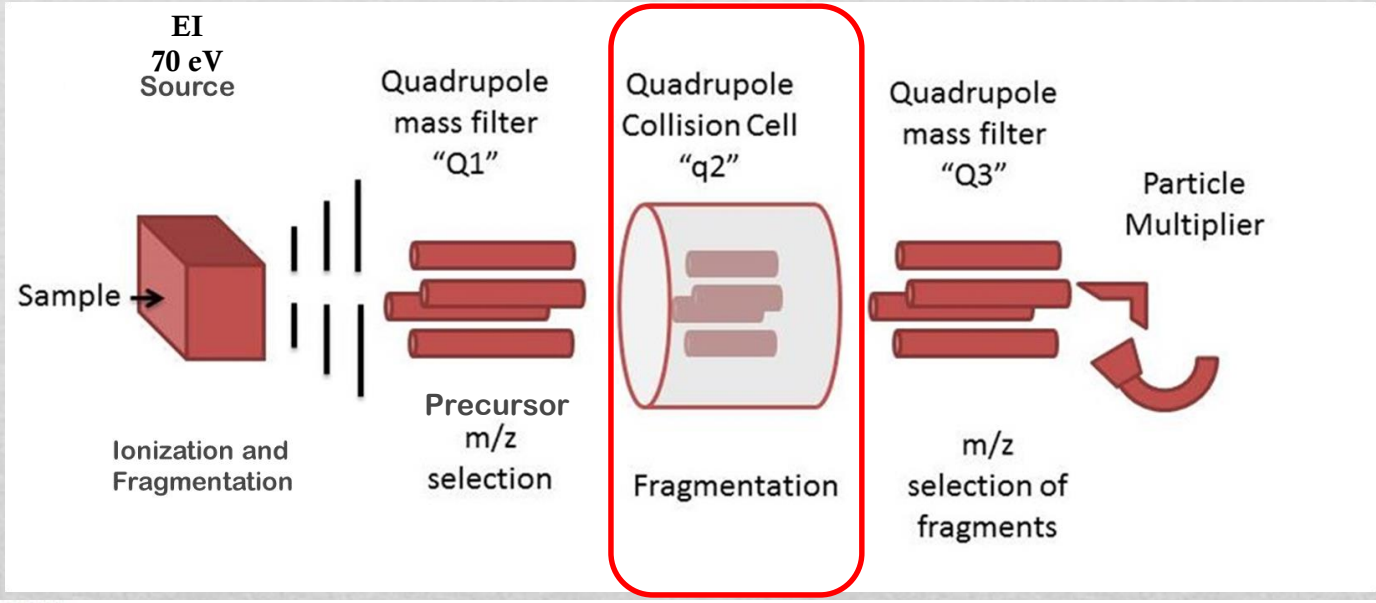


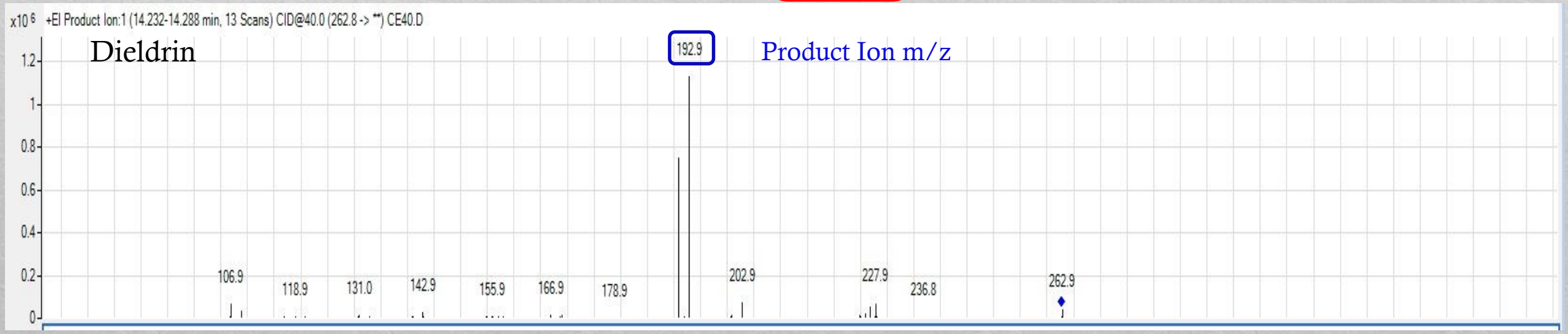


MSMS Overview

Structure Related Selectivity

Collision energies are optimized for each target precursor to generate the greatest abundance of a desired product m/z(s). Precursor ions contributed from different compounds (target vs matrix) will have a unique fragmentation pattern based on their structures.

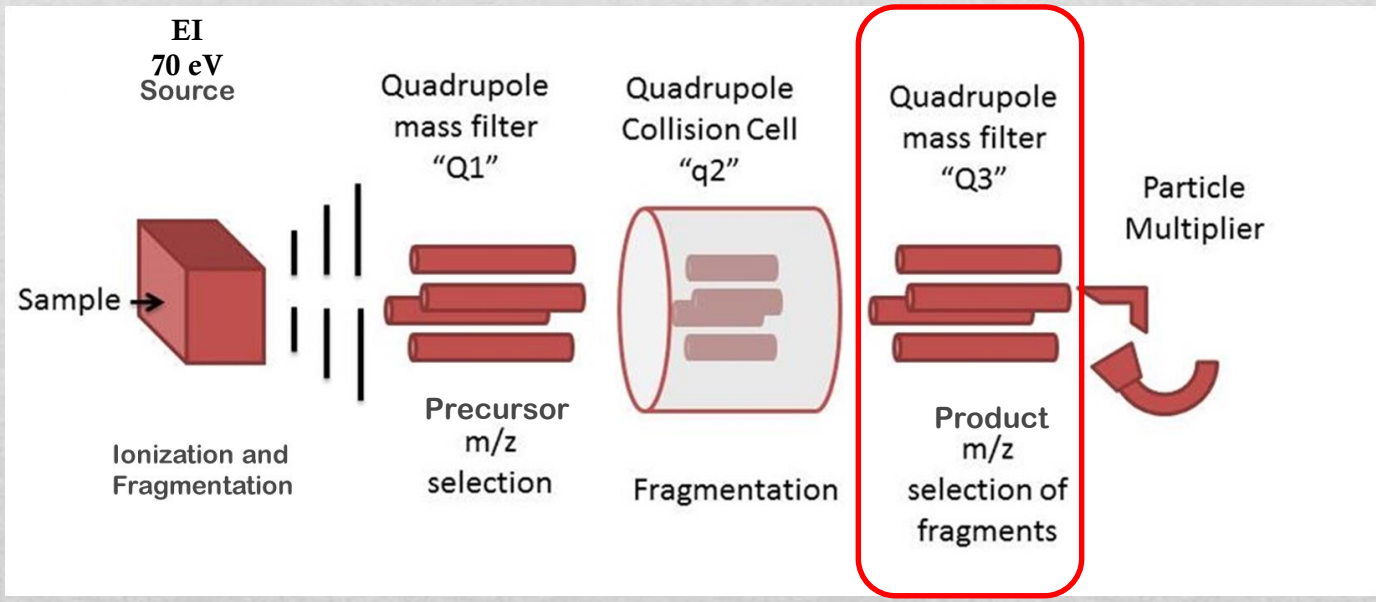


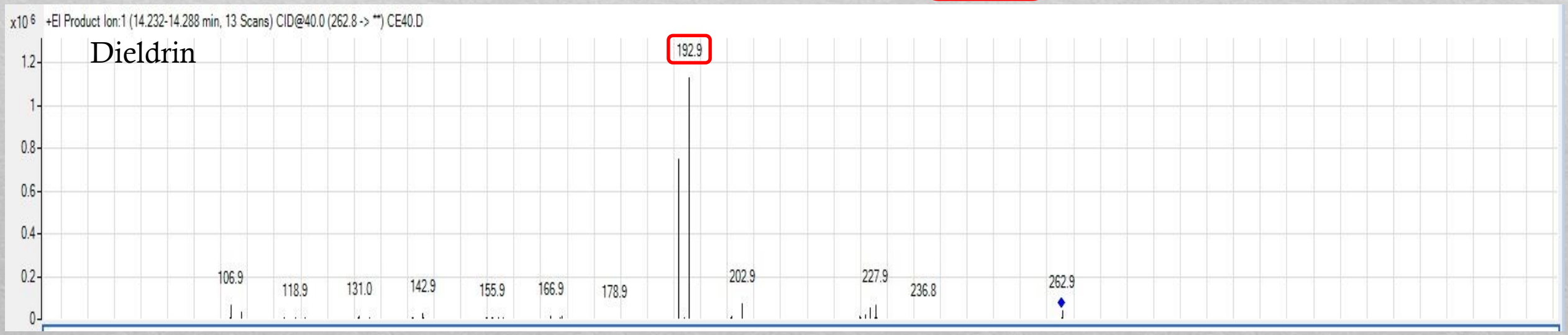


MSMS Overview

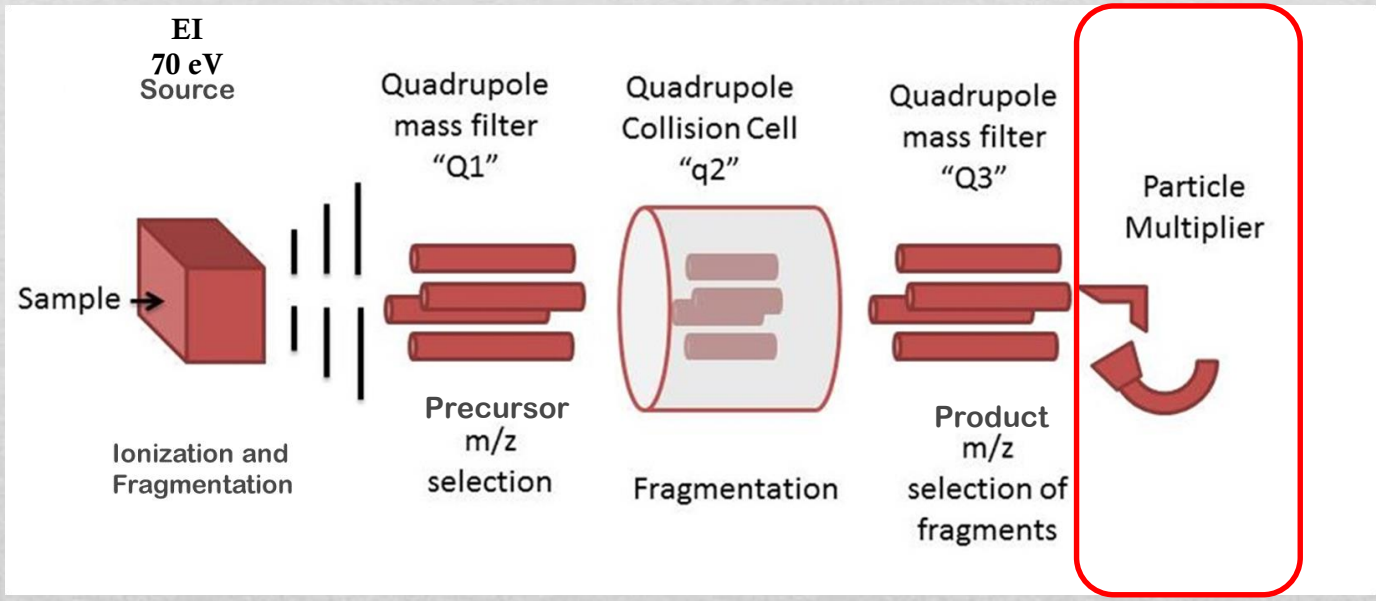
Structure Related Selectivity

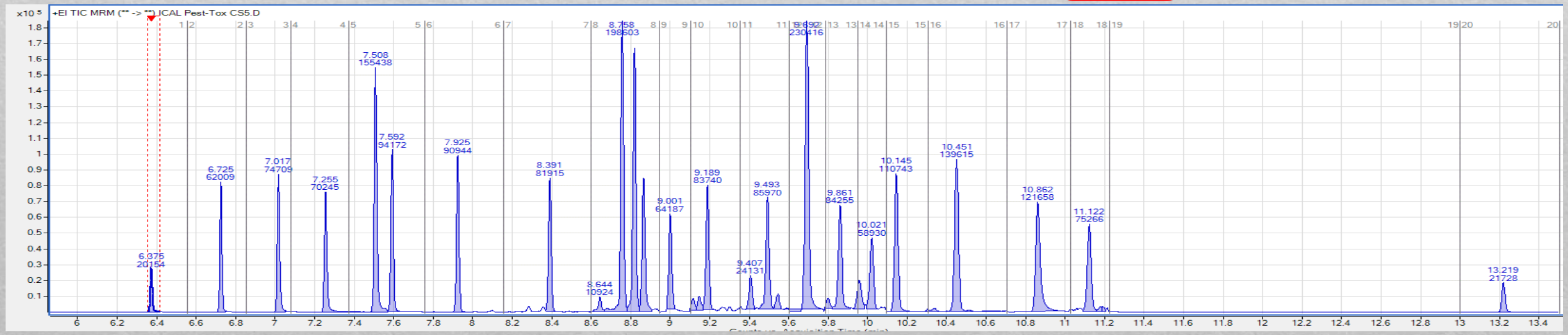
The Q3 mass filter selects for product ions that were determined by the target structure. Therefore, the resultant product ion spectrum is (almost) entirely due to the target precursor ion and not the chemical background.





MSMS Overview





Performance of MSMS Method - Consistency

MSMS method as a confirmatory analysis

- ❖ Since instrumental method development was completed, all samples with reportable levels of any pesticides from ECD analysis have been confirmed by GC/MSMS.
- ❖ MSMS and ECD analyses consistently agree in the identification of target compounds present in samples.
- ❖ MSMS analysis regularly yielded reportable concentrations of additional target pesticides in samples, which were masked in ECD chromatograms by interference.
- ❖ The extent of comparative data gives a high level of confidence in the ability for MS/MS to generate data of equal or higher quality to that of ECD across a variety of sample matrices and project sites.

Performance of MSMS Method - Sensitivity

- With virtually no chemical interferences reaching the detector, S/N is dramatically improved.
- Because there is very little noise to be amplified, increasing the electron multiplier gain or voltage to boost response of poor performing compounds is much more effective, reducing the need to inject larger sample volumes on column, reduce extract volume, etc.
- EM voltage/gain can be independently adjusted for each RT window, which can help to "normalize" responses of target compounds.

Time segments

	Time	Scan type	Electron energy	Delta EMV	Calculated EMV	Gain
1	5.00	MRM			1682.2	100
2	5.70	MRM			1492.7	30
3	6.05	MRM			1515.7	35
4	6.30	MRM			1554.0	45
5	6.53	MRM			1570.3	50
6	6.90	MRM			1623.7	70
7	7.30	MRM			1645.4	80
8	7.70	MRM			1664.7	90
9	8.05	MRM			1515.7	35
10	8.20	MRM			1682.2	100
11	8.44	MRM			1682.2	100
12	8.66	MRM			1570.3	50
13	8.96	MRM			1682.2	100
14	9.08	MRM			1515.7	35
15	9.27	MRM			1623.7	70
16	9.64	MRM			1554.0	45
17	10.20	MRM			1535.9	40
18	10.80	MRM			1682.2	100

Performance of MSMS Method - Linearity

	Compound	2	5	25	50	100	300	500	Avg RF	%RSD
I	13C6 δ-BHC				---------- ISTD ----------					
S	TCMX	1.6113	1.6627	1.7352	1.7696	1.7283	1.7497	1.7287	1.7122	3.234
T	α-BHC	0.8130	0.7484	0.7572	0.7545	0.7241	0.7316	0.7314	0.7515	3.985
T	γ-BHC	1.0290	0.9170	0.9240	0.9486	0.9129	0.9373	0.9388	0.9440	4.196
T	β-BHC	0.8520	0.8070	0.8401	0.8343	0.8257	0.8607	0.8550	0.8392	2.239
T	δ-BHC	1.1980	1.1147	1.0668	1.0501	1.0566	1.1155	1.1421	1.1063	4.817
T	Heptachlor	1.4528	1.5150	1.4677	1.5549	1.5270	1.6622	1.7339	1.5591	6.615
T	Aldrin	1.1103	0.9964	1.0274	1.0006	1.0014	1.0295	1.0323	1.0283	3.818
T	Heptachlor epoxide	1.2852	1.1126	1.1915	1.2333	1.2787	1.3725	1.3984	1.2675	7.859
T	γ-Chlordane	2.5366	2.1210	2.3351	2.3120	2.3755	2.4188	2.4876	2.3695	5.729
T	α-Chlordane	2.2502	1.8944	1.9751	2.0315	2.1146	2.1681	2.1600	2.0849	5.938
T	Endosulfan I	0.8072	0.8667	0.9308	0.9286	0.9313	1.0200	1.0432	0.9325	8.754
I	13C12 4,4'-DDD				---------- ISTD ----------					
T	4,4'-DDE	0.5653	0.5744	0.5200	0.5450	0.5411	0.5238	0.5027	0.5389	4.731
T	Dieldrin	0.5861	0.5111	0.5057	0.5437	0.5399	0.5428	0.5406	0.5385	4.883
T	Endrin	0.4474	0.4704	0.4629	0.5375	0.5007	0.5345	0.5729	0.5038	9.183
T	4,4'-DDD	1.0701	1.0142	1.0141	1.0779	1.0896	1.0776	1.0918	1.0622	3.166
T	Endosulfan II	0.4159	0.5029	0.4357	0.4821	0.4899	0.5162	0.5219	0.4807	8.391
T	Endrin aldehyde	0.2508	0.3328	0.2815	0.3470	0.3367	0.3968	0.4186	0.3378	17.458
T	4,4'-DDT	0.7208	0.8210	0.7636	0.8435	0.8741	0.9099	0.9234	0.8366	8.908
T	Endosulfan sulfate	0.7496	0.7710	0.7270	0.8107	0.8202	0.8373	0.8585	0.7963	6.054
T	p,p'-Methoxychlor	0.4054	0.5036	0.5939	0.6891	0.7101	0.8048	0.8663	0.6533	25.014
T	Endrin ketone	0.3534	0.3530	0.3211	0.3182	0.3713	0.4061	0.4043	0.3610	9.834
T	Toxaphene	0.0590	0.0713	0.0658	0.0733	0.0741	0.0758	0.0756	0.0707	8.752
S	DCB	0.3118	0.3168	0.2934	0.3001	0.2875	0.2762	0.2750	0.2944	5.536

Compound		Curve Fit	Curve Fit Formula	Curve Fit R2
T	Endrin aldehyde	Quadratic	y = 0.020664 * x ^ 2 + 0.322278 * x - 0.001219	0.994737
T	p,p'-Methoxychlor	Quadratic	y = 0.048833 * x ^ 2 + 0.640860 * x - 0.005060	0.997885

(RedFont and #) = Outlier Flag; (I) = Internal Standard; (T) = Target; (S) = Surrogate; (M) = Matrix Spike

Performance of MSMS Method - Accuracy

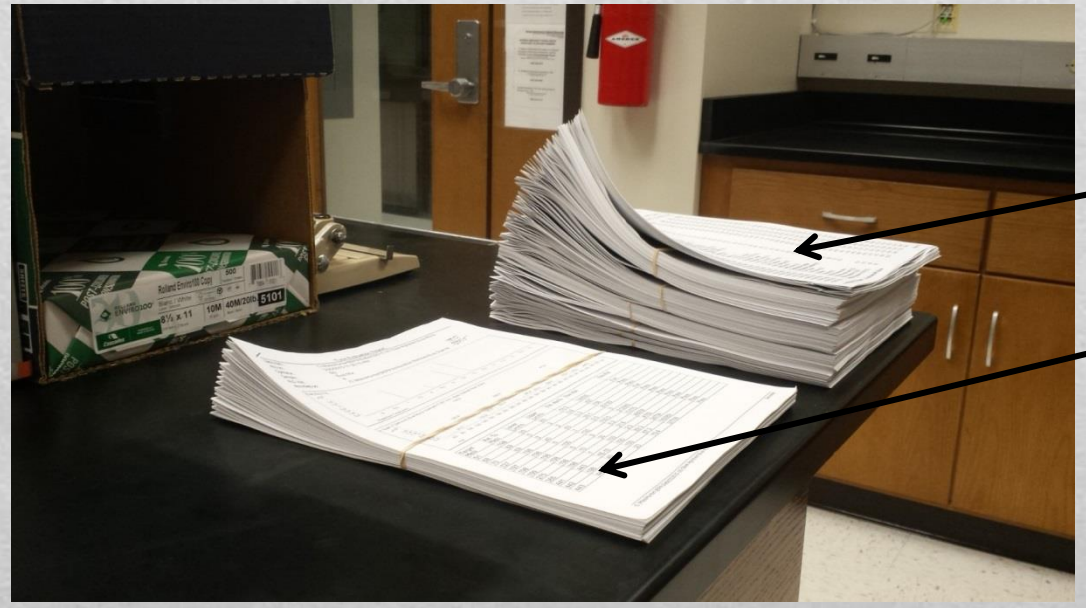
Results of "unofficial" analysis of a pesticide performance test sample (in µg/L).

E113807-13 (WP-200)

Compound	*QQQ*	*True Value*	*%D*	*Acceptance Limits*
alpha-BHC	6.26	6.94	9.75%	2.99 - 9.48
beta-BHC	12.44	12.70	2.02%	5.32 -17.1
gamma-BHC	7.06	7.96	11.26%	3.26 -11.0
delta-BHC	7.03	7.62	7.71%	2.83 - 10.6
Heptachlor	5.33	5.38	1.01%	1.74 - 7.37
Aldrin	3.80	4.69	19.00%	1.33 - 6.48
Heptachlor Epoxide	6.08	6.30	3.43%	3.13 - 8.80
gamma-Chlordane	3.54	3.78	6.35%	1.58 - 5.20
Endosulfan I	3.42	4.24	19.31%	0.920 - 5.82
alpha-Chlordane	1.49	1.40	6.21%	0.616 - 2.05
DDE	4.72	6.36	25.71%	2.82 - 8.24
Dieldrin	7.23	7.06	2.42%	3.45 - 9.61
Endrin	3.28	3.46	5.25%	1.34 - 5.28
DDD	2.86	3.15	9.24%	1.22 - 4.61
Endosulfan II	9.23	10.20	9.54%	3.22 - 13.6
Endrin Aldehyde	6.44	4.96	29.74%	1.26 - 7.84
DDT	2.16	2.38	9.42%	0.895 - 3.51
Endosulfan Sulfate	3.66	4.12	11.09%	1.51 - 6.02
Methoxychlor	8.95	7.73	15.80%	2.03 - 12.3
Endrin Ketone	4.81	5.27	8.67%	2.90 - 7.64

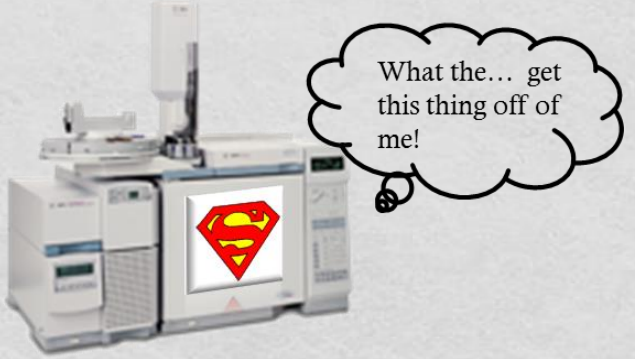
Performance of MSMS Method - Efficiency

Better data quality, easier data processing, fewer calibration standards, less sample dilutions, and a LOT less paper.



GC/MSMS is a great instrument for targeted analyses in dirty samples, **BUT…**

GC/MSMS is Not a Panacea



Regardless of the analytical approach, target analysis of OC pesticides in environmental samples presents significant analytical challenges due to a need to address both trace level reporting limits and complex sample matrices.

- Sample cleanup is still a vital part of generating quality data! The analysis relies on retention time windows, which means that samples still need to be clean enough to chromatograph well, consistently. Interferences are still there, just masked from the detector.
- The reduction in chemical noise is great for targeted analyses, but it comes at a price- the target product ion abundances are all you get. No other spectral or chromatographic information is collected, so suspected issues will require re-analyses to investigate.
- Target sensitivity is low for structurally labile compounds that experience excessive fragmentation upon ionization or collision activated dissociation, particularly with some of the bicyclic pesticides (endrin, dieldrin).
- MRM transition databases are an expensive, quick way to set up an MSMS method, but there is no substitute for running the MRM experiments on the instrument, with the whole target compound list together. Additionally, many compound transitions can be found online for free.

Current Status of GC-MSMS Pesticides Method

❖Initial test method evaluation (ITME) and limit of detection (LOD) studies for all targets compounds was completed for both water and soil matrices in January 2015.

❖A standard operating procedure based on Methods 8270 and 8081 was finalized and posted in June 2015. Analysis of SESD's routine pesticide list by GC/MSMS is now available as an alternative to the Method 8081 ECD analysis.

Future Development of GC/MSMS Pesticides Method

- ❖ Method is scheduled for inclusion in the next round of routine performance test analyses, with the intention of adding to our laboratory's ISO 17025 accreditation in the near future.
- ❖ Evaluating the use of softer chemical ionization for improved instrument sensitivity of labile target compounds
- ❖ Take advantage of the expanded capabilities of GC/EI or GC/CI MSMS to analyzes for any non-halogenated pesticides of interest to Region 4, and more broadly, any compounds of interest to the Region that are amenable to MSMS analysis

Thank You!

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Questions?