GC-MS/MS Approaches for Chlorinated POPs Analysis: What's Next after Dioxins and Furans. Validation and Comparative Data Evaluations

Speaker: Bharat Chandramouli, SGS-AXYS

- **Webinar Starts at 11:45 am Eastern Daylight Time**
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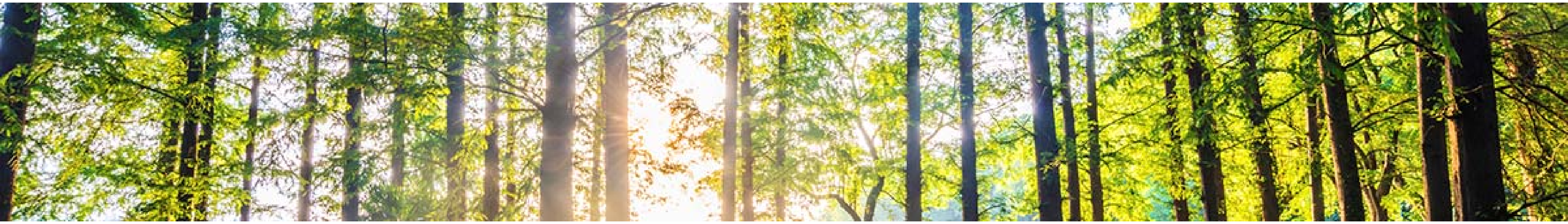

GC-MS/MS Approaches for Chlorinated POPs Analysis: What's Next After Dioxins and Furans. Validation and Comparative Data Evaluation

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SGS AXYS

SGS AXYS

BACKGROUND

1. EPA needs alternatives to GC-HRMS for dioxins
2. EPA, SGS AXYS, Waters and Agilent collaboration
3. Tier 3 Validation of 1613B by SGS AXYS on both platforms
4. Robustness testing on real samples
5. Alternate test procedure submission

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DEVELOPMENT OF A METHOD FOR PCDD/F ANALYSIS BY GC-MS/MS

- Focus only on detector system (MS/MS)
- Verify operating parameters: Instrument set up and control, MRM transitions, product ions ratios
- Confirm QC criteria that are not HRMS-specific, e.g. sensitivity, linearity
- Adapt HR-specific QC Protocols to MS/MS e.g. detector specificity and stability (mass resolution and lock mass) monitoring, qualitative identification criteria
- Investigate potential interferences – PCBs, chlorodiphenyl ethers

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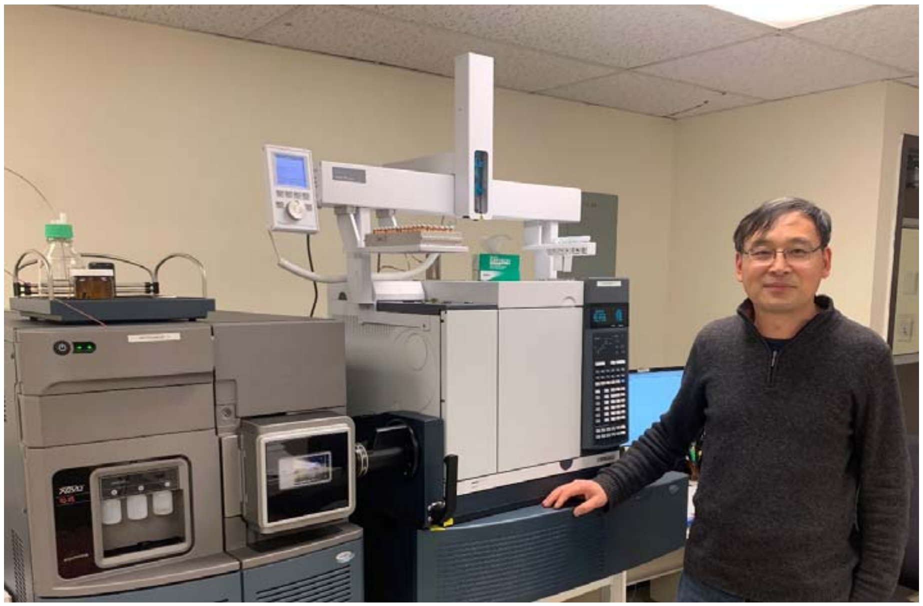
THE INSTRUMENT: WATERS APGC-XEVO TQ-XS



Lead development scientist Xinhui Xie with the Waters APGC-Xevo TQ-XS

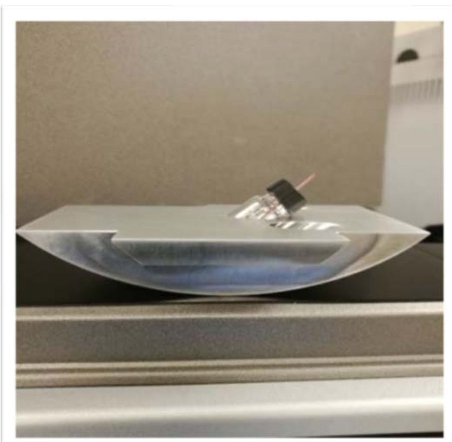
GC: Atmospheric pressure chemical ionization source

- Low energy (20 to 30 V collision energy)
- Less fragmentation
- More sensitivity and selectivity than an EI source

MS/MS: Xevo TQ-XS

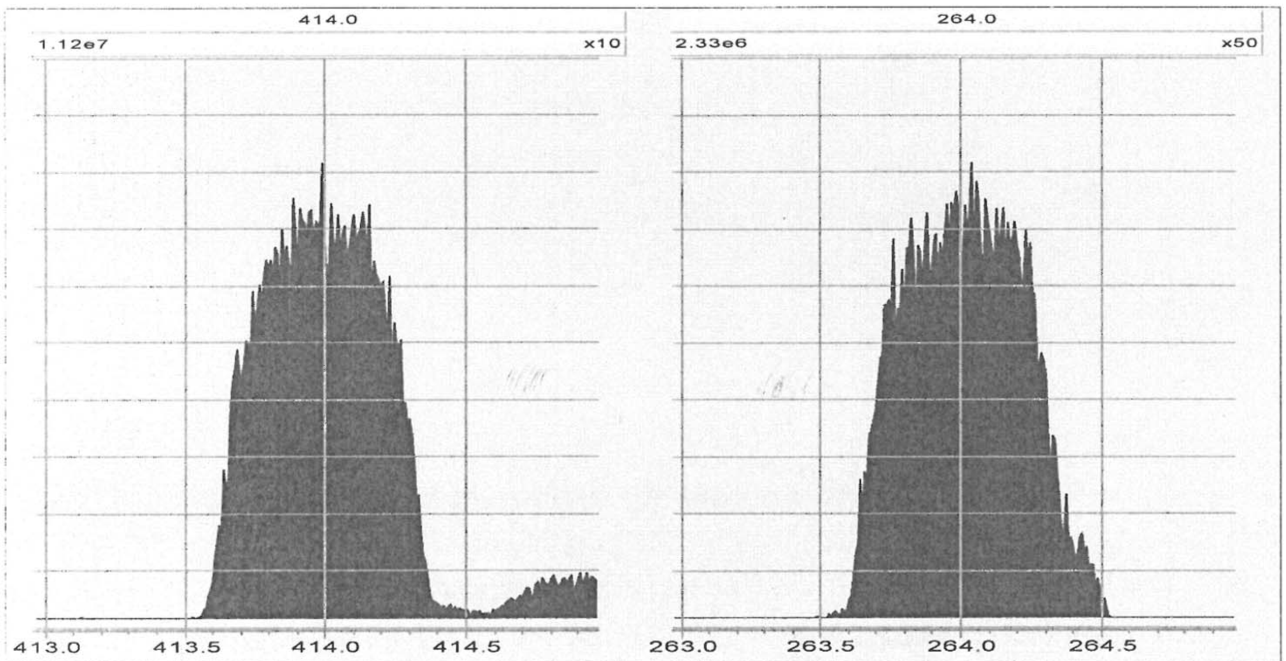
- High sensitivity
- Large linear range
- Tandem quadrupole detection system optimizes selectivity

USE OF A REFERENCE COMPOUND TO MONITOR DETECTOR RESPONSE STABILITY



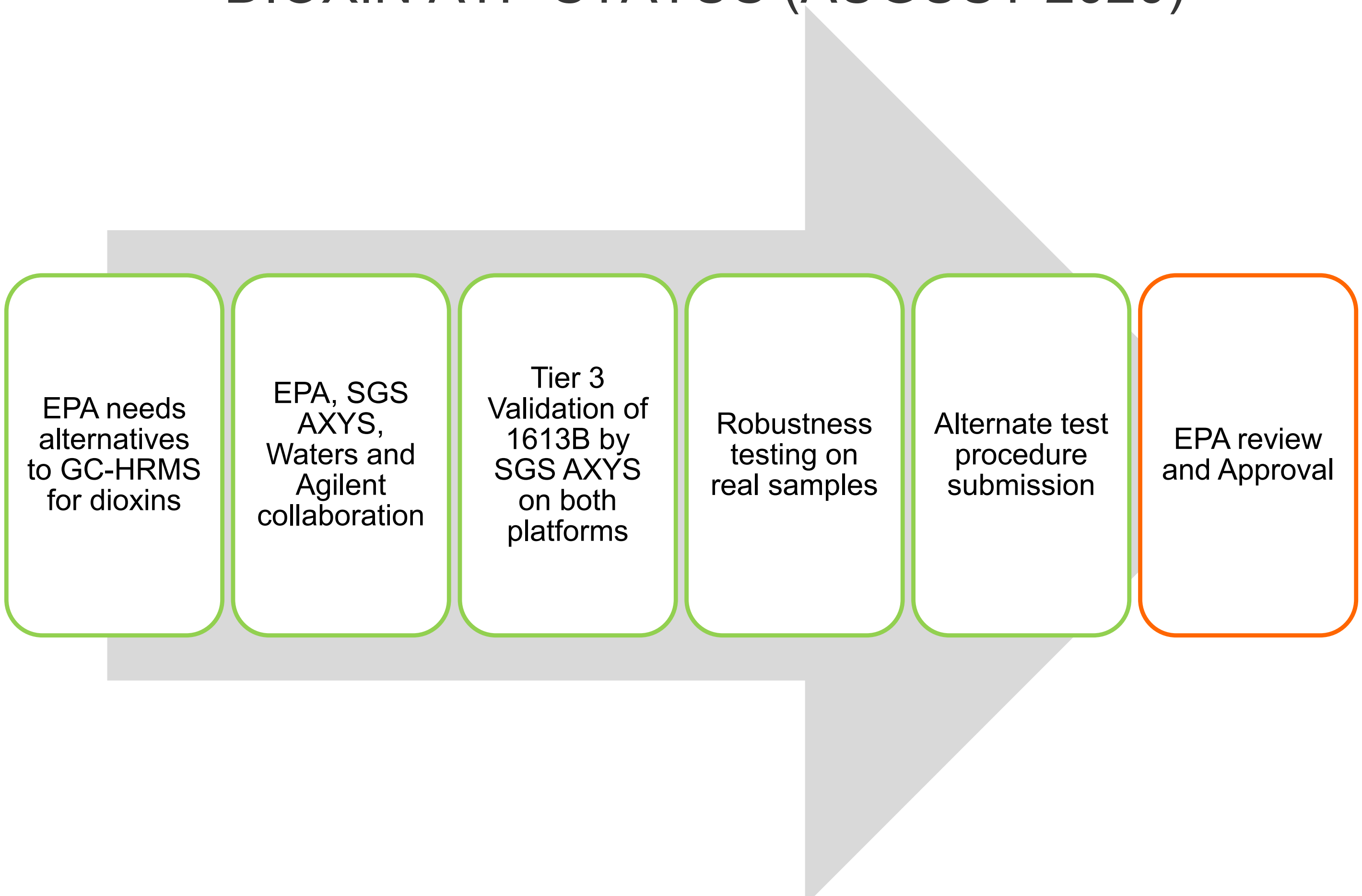
- To detect suppression of detector response by sample matrix – replacement for HRMS lock mass monitoring requirement
- Bleed a reference compound into the detector system throughout run and monitor its response. Expect it to be constant
- Select PFTBA (the tuning compound). Large number of masses available
- Select 414.0 > 264.0 transition due to good response and appropriate mass range. Monitor 264.0 response continuously

MASS RESOLUTION AND MASS ACCURACY



- Demonstrate unit mass resolution and mass accuracy on an on-going basis.
- How: PFTBA transition 414.0 > 264.0. Scan MS1 and MS2
- Every 12 hours
- Criteria for resolution: resolution of 414.0 from 415.0 and peak width at base is 1 ± 0.2 amu

DIOXIN ATP STATUS (AUGUST 2020)



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STUDY OBJECTIVES

- Build on our 2019 development and validation alternate method on for PCDD/PCDF using tandem quadrupole mass spectrometry (MS/MS) rather than high resolution mass spectrometry (HRMS)
- Validate alternate procedure to EPA 1668
- Validate alternate procedure for high-sensitivity chlorinated pesticides

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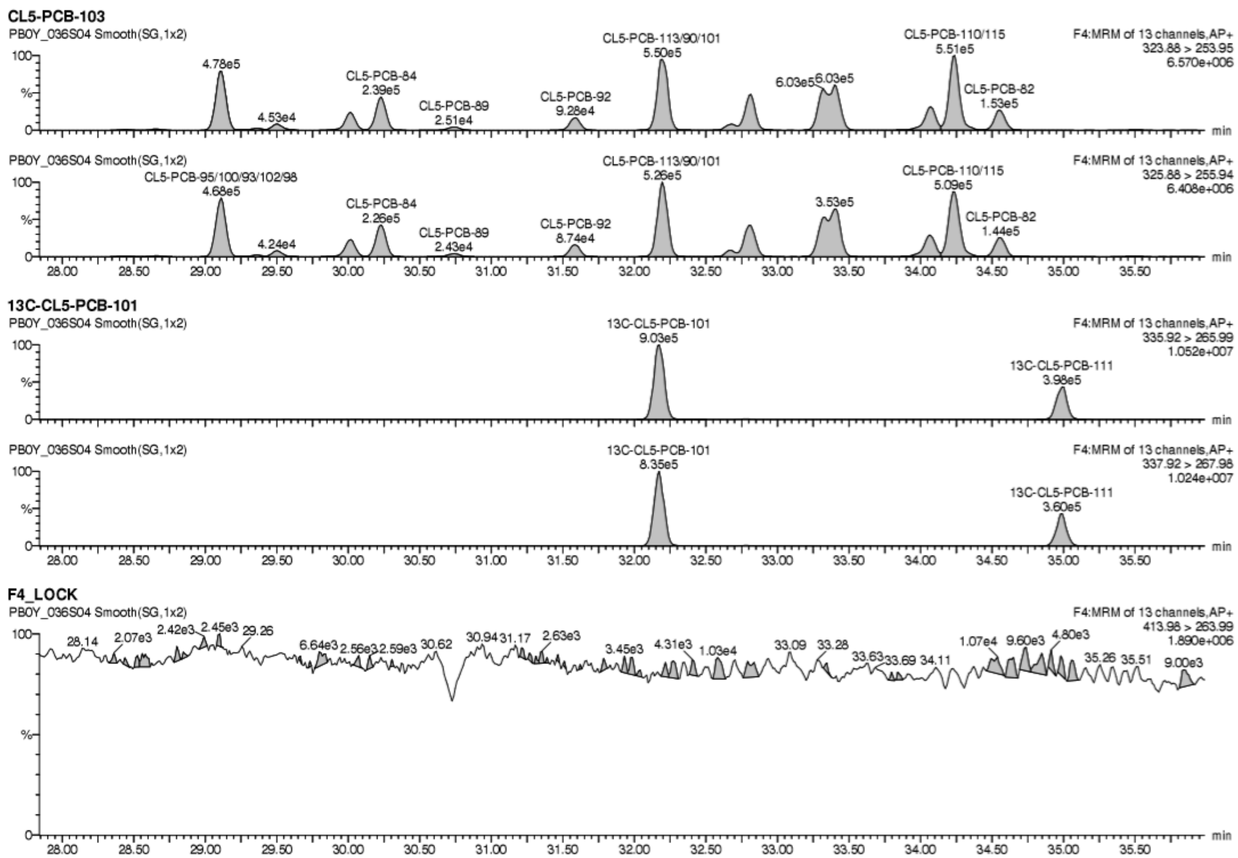
PCB METHOD DEVELOPMENT AND VALIDATION

- No changes needed to lab extraction and cleanup procedures
- No changes on GC acquisition protocols as validated for EPA 1668
- All new MS-MS instrument protocol development for primary and secondary precursor and products
- Typical validation package with calibration, Initial Demonstrations of Capability, MDLs and real sample analysis
- Accreditation with NELAP and ISO-17025

Compound	Primary MRM Transition Precursor Ion	Primary MRM Transition Product Ion	Transition (Quan Trace)	Secondary MRM Transition Precursor Ion	Secondary MRM Transition Product Ion	Transition (Target Trace)
MCB	$C_{12}H_9Cl$ (m)	$C_{12}H_9$ (m)	188.0 > 153.0	$C_{12}H_9{}^{37}Cl$ (m+2)	$C_{12}H_9$ (m)	190.0 > 153.0
DiCB	$C_{12}H_8Cl_2$ (m)	$C_{12}H_8$ (m)	222.0 > 152.1	$C_{12}H_8{}^{35}Cl^{37}Cl$ (m+2)	$C_{12}H_8$ (m)	224.0 > 152.1
TrCB	$C_{12}H_7Cl_3$ (m)	$C_{12}H_7Cl$ (m)	256.0 > 186.0	$C_{12}H_7{}^{35}Cl_2{}^{37}Cl$ (m+2)	$C_{12}H_7Cl$ (m)	258.0 > 186.0
TeCB	$C_{12}H_6Cl_4$ (m)	$C_{12}H_6Cl_2$ (m)	289.9 > 220.0	$C_{12}H_6{}^{35}Cl_3{}^{37}Cl$ (m+2)	$C_{12}H_6{}^{35}Cl^{37}Cl$ (m+2)	291.9 > 222.0
PeCB	$C_{12}H_5Cl_5$ (m)	$C_{12}H_5Cl_3$ (m)	323.9 > 254.0	$C_{12}H_5{}^{35}Cl_4{}^{37}Cl$ (m+2)	$C_{12}H_5{}^{35}Cl_2{}^{37}Cl$ (m+2)	325.9 > 255.9
HxCB	$C_{12}H_4{}^{35}Cl_5{}^{37}Cl$ (m+2)	$C_{12}H_4{}^{35}Cl_3{}^{37}Cl$ (m+2)	359.8 > 289.9	$C_{12}H_4Cl_6$ (m)	$C_{12}H_4Cl_4$ (m)	357.8 > 287.9
HpCB	$C_{12}H_3{}^{35}Cl_6{}^{37}Cl$ (m+2)	$C_{12}H_3{}^{35}Cl_4{}^{37}Cl$ (m+2)	393.8 > 323.9	$C_{12}H_3{}^{35}Cl_5{}^{37}Cl_2$ (m+4)	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2$ (m+4)	395.8 > 325.9
OCB	$C_{12}H_2{}^{35}Cl_7{}^{37}Cl$ (m+2)	$C_{12}H_2{}^{35}Cl_5{}^{37}Cl$ (m+2)	427.8 > 357.8	$C_{12}H_2{}^{35}Cl_6{}^{37}Cl_2$ (m+4)	$C_{12}H_3{}^{35}Cl_4{}^{37}Cl_2$ (m+4)	429.8 > 359.8
NCB	$C_{12}H^{35}Cl_8{}^{37}Cl$ (m+2)	$C_{12}H^{35}Cl_6{}^{37}Cl$ (m+2)	461.7 > 391.8	$C_{12}H^{35}Cl_7{}^{37}Cl_2$ (m+4)	$C_{12}H^{35}Cl_5{}^{37}Cl_2$ (m+4)	463.7 > 393.8
DeCB	$C_{12}{}^{35}Cl_8{}^{37}Cl_2$ (m+4)	$C_{12}H^{35}Cl_6{}^{37}Cl_2$ (m+4)	497.7 > 427.7	$C_{12}H^{35}Cl_7{}^{37}Cl_3$ (m+6)	$C_{12}H^{35}Cl_5{}^{37}Cl_3$ (m+6)	499.7 > 429.7
13C12-MCB	${}^{13}C_{12}H_9Cl$ (m)	${}^{13}C_{12}H_9$ (m)	200.1 > 165.1	${}^{13}C_{12}H_9{}^{37}Cl$ (m+2)	${}^{13}C_{12}H_9$ (m)	202.1 > 165.1
13C12-DiCB	${}^{13}C_{12}H_8Cl_2$ (m)	${}^{13}C_{12}H_8$ (m)	234.0 > 164.1	${}^{13}C_{12}H_8{}^{35}Cl^{37}Cl$ (m+2)	${}^{13}C_{12}H_8$ (m)	236.0 > 164.1
13C12-TrCB	${}^{13}C_{12}H_7Cl_3$ (m)	${}^{13}C_{12}H_7Cl$ (m)	268.0 > 198.1	${}^{13}C_{12}H_7{}^{35}Cl_2{}^{37}Cl$ (m+2)	${}^{13}C_{12}H_7Cl$ (m)	270.0 > 198.1
13C12-TeCB	${}^{13}C_{12}H_6Cl_4$ (m)	${}^{13}C_{12}H_6Cl_2$ (m)	301.9 > 232.0	${}^{13}C_{12}H_6{}^{35}Cl_3{}^{37}Cl$ (m+2)	${}^{13}C_{12}H_6{}^{35}Cl^{37}Cl$ (m+2)	304.0 > 234.0
13C12-PeCB	${}^{13}C_{12}H_5Cl_5$ (m)	${}^{13}C_{12}H_5Cl_3$ (m)	335.9 > 266.0	${}^{13}C_{12}H_5{}^{35}Cl_4{}^{37}Cl$ (m+2)	${}^{13}C_{12}H_5{}^{35}Cl_2{}^{37}Cl$ (m+2)	337.9 > 268.0
13C12-HxCB	${}^{13}C_{12}H_4{}^{35}Cl_5{}^{37}Cl$ (m+2)	${}^{13}C_{12}H_4{}^{35}Cl_3{}^{37}Cl$ (m+2)	371.9 > 301.9	${}^{13}C_{12}H_4Cl_6$ (m)	${}^{13}C_{12}H_4Cl_4$ (m)	369.9 > 300.0
13C12-HpCB	${}^{13}C_{12}H_3{}^{35}Cl_6{}^{37}Cl$ (m+2)	${}^{13}C_{12}H_3{}^{35}Cl_4{}^{37}Cl$ (m+2)	405.8 > 335.9	${}^{13}C_{12}H_3{}^{35}Cl_5{}^{37}Cl_2$ (m+4)	${}^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2$ (m+4)	407.8 > 337.9
13C12-OCB	${}^{13}C_{12}H_2{}^{35}Cl_7{}^{37}Cl$ (m+2)	${}^{13}C_{12}H_2{}^{35}Cl_5{}^{37}Cl$ (m+2)	439.8 > 369.9	${}^{13}C_{12}H_2{}^{35}Cl_6{}^{37}Cl_2$ (m+4)	${}^{13}C_{12}H_3{}^{35}Cl_4{}^{37}Cl_2$ (m+4)	441.8 > 371.9
13C12-NCB	${}^{13}C_{12}H^{35}Cl_8{}^{37}Cl$ (m+2)	${}^{13}C_{12}H^{35}Cl_6{}^{37}Cl$ (m+2)	473.8 > 403.8	${}^{13}C_{12}H^{35}Cl_7{}^{37}Cl_2$ (m+4)	${}^{13}C_{12}H^{35}Cl_5{}^{37}Cl_2$ (m+4)	475.8 > 405.8
13C12-DeCB	${}^{13}C_{12}{}^{35}Cl_8{}^{37}Cl_2$ (m+4)	${}^{13}C_{12}H^{35}Cl_6{}^{37}Cl_2$ (m+4)	509.7 > 439.8	${}^{13}C_{12}H^{35}Cl_7{}^{37}Cl_3$ (m+6)	${}^{13}C_{12}H^{35}Cl_5{}^{37}Cl_3$ (m+6)	511.7 > 441.8

TYPICAL PCB CHROMATOGRAM

Quantitation based on sum of transitions



← Transition 1

← Transition 2

← Surr Transition 1

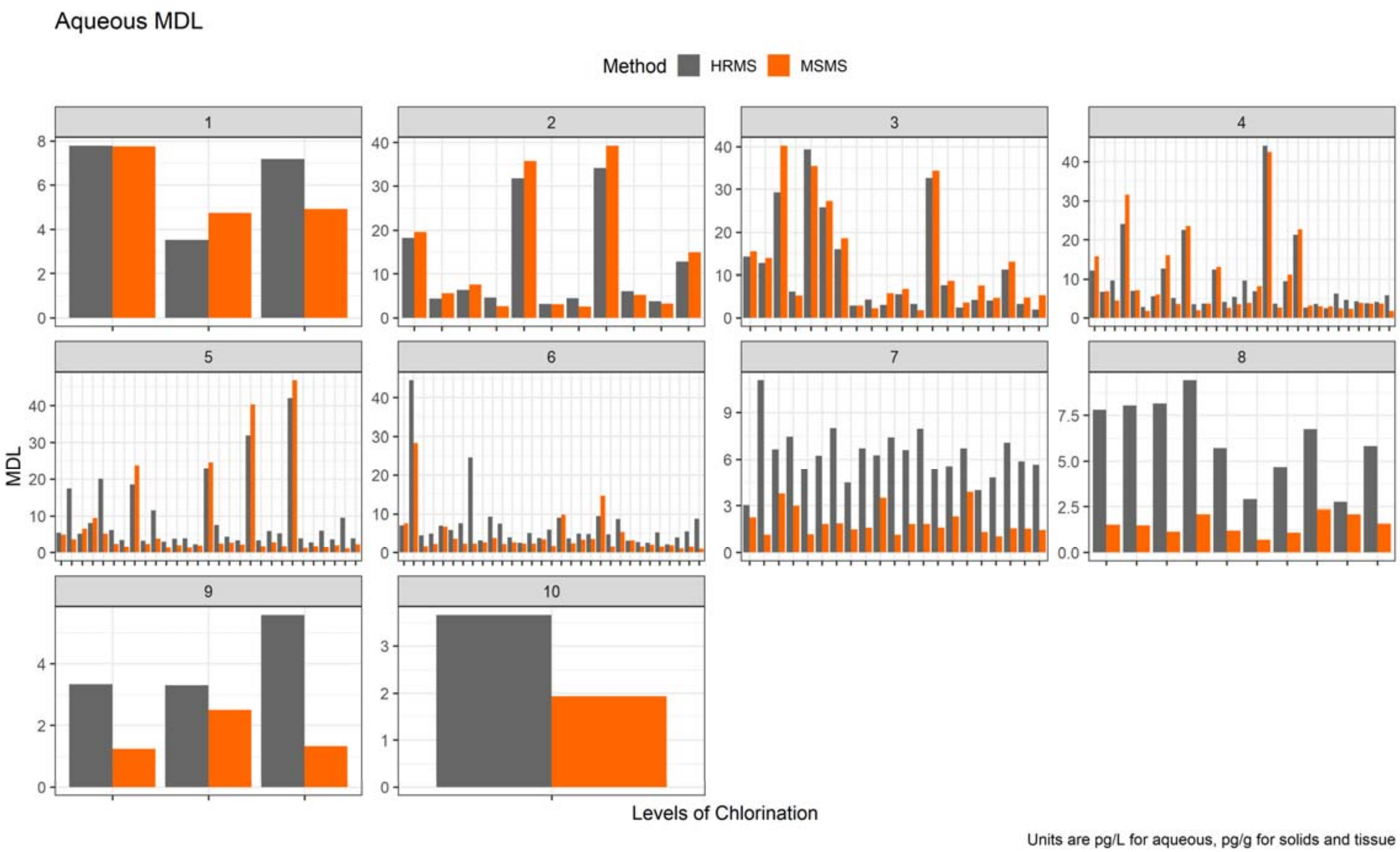
← Surr Transition 2

← Lock mass

CALIBRATION AND INITIAL VALIDATION

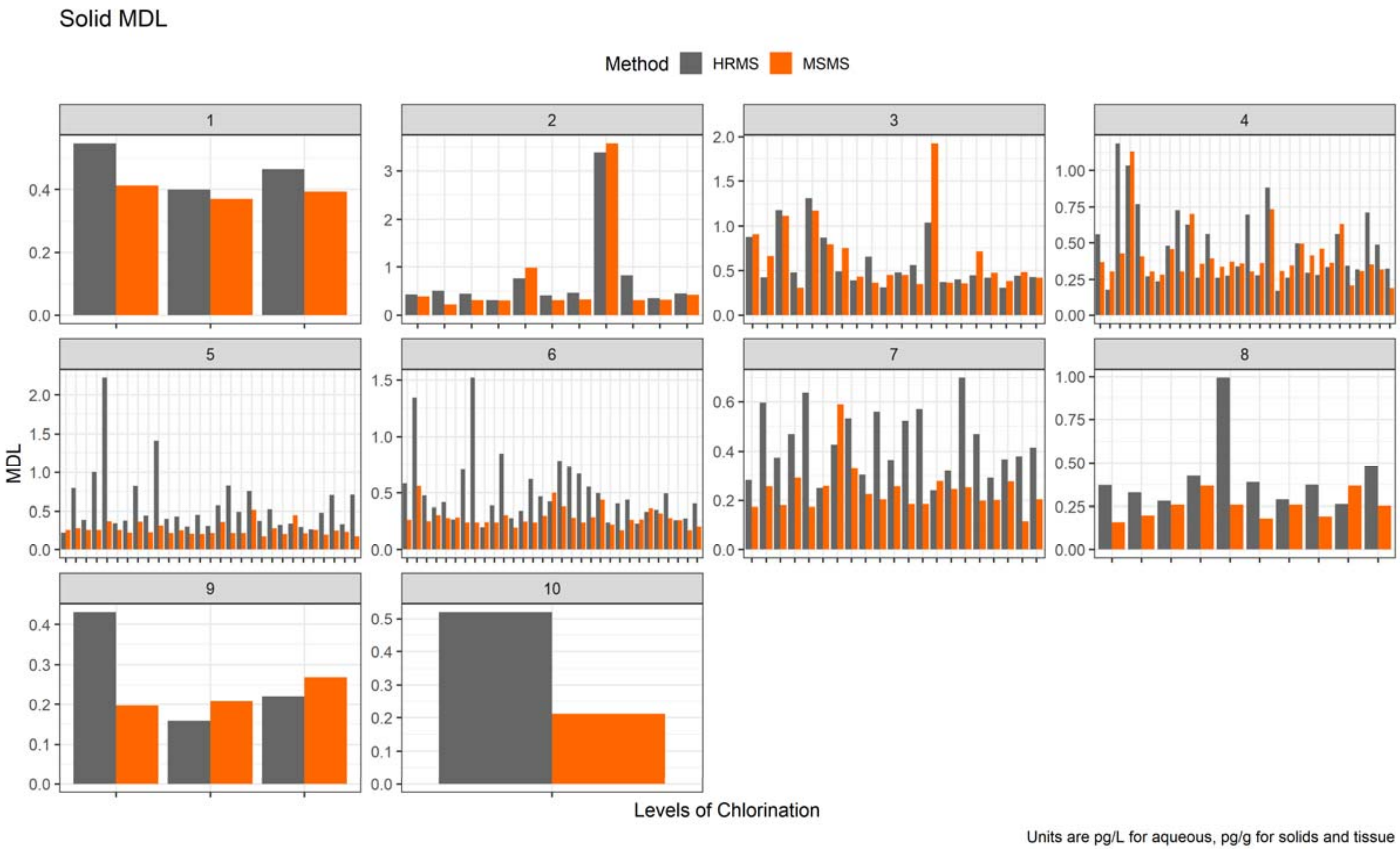
- 0.2 ng/mL – 6000 ng/mL (2000 for mono- and di-chlorinated): Same as GC-HRMS
- Calibrations meet this linear range comfortably
- Initial validations with clean matrices for water, solids and tissue were routine, no issues encountered, met all 1668 specifications
- Methods currently accredited under ISO-17025 (CALA) and NELAP TNI (Fl DOH)

AQUEOUS MDL (PG/L)



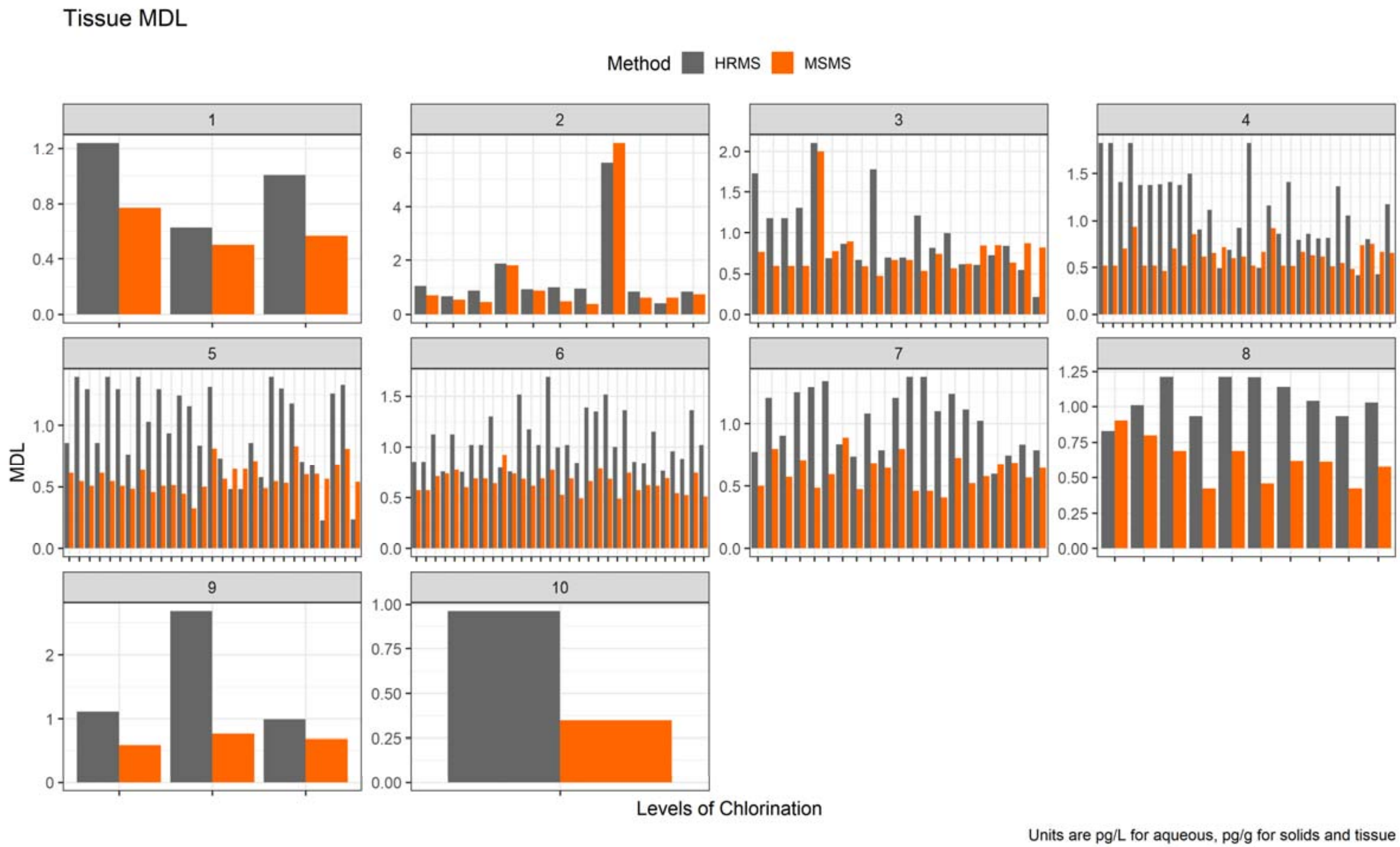
- Most PCBs, especially lower chlorinated (<Cl6) tend to be blank limited rather than sensitivity limited
- Superior instrument sensitivity evident Cl6 and onwards as background is less of a concern

SOLID MDL (PG/G)



- Similar to the aqueous MDL on trends, overall, about 30% better than the GC-HRMS

TISSUE MDL (PG/G)



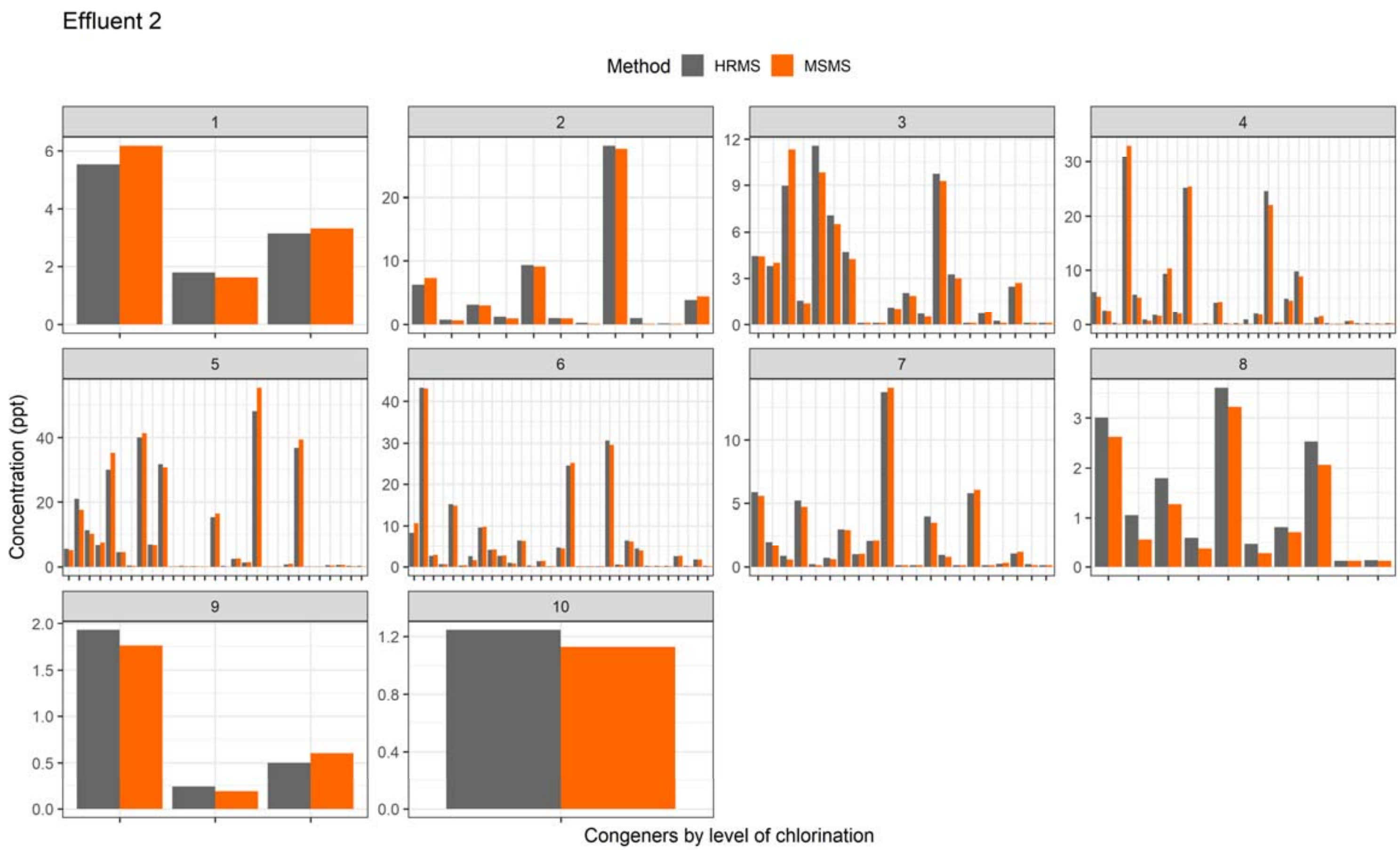
- Tissue backgrounds are lower, so sensitivity improvements evident Cl4 onwards
- Ability to get actionable data from limited tissue sample key is a key differentiator

PCB SAMPLE COMPARISONS

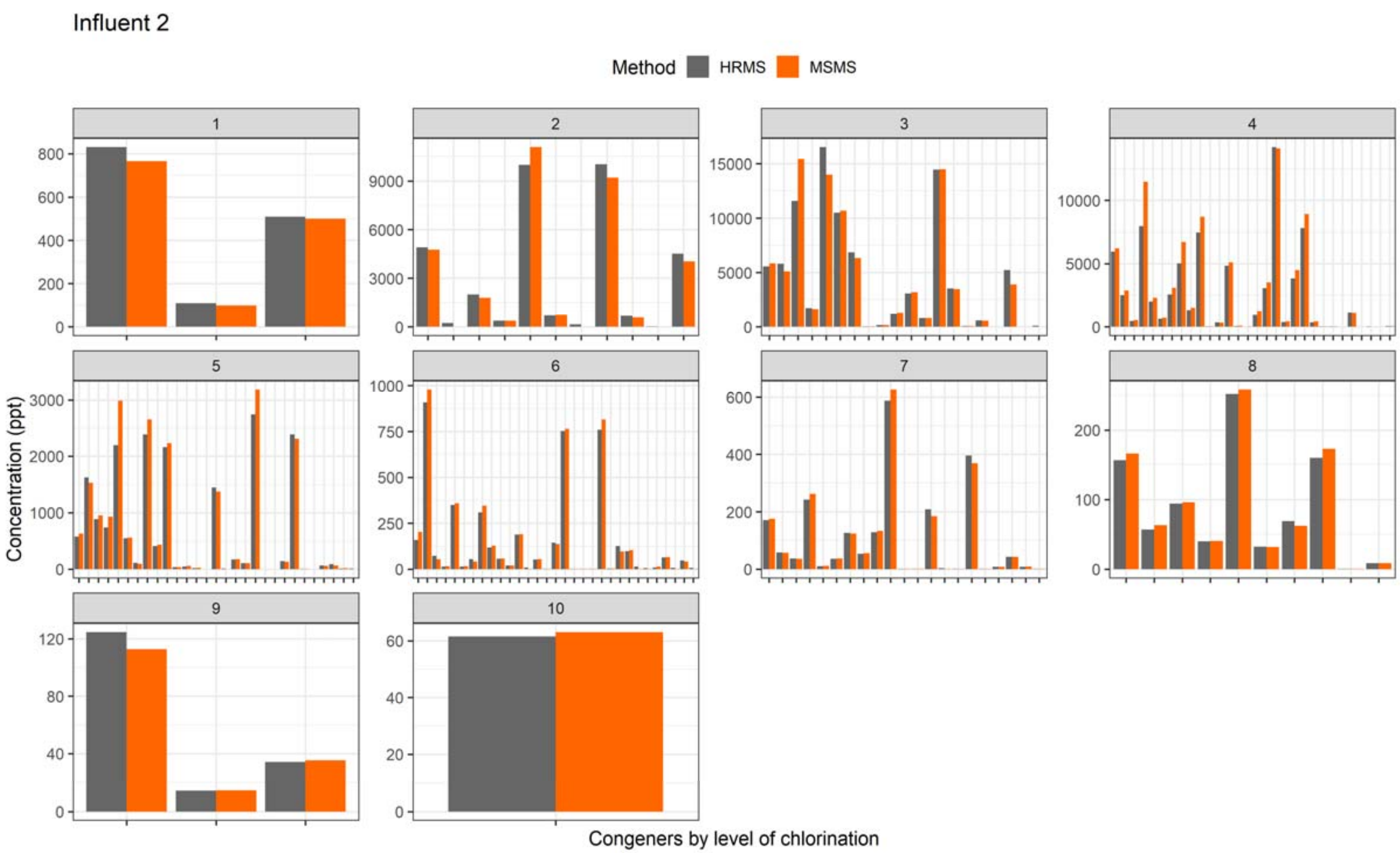
Matrix	#
WWTP Influent	2
WWTP Effluent	2
WWTP biosolids	1
Whale poop	1
Tissue	2
Serum	2

- Pick sample extracts within the past year extracted by EPA method 1668 GC-HRMS
- Re-run extracts by GC-MS/MS
- Except extract hold time, all other factors identical

COMPARATIVE DATA – EFFLUENT EXAMPLE



WWTP INFLUENT



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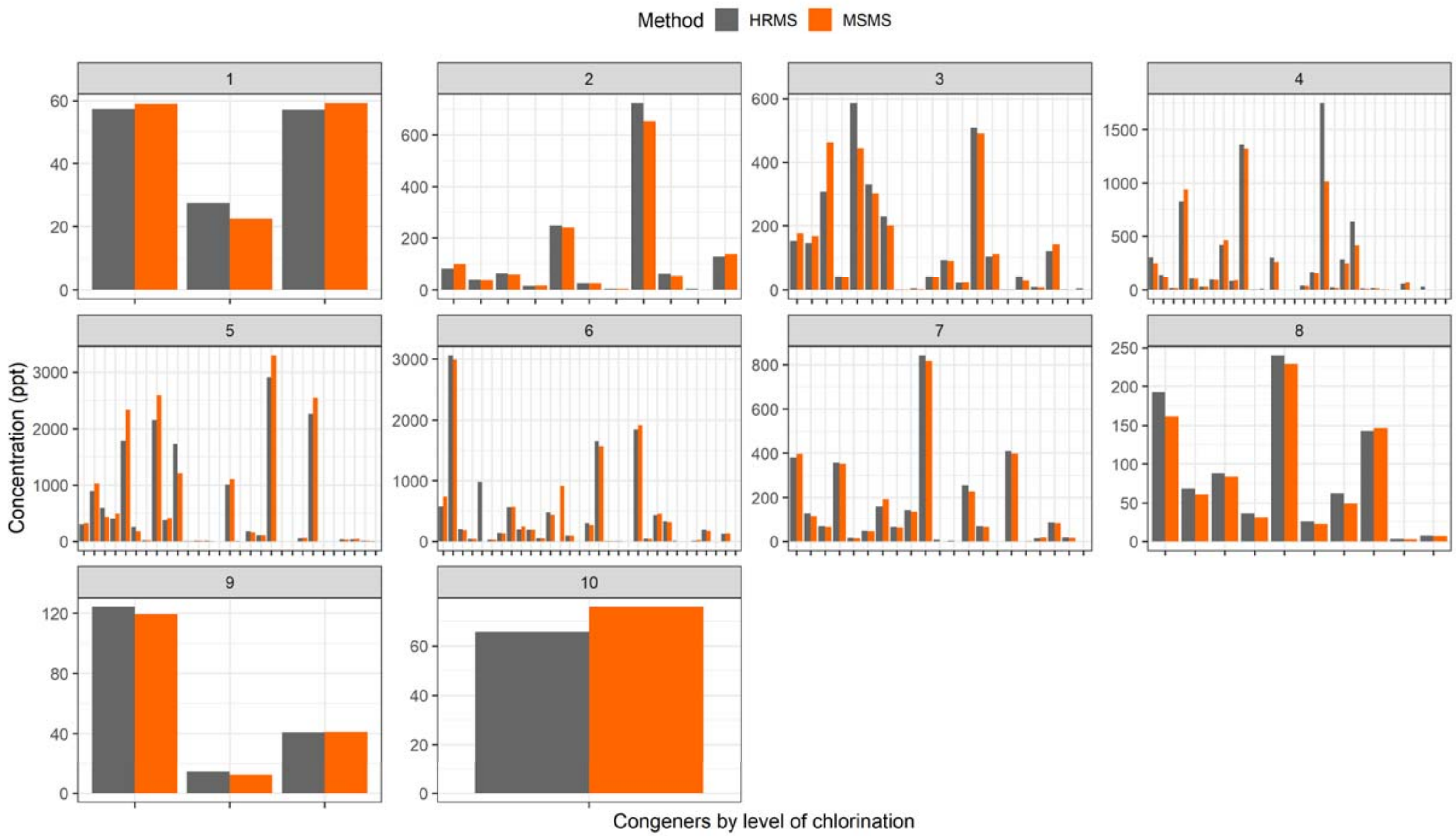
BIOSOLID

Biosolid 1



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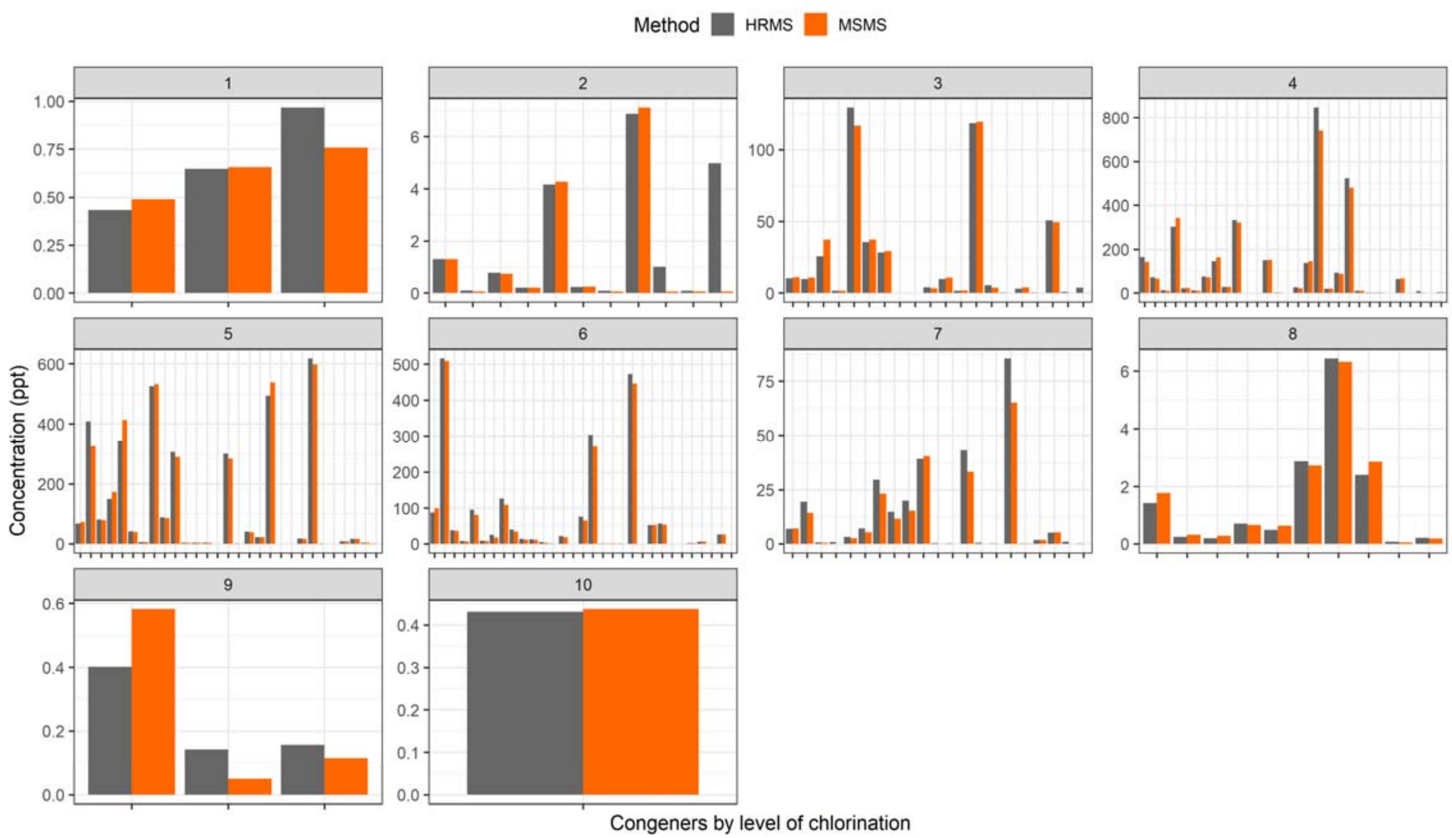
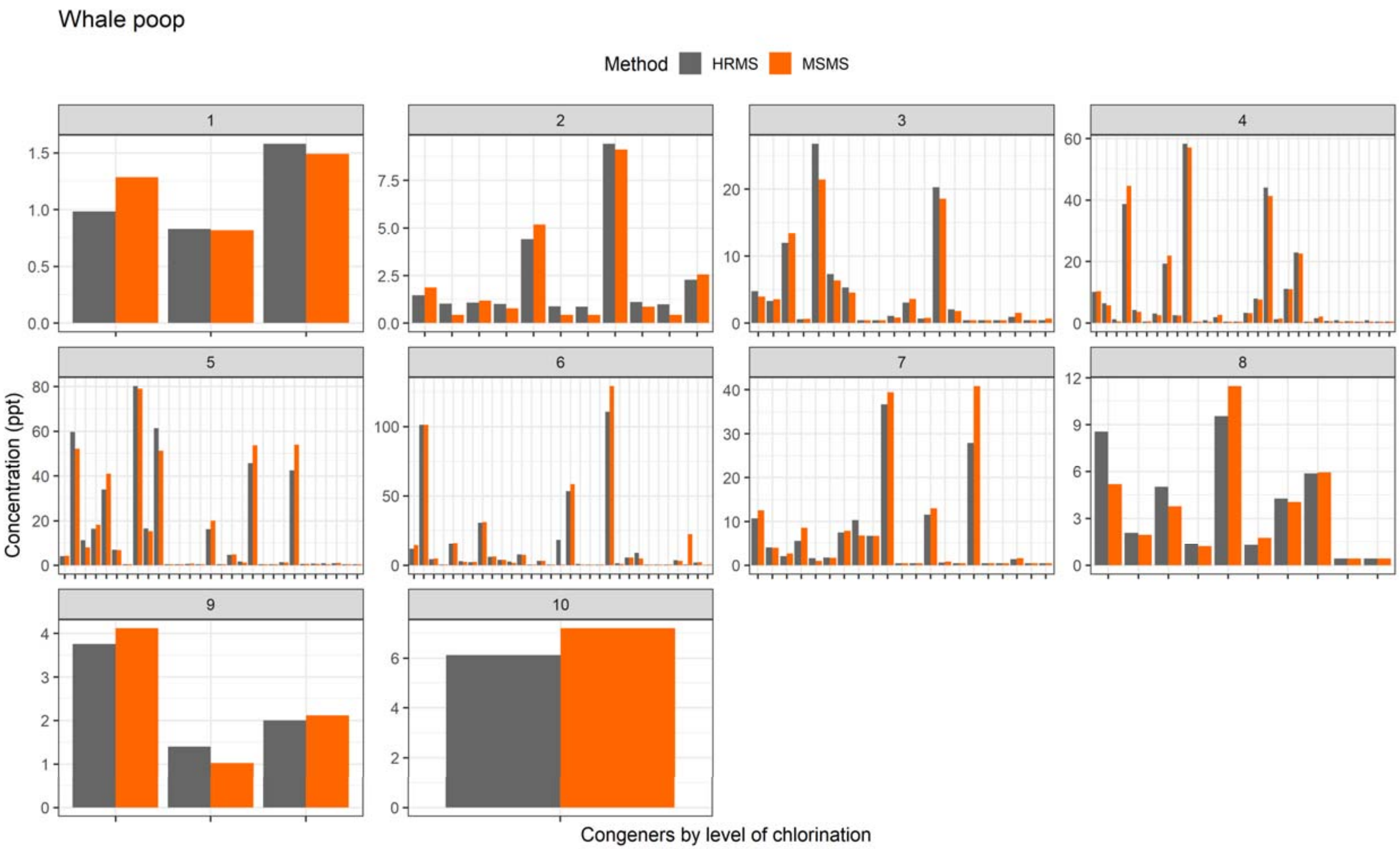
CB(1 Chandramouli, Bharat (Sidney), 8/12/2020

TISSUE

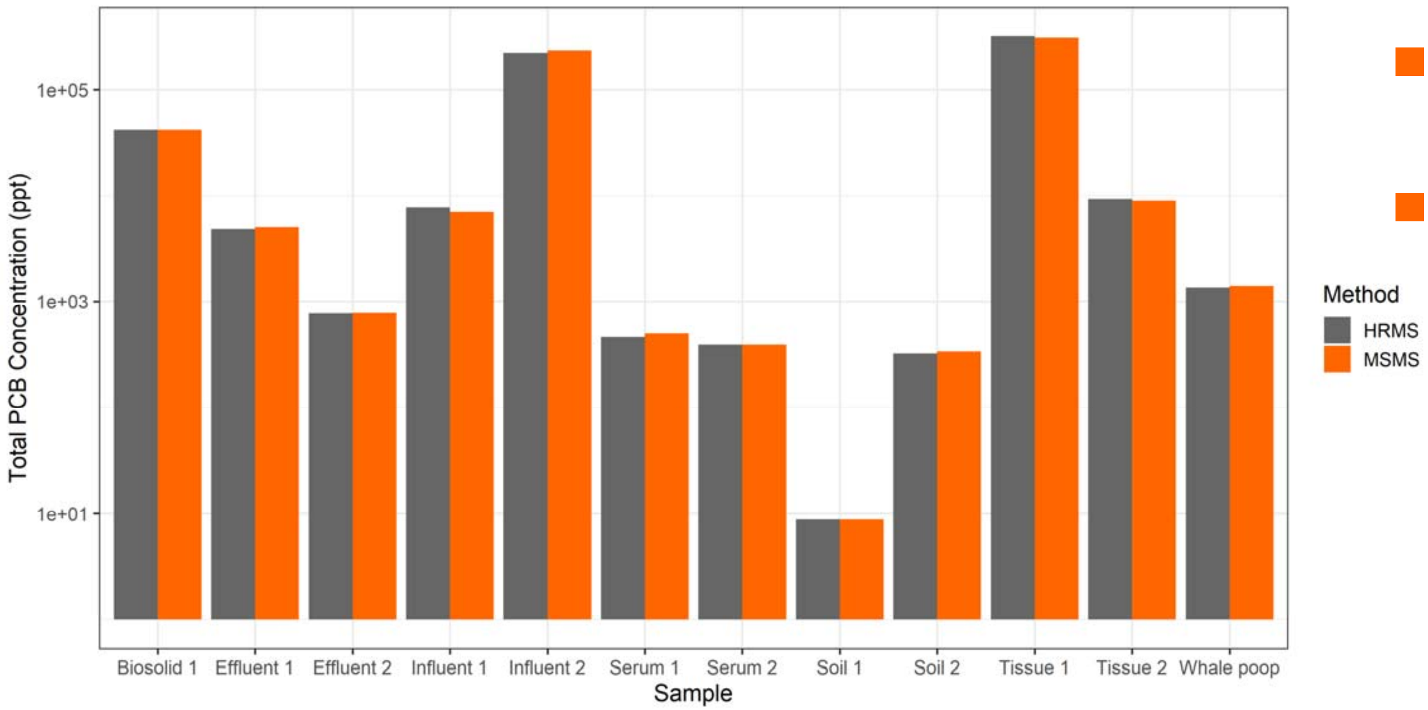
Tissue 2



WHALE POOP


Whale poop
Method
HRMS
MSMS
1
2
3
4
5
6
7
8
9
10
Concentration (ppt)
Congeners by level of chlorination

PCB COMPARISON – INITIAL TAKEAWAYS



- Totals show no systematic issues
- Out of the > 1500 points of comparison between the methods, 19 instances identified for further attention based on differences (approx. 1%)

CHLORINATED PESTICIDES

- Long standing internal method initially converted from GC-LRMS and GC-ECD to GC-HRMS in order to provide better data on challenging samples
- Specificity of GC-HRMS compensates for interferences that would not be resolved otherwise
- Issues with GC-HRMS method
  - Large m/z scanning range not optimal for magnetic sector instrument
  - Need two separate runs in order to produce acceptable data
- **Objective: GC-MS/MS method that measures all chlorinated pesticides in one run with better sensitivity and better performance on challenging samples and sample-limited projects**

PESTICIDE TARGET LIST

1	2	3	4	5	6
ALPHA-HCH	2,4'-DDT	ALPHA-Endosulphan	Aldrin	HCB	Heptachlor
BETA-HCH	4,4'-DDT	BETA-Endosulphan	Dieldrin	Oxychlordane	Heptachlor epoxide
GAMMA-HCH	2,4'-DDD	Endosulphan sulphate	Endrin	Trans-Chlordane	Methoxychlor
DELTA-HCH	4,4'-DDD		Endrin aldehyde	Cis-Chlordane	Trans-Nonachlor
	2,4'-DDE		Endrin ketone	Mirex	Cis-Nonachlor

- Also validated several other targets including di, tri, tetra and pentachlorobenzenes, hexachlorobutadiene, octachlorostyrene oxadiazon and dacthal

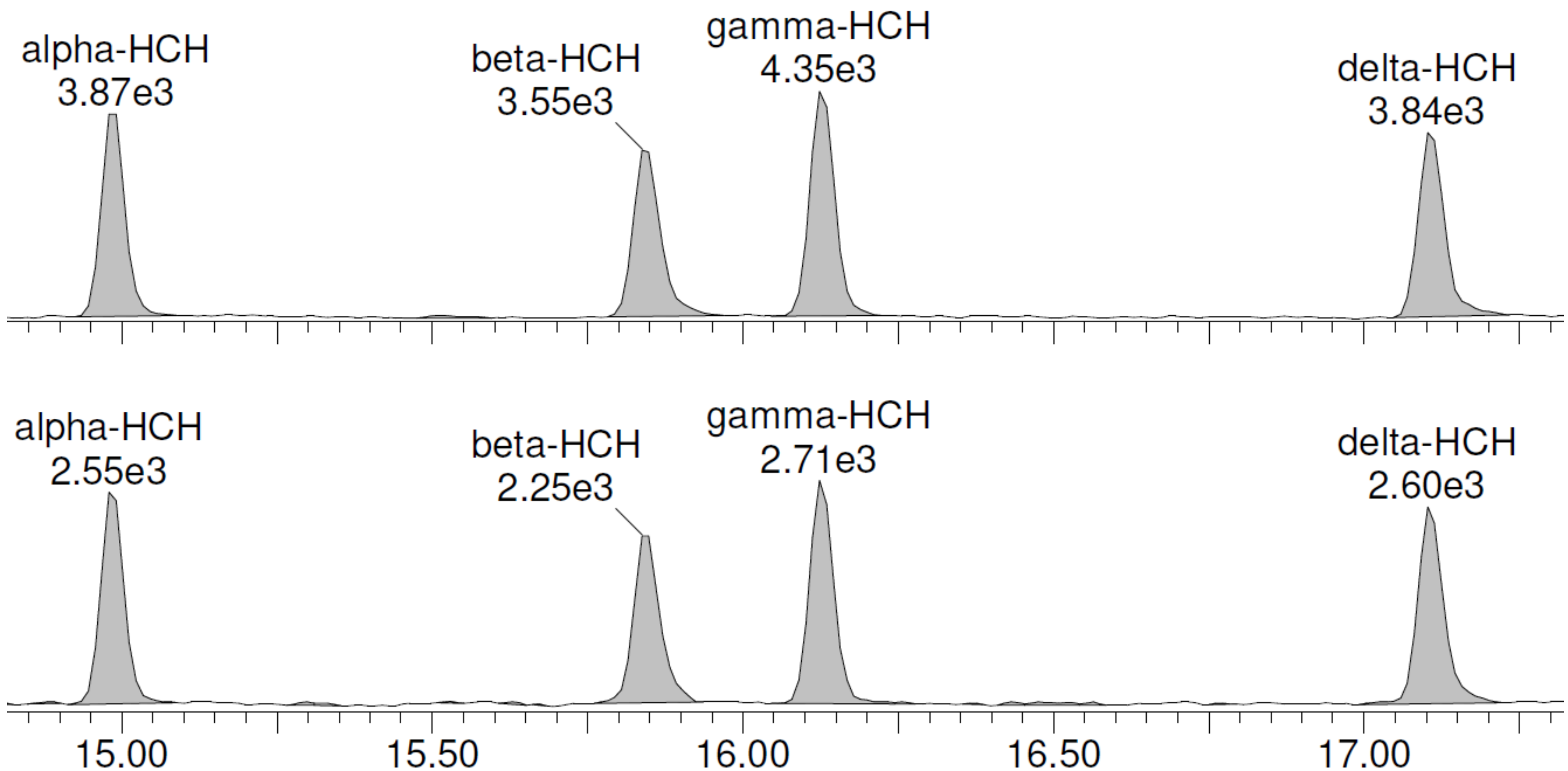
SAMPLE PREPARATION AND GC DEVELOPMENT

- New development to combine two run program into 1 run
- Eliminated florisil fractionation
- DB-5MS column – 32 minute run – more than doubling current throughput
- Splitless injection for maximum sensitivity
- Endrin and DDT breakdown equivalent to GC-HRMS
- Methods currently accredited under ISO-17025 (CALA) and NELAP TNI (FI DOH)

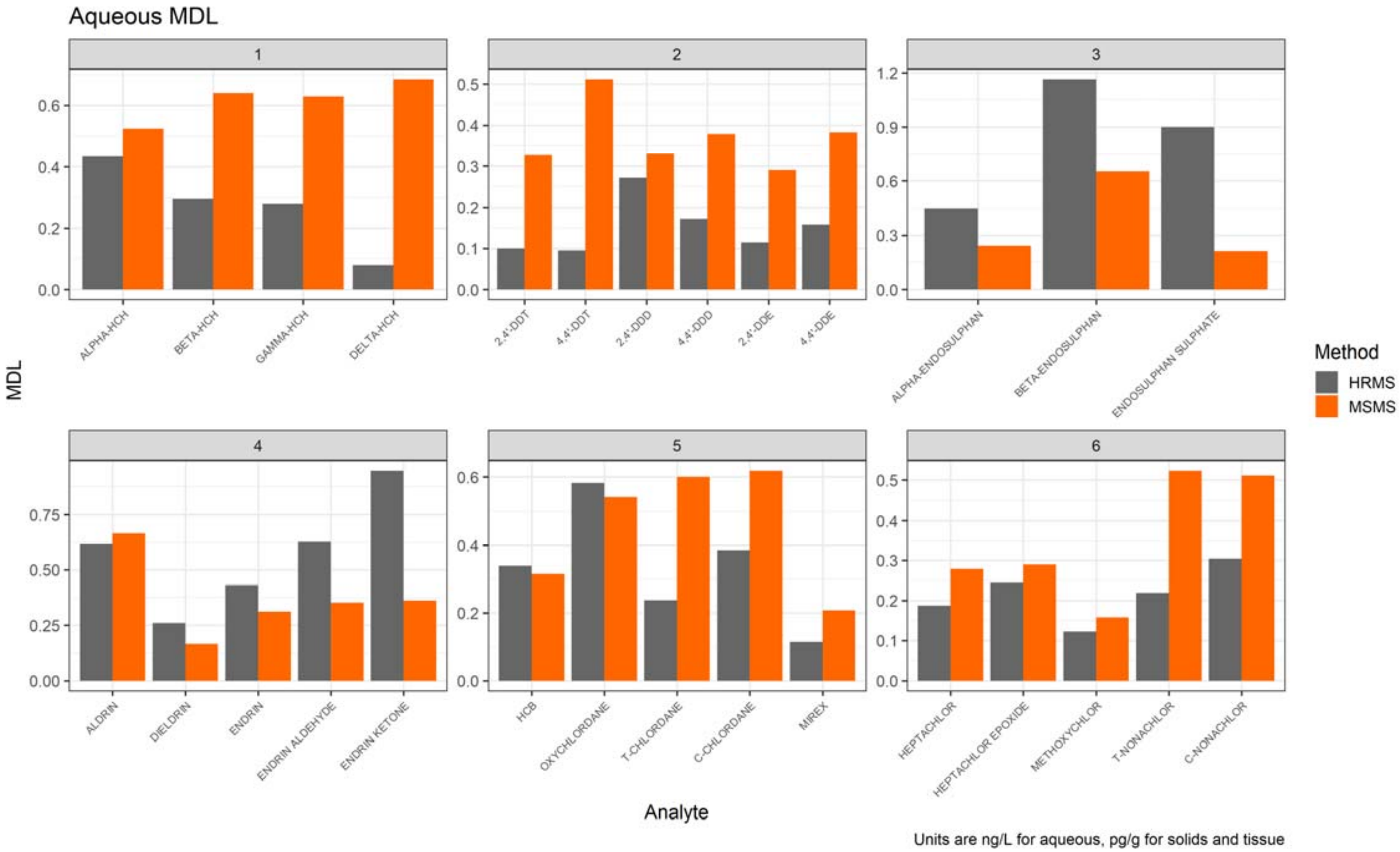
MS/MS DEVELOPMENT

- Optimization of source parameters, pesticides don’t ionize like PCBs or dioxins
- Just as in PCBs, all new development for two precursor -> product transitions per analyte based on chlorine isotope differences
- Ratio checks against theoretical
- Same procedure for lock mass checks and resolution checks

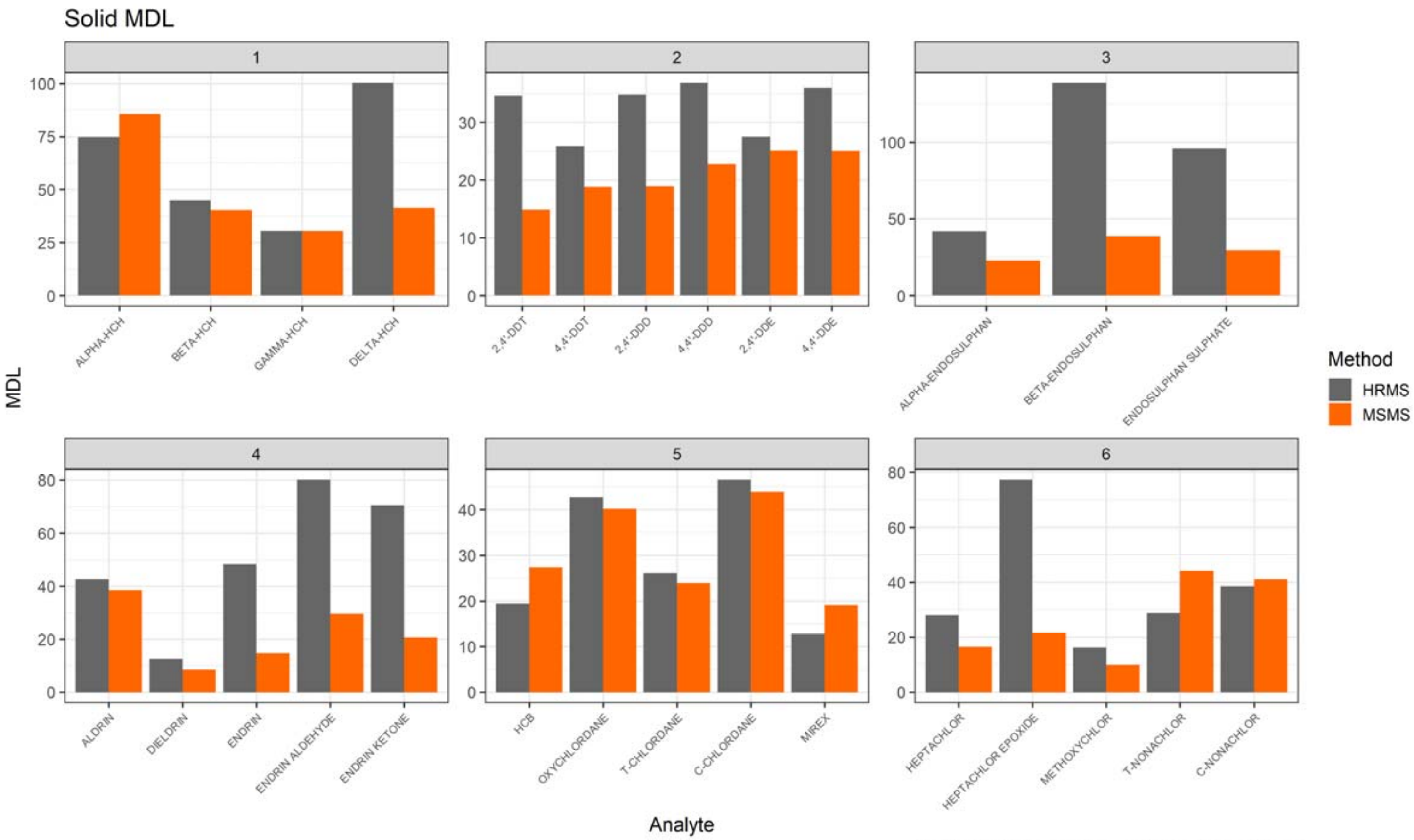
PESTICIDE SENSITIVITY AT LOWEST CALIBRATION



AQUEOUS MDL (NG/L)

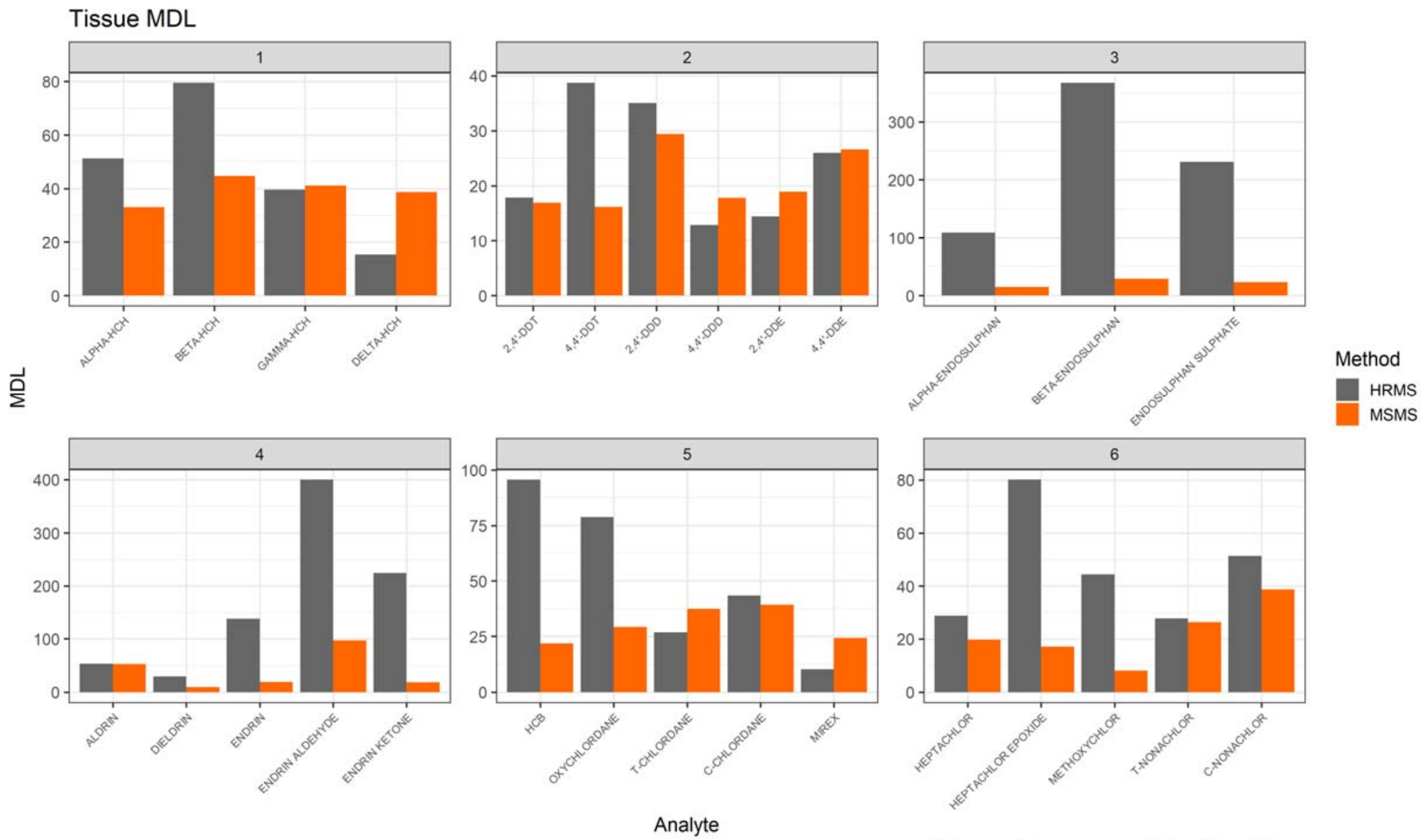


SOLID MDL (PG/G)



Units are ng/L for aqueous, pg/g for solids and tissue

TISSUE MDL (PG/G)



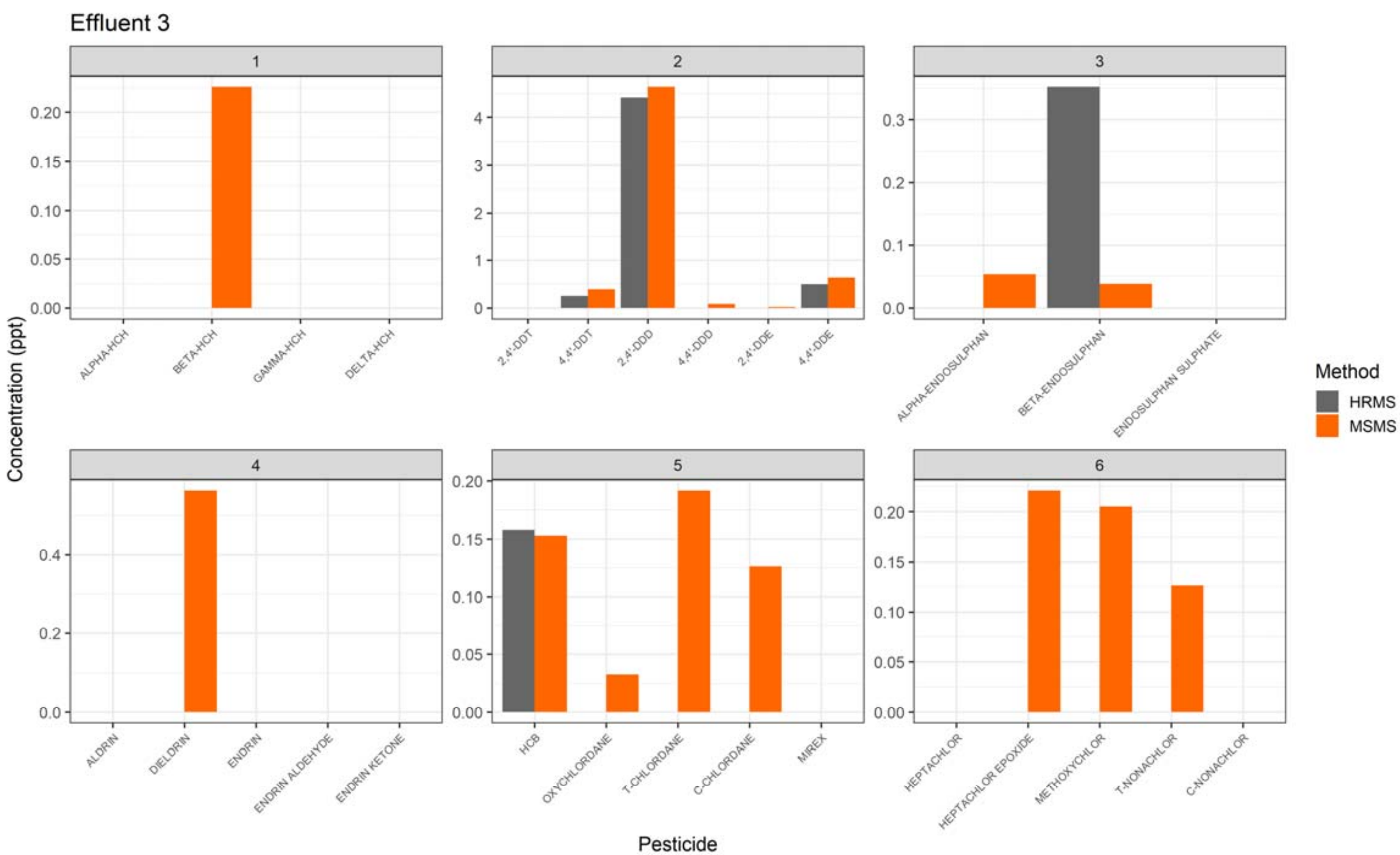
Units are ng/L for aqueous, pg/g for solids and tissue

PESTICIDE COMPARISON SAMPLES

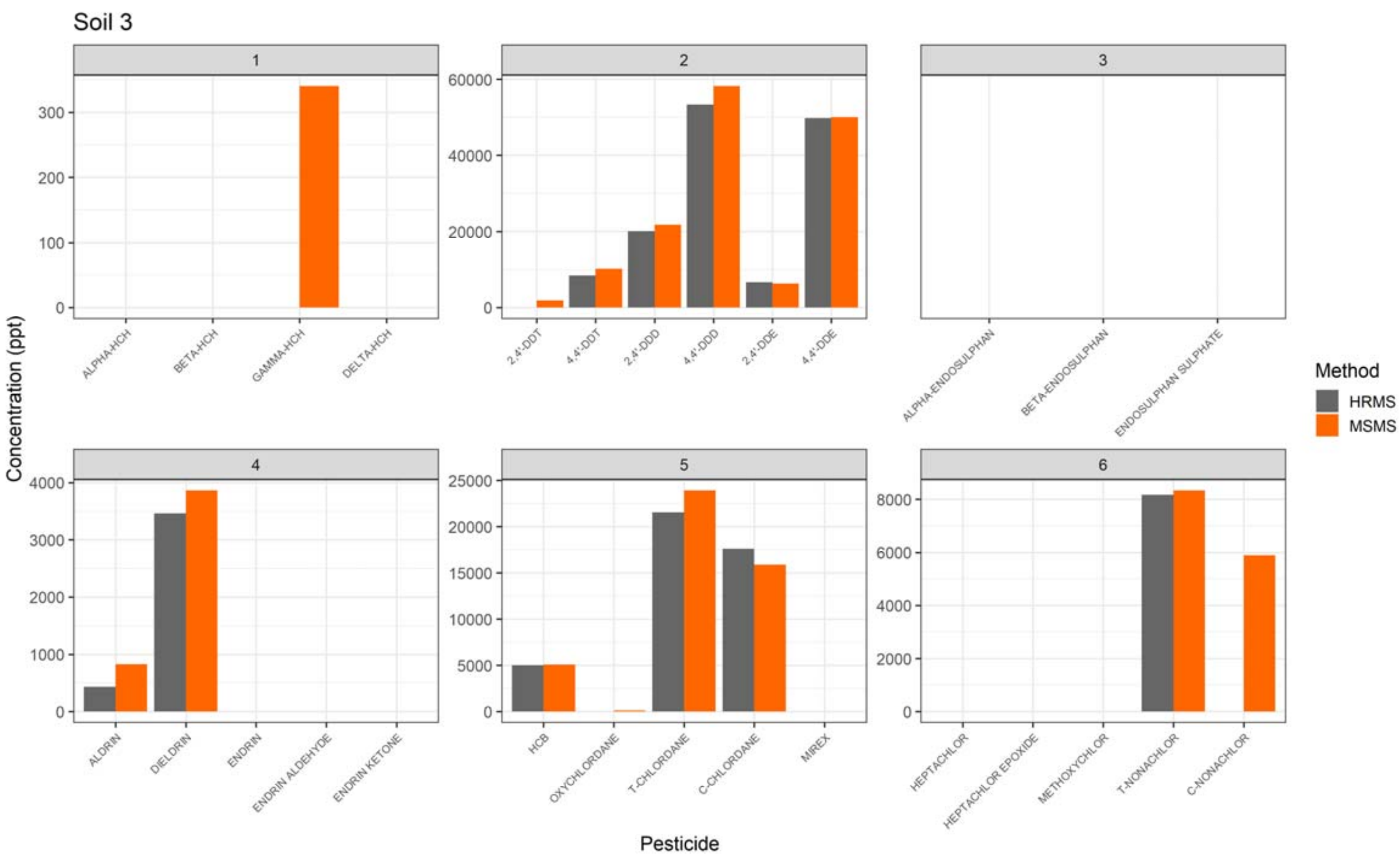
Sample Type	#
WWTP Effluent	1
Soil	3
Tissue	3
Serum	2

- Again based on extracts from GC-HRMS projects
- Extracts from two GC-HRMS runs were combined and reanalyzed using GC-MS/MS

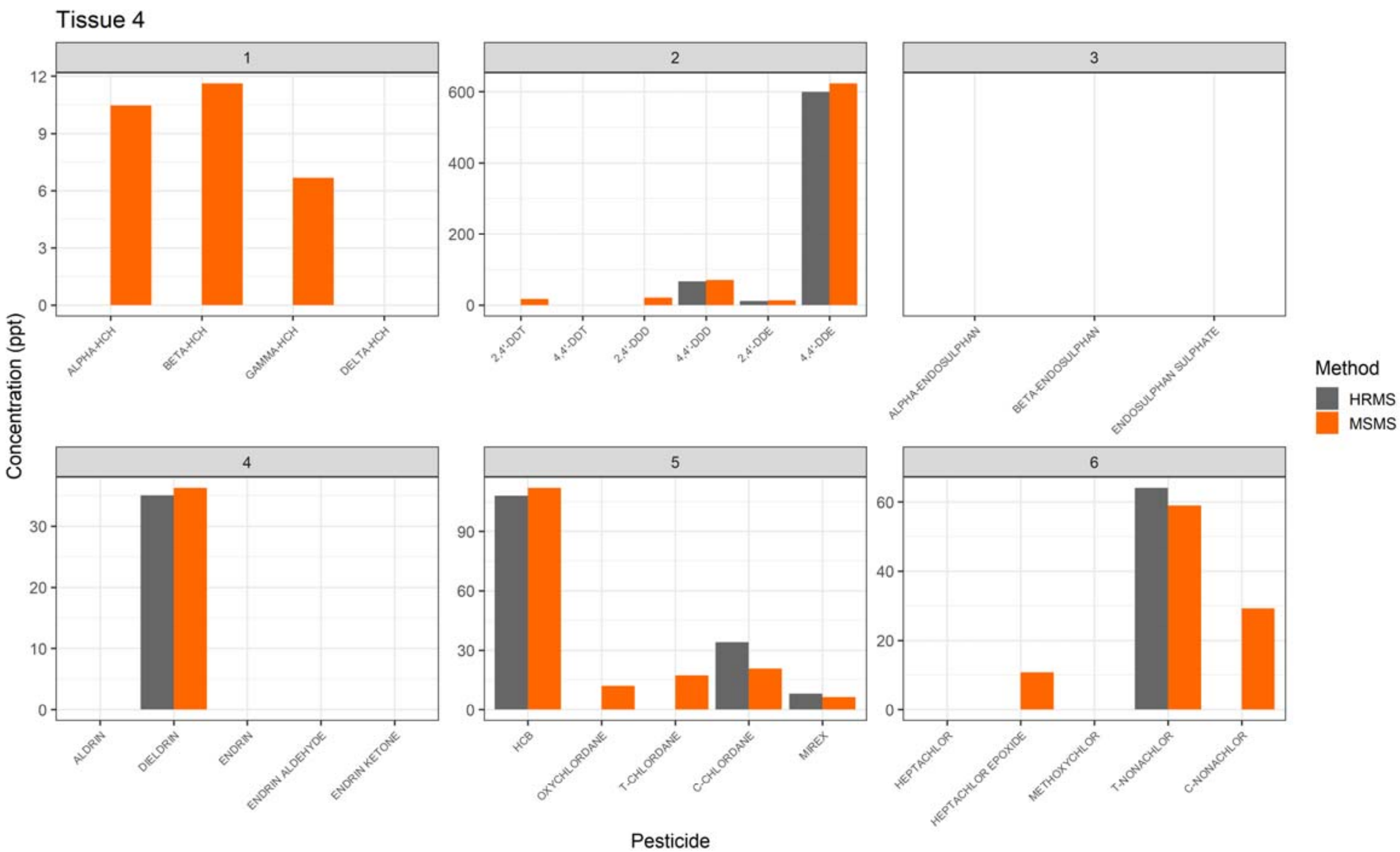
WWTP EFFLUENT



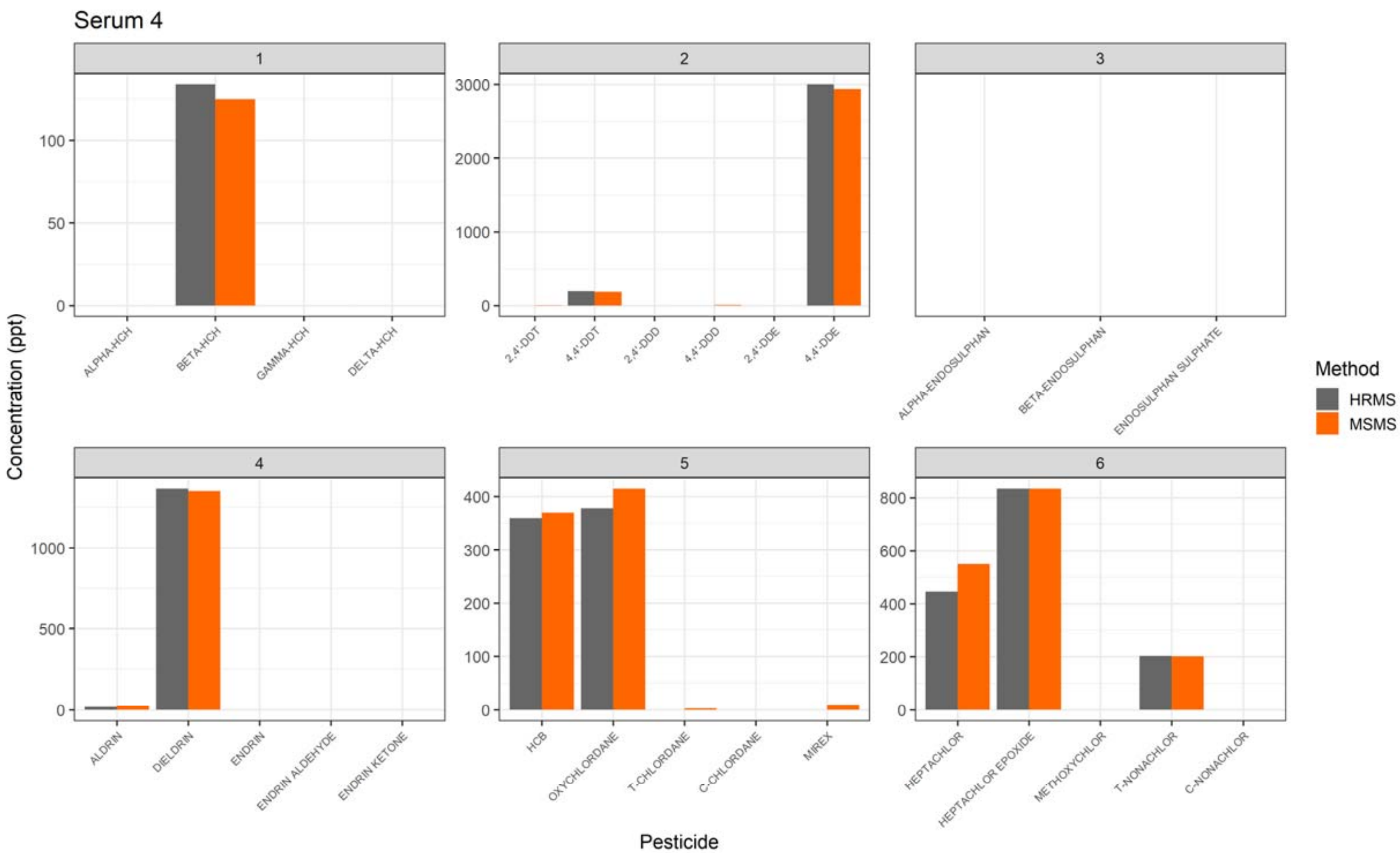
SOIL COMPARISON DATA



LOW LEVEL TISSUE COMPARISON DATA



SERUM COMPARATIVE DATA



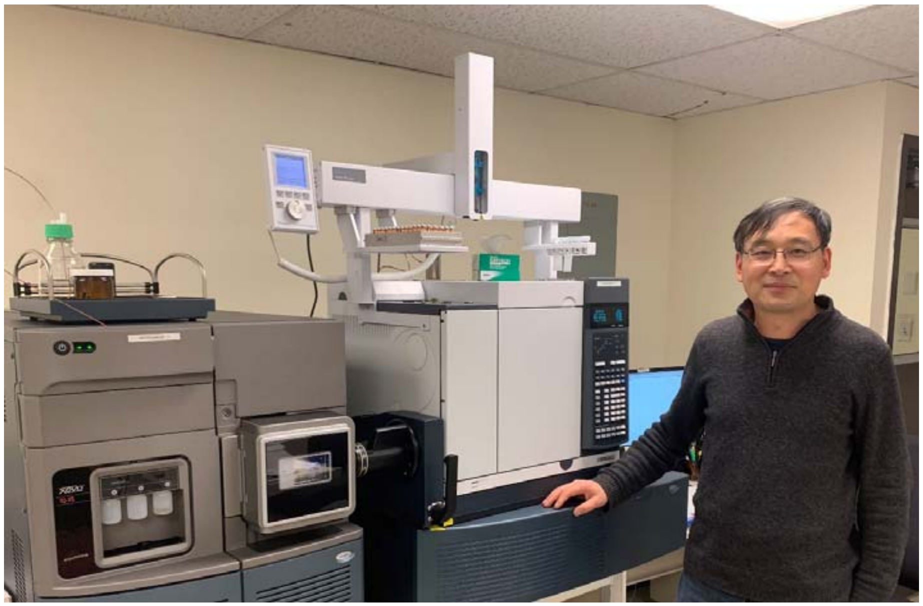
PESTICIDE TAKEAWAYS

- Excellent correlation with GC-HRMS results
- Instrument is more sensitive, meaning smaller sample sizes can be used
- Initial sample testing indicates promise on robustness
- More challenging samples needed to really test the instruments (don’t let our operations team hear this ☺)

OVERALL PROGNOSIS

- Instrument has performed very well
- Initial use cases are non-regulatory projects with limited sample and multiple analyses – Dioxins, PCBs and Pesticides from 2g of tissue!
- ATP approval from EPA showing equivalency for dioxins will also increase acceptance for PCBs and pesticide analysis

ACKNOWLEDGEMENTS



- Waters: Joe Romano and Rhys Jones for great technical expertise
- EPA: Lem Walker
- SGS AXYS Senior Scientist Coreen Hamilton: The brains
- SGS AXYS Senior Chemists Xinhui Xie, Angie Schlak and Jason Mackenzie