Innovative methodology based on a non-targeted screening approach combined with activity patterns for estimation of soil and dust ingestion rate in children.

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Co-PIs: Daniel Bagner, Florence George and Piero Gardinali

Disclaimer

- This project is funded by the following award from the Environmental Protection Agency (EPA) to Florida International University

  EPA-G2020-STAR-D, Estimating Children's Soil and Dust Ingestion Rates for Exposure Science

The opinions, findings, and conclusions hereby presented are those of the authors and do not necessarily reflect those of the Environmental Protection Agency (EPA)

Outline

- Introduction
- Study Aims
- Research Group
- Methodology
- Initial Findings
- Future work

NTA Solution
PROJECT

Website: https://case.fiu.edu/research/nta-solution/

Introduction

- Vulnerability and susceptibility of young children from 6 months through 6 years who are daily exposed to a variety of chemicals through outdoor activities, but also indoors in their homes and schools

- A better understanding of the contaminants ingested from soil and dust ingested is needed

- Issues on current soil and dust ingestion assessments

  Designation of interviews or surveys

  Misinterpretation, language, lack of attention.

  Incorrect assumptions on modeling

Addressing some gaps…

- Flexible remote appointments for surveys and interviews, without children interference, could increase the reliability of the information obtained.
- We are increasing representativeness for southeastern U.S territories with different climate and soil types
- Our study area is Miami, South Florida, with the majority of the population being Hispanic (Latino) and Black increasing underrepresented races and ethnicities in the soil and dust ingestion rate estimates.
- Combination of NTA to identify unique chemicals in soil and dust and information on children activities patterns (mouthing behavior, frequency of hand washing, time spent in specific microenvironments, among others) will improve soil and dust ingestion rates, reducing bias and providing long-term exposure data.

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Why non-target analysis?

- Non-target analysis assumes no prior knowledge of the compounds, providing a far more comprehensive picture of the chemical composition

- Our goal is to establish an NTA method to assess the organic contaminants for different matrices

- Seek potential tracers that can be potentially used to study the soil and dust ingestion rate

NTA Workflow

Sampling
- Grab samples
- Passive Samplers

Sample Preparation and Analysis
- Filtration
- Extraction/clean-up
- Dilution/concentration
- LC-HRMS

Data Pre-processing
- Detect peaks
- Align RT
- Subtract Blanks

Prioritization and Identification
- Isotope patterns
- Mass errors/defects
- Merge and grouping
- Assign Formulas
- Fragments (MS2)
- Database search

Study Aims

Aim 1: Development of a non-targeted analysis (NTA) method to estimate soil and dust ingestion by children

Aim 2: Activity pattern study to estimate soil and dust ingestion by children.

Aim 3: Statistical calculations combining both approaches for accurate estimation of soil and dust ingestion rate by children.

- ✓ IRB Protocol Approval #: IRB-21-0385 (Approval Date: 08/23/21 until 08/23/24)

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Study Aims

Aim 1: Development of a non-targeted analysis (NTA) method to estimate soil and dust ingestion by children

- ✓ The QAPP document was submitted and has been approved
- ✓ Non-targeted screening methods for different matrices (urine, soil, dust, food and water provided by parents/caregivers) have been established and optimized
- ✓ Identify specific chemicals that could be used as tracers for soil and dust ingestion

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Study Aims

Aim 2: Activity pattern study to estimate soil and dust ingestion by children.

- ✓ Goal to recruit 81-90 children aged 6 months to 6 years from families in the greater Miami area ->Pediatric Care Center (PCC) at Nicklaus (Miami) Children's Hospital (NCH), including a database of children of 12- to 18-month-old from Dr. Bagner.
- ✓ Most of the population from underrepresented races and ethnicities
- ✓ Children's behavior, activities, and demographic information will be assessed by remote surveys and/or interviews-> Surveys and questionnaires were created and setup in REDCap

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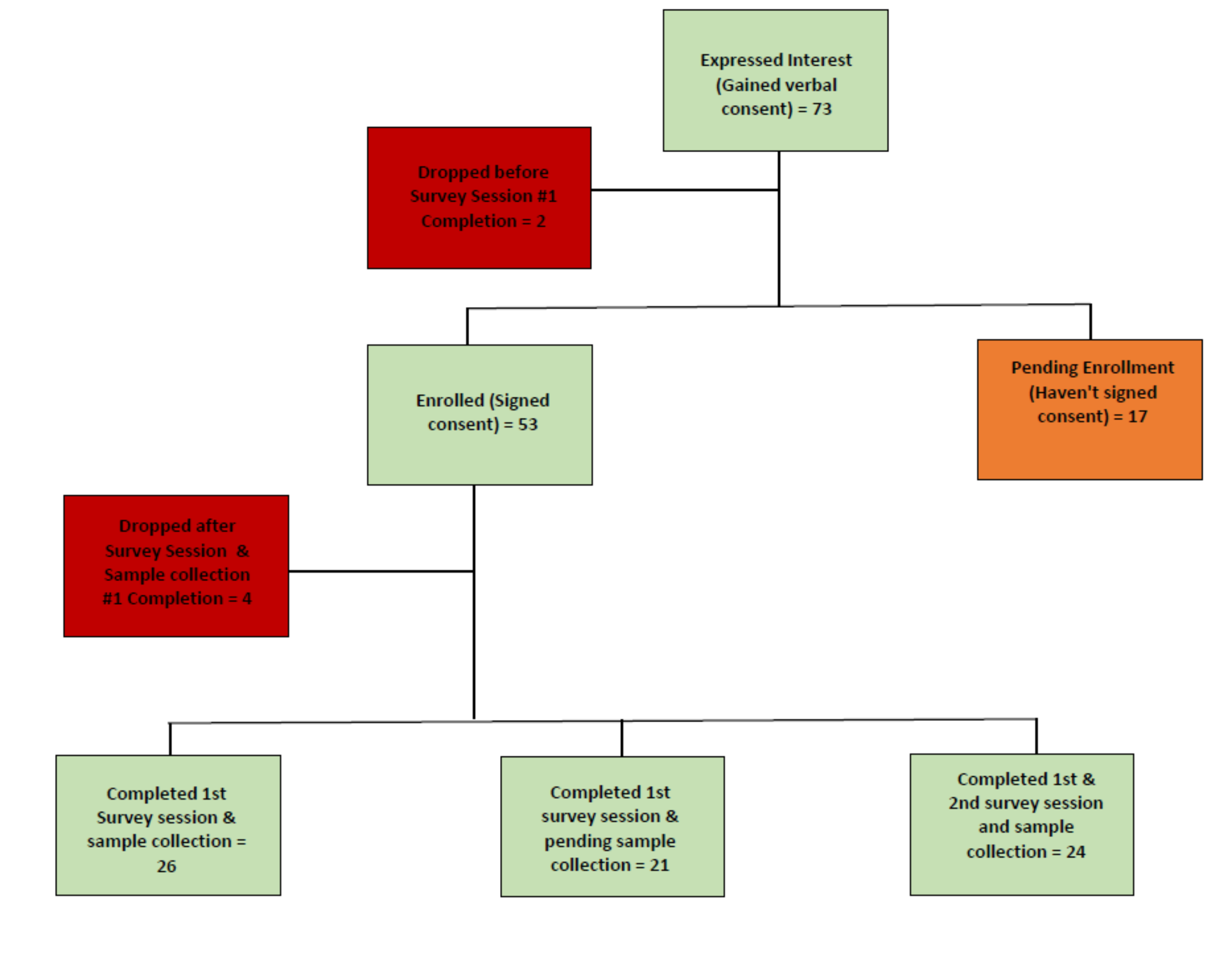
Recruitment Rate Progress



- ✓ We are close to our targeted enrollment (i.e., at least 81 families), and a total of 24 families have completed the first two sets of sample collection and surveys
- ✓ Hire and Training of undergraduate students to help with family recruitment at Nicklaus Children's Hospital

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Surveys

- ✓ A short demographic questionnaire
- ✓ Surveys on children's activities and microenvironment (how many times the child put their hands in their mouth, what is the time spent inside the house or outside in nearby playground areas, etc.)
- ✓ Surveys about the child's development and behavior (BITSEA for 12-18 months and CBCL for children 18 months- 5 years)
- ✓ Surveys about parenting style and behavior
- ✓ Sampling procedure and materials (document and instruction video) provided to parents/caregivers

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Surveys

NTA SOLUTION PROJECT

Understanding children exposure by chemicals in the environment

1-Recruitment/ Screening

Per email/phone
Duration: 1 hour

2- Remote Survey/ Interviews

4 sessions per year
Each about 1 hour long

3-Collection of samples at home for analysis (household dust, soil, water, food items and child's urine)

4 times per year
Total possible compensation: $900

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Study Aims

Aim 3: Statistical calculations combining both approaches for accurate estimation of soil and dust ingestion rate by children.

- ✓ Goal to combine statistical calculations derived from the tracer chemical studies and the data obtained from the activity pattern studies applied to the SHEDS-HT model
- ✓ We will use an overlapping index for quantifying similarities or differences between the soil and dust ingestion distributions estimated by the calculations derived from the tracer studies and SHEDs models.
- ✓ Multiple linear regression will be performed to evaluate differential soil and dust ingestion rate in children of different socio-economic status and race/ethnicities.
- ✓ Age-dependent distributions as well as seasonal variations for soil/dust ingestion rates will be evaluated in this study

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Research Team

Co-PIs

Daniel Bagner

Florence George

Piero Gardinali

Postdocs

Danni Cui

Joe Cox

Emily Meijas,

Project Coordinator

Mymuna Monem ,

Graduate student

Kevin Drai,

Research intern

Overall Workflow

Recruitment and Consent

Sampling

Collection

Sample Prep

Extraction/ Cleanup

Analysis on Orbitrap

Analysis using Compound Discoverer

Manual Post Processing

List of Features

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Methods – Instrument / Screening

Liquid Chromatography- Orbitrap MS (Thermo Q-Exactive)

Analytical Column: Hypersil GOLD aQ Dim (mm) 100 x 2.1x 1.9 µm

Mobile Phase:
Acetonitrile and 0.1% Formic Acid

- Source : H-ESI
- Resolution: 140,000
- Full scan: 100-800 m/z
- Positive and Negative modes
- MS2 for confirmation: NCE 30
- Mass tolerance: <5 ppm

Spray Voltage (V)	5000
Capillary Temperature (˚C)	350
Sheath Gas (arbitrary units)	30
Aux Gas (arbitrary units)	2
S-Lens RF Level	50

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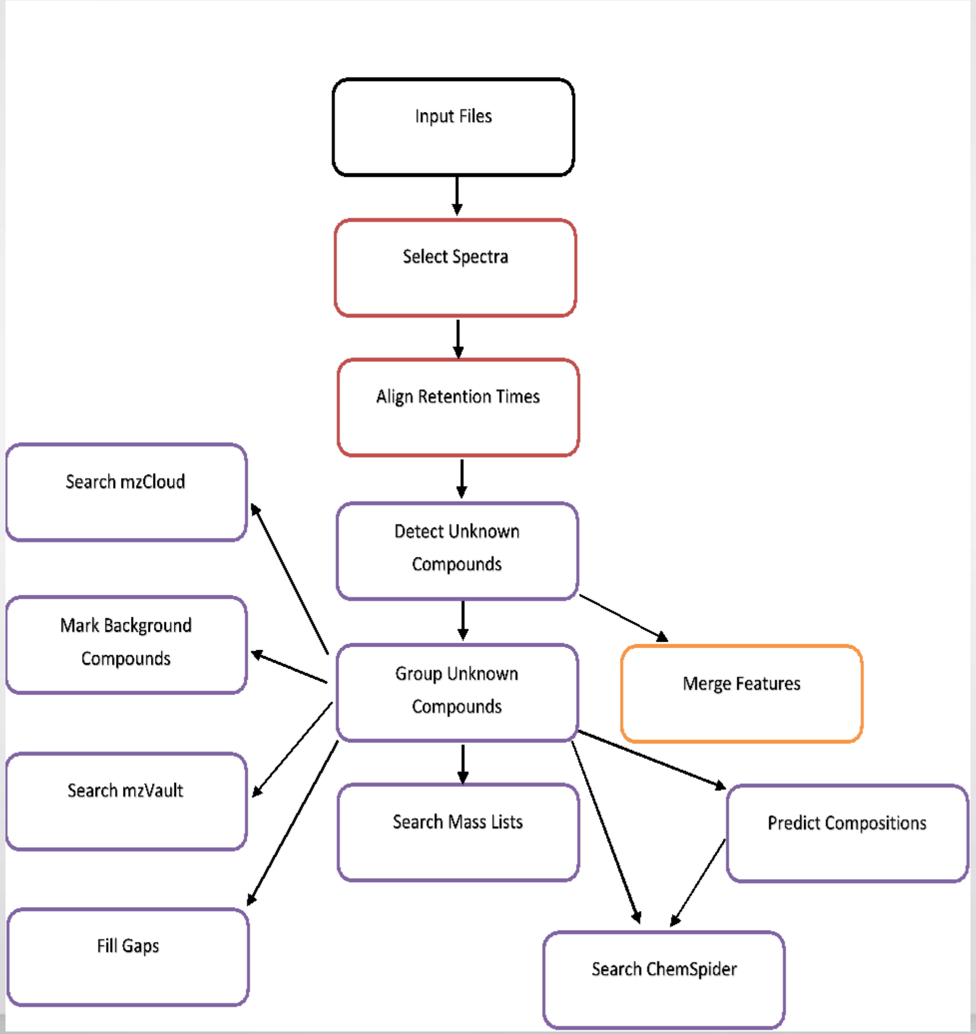
Data processing for NTA



The data analysis usually includes steps such as peak-picking, blank subtraction, componentization, molecular formula generation, isotopic pattern comparison, evaluation of adducts, and the assessment and comparison of fragmentation patterns.

Databases:

- ACToR: Aggregated Computational Toxicology Resource
- DrugBank
- EAWAG Biocatalysis/Biodegradation Database
- EPA DSSTox
- EPA Toxcast
- FDA UNII - NLM

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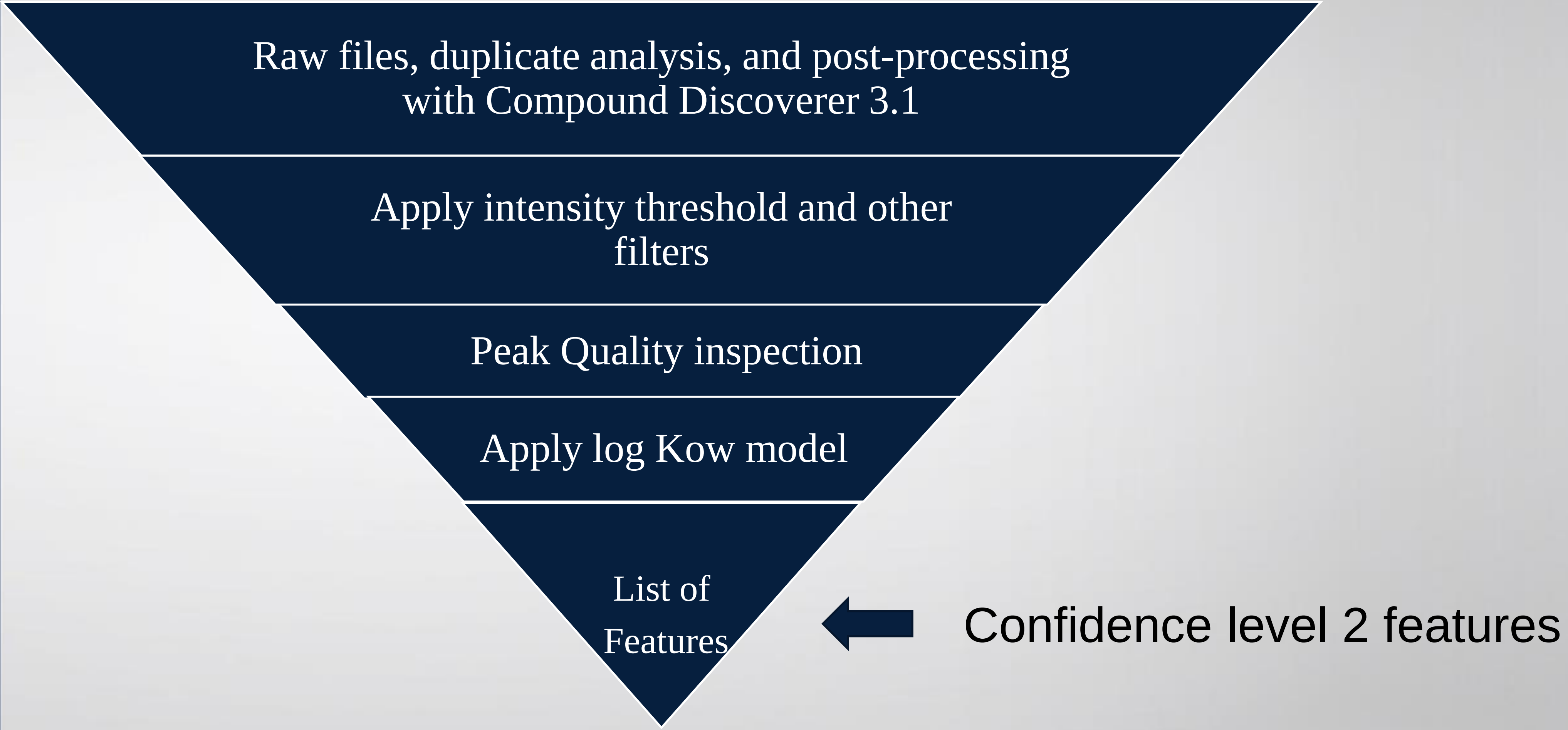
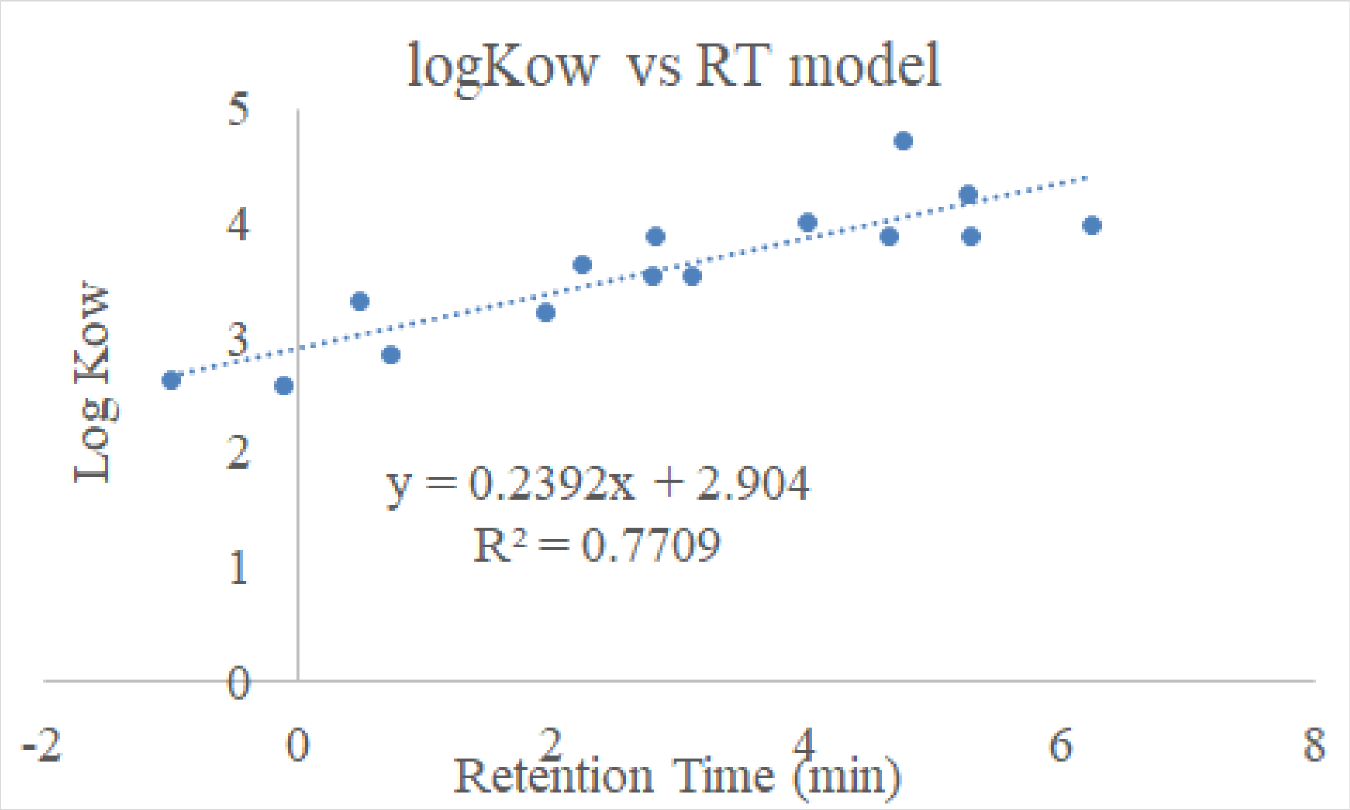
Detailed Screening Process



Schymanski, et.al (2014). Identifying small molecules via high resolution mass spectrometry: Communicating confidence. *Environmental Science and Technology*, *48*(4), 2097–2098.

Quality Control

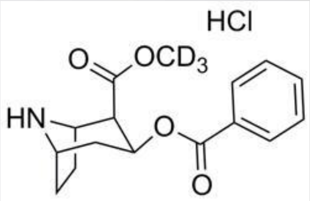
Compound	log Kow
Caffeine	0.16
lincomycin	0.29
sulfamethoxazole	0.48
trimethoprim	0.73
carbamazepine	2.25
diltiazem	2.79
atrazine	2.82
diphenhydramine	3.11
sucralose	-1
hydrochlorothiazide	-0.1
acetaminophen	0.22
diclofenac	4.02
gemfibrozil	4.77
mefenamic acid	5.28



Ng B, Quinete N, Gardinali PR. 2020 *Sci Total Environ* 713:136568.

Selection of internal standards

Internal Standard Name	# of Samples Detected
aetaminophen-d3 (+)	19
duktuazen-d3 (+)	6
norethindrone-d6 (+)	20
paroxetine-d4 (+)	5
sertraline-d3 (+)	7
triamterene-d5 (+)	20
sulfamethoxazole-d4 (+)	20
trimethoprim-d9 (+)	22
albuterol-d9 (+)	22
amitriptyline-d6 (+)	20
atenolol-d7 (+)	22
carbamazepine-d8 (+)	18
fluoxetine-d5 (+)	13
metoprolol-d5 (+)	20
valsartan-d3 (+)	22
verapamil-d6 (+)	11
almlodipine-d4 (+)	7
ibuprofen-d3 (-)	7
glipizide-d11 (-)	19
hydrochlorothiazide-13C, d2 (-)	10
warfarin-d5 (-)	21
gemfibrozil-d6 (-)	18



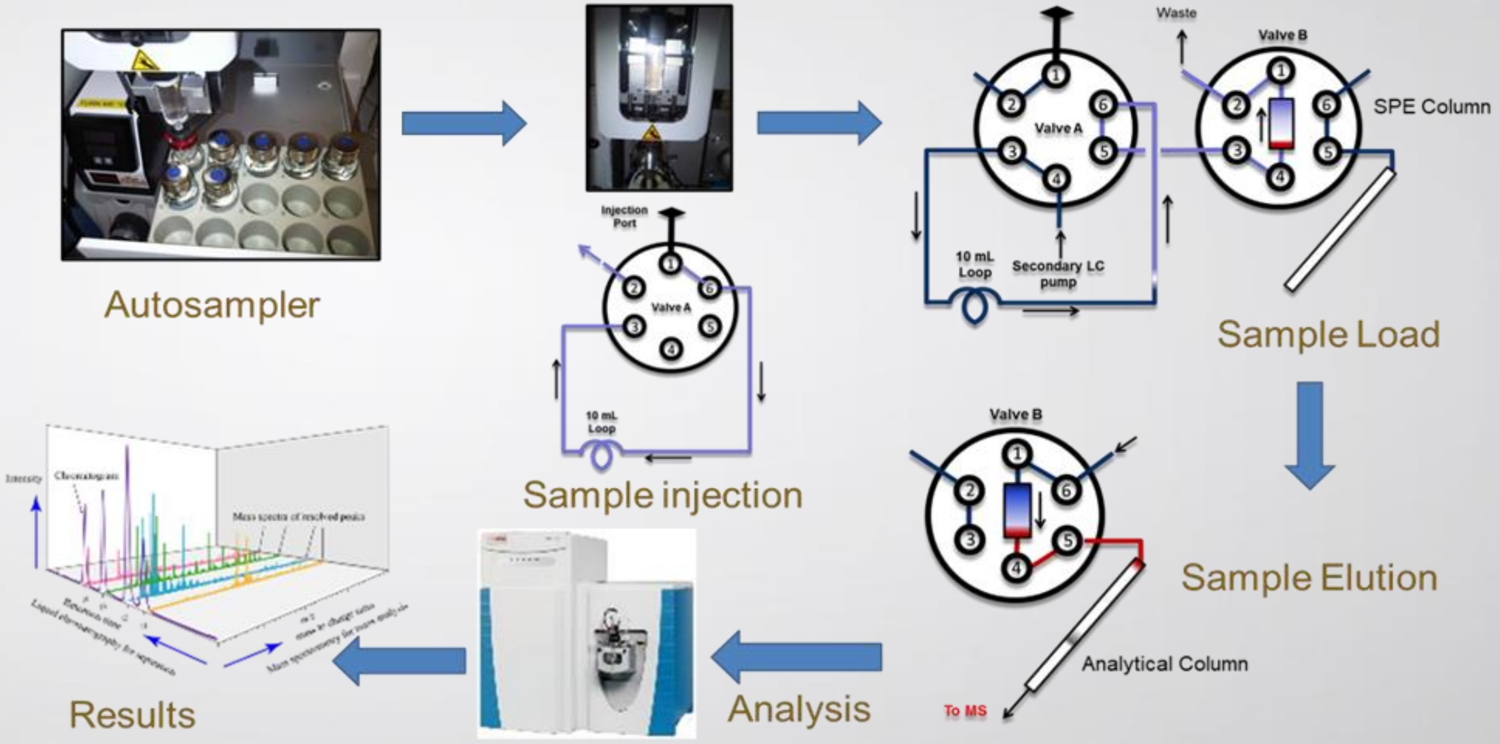
D3-Norcocaine

Online-SPE vs Direct Inject

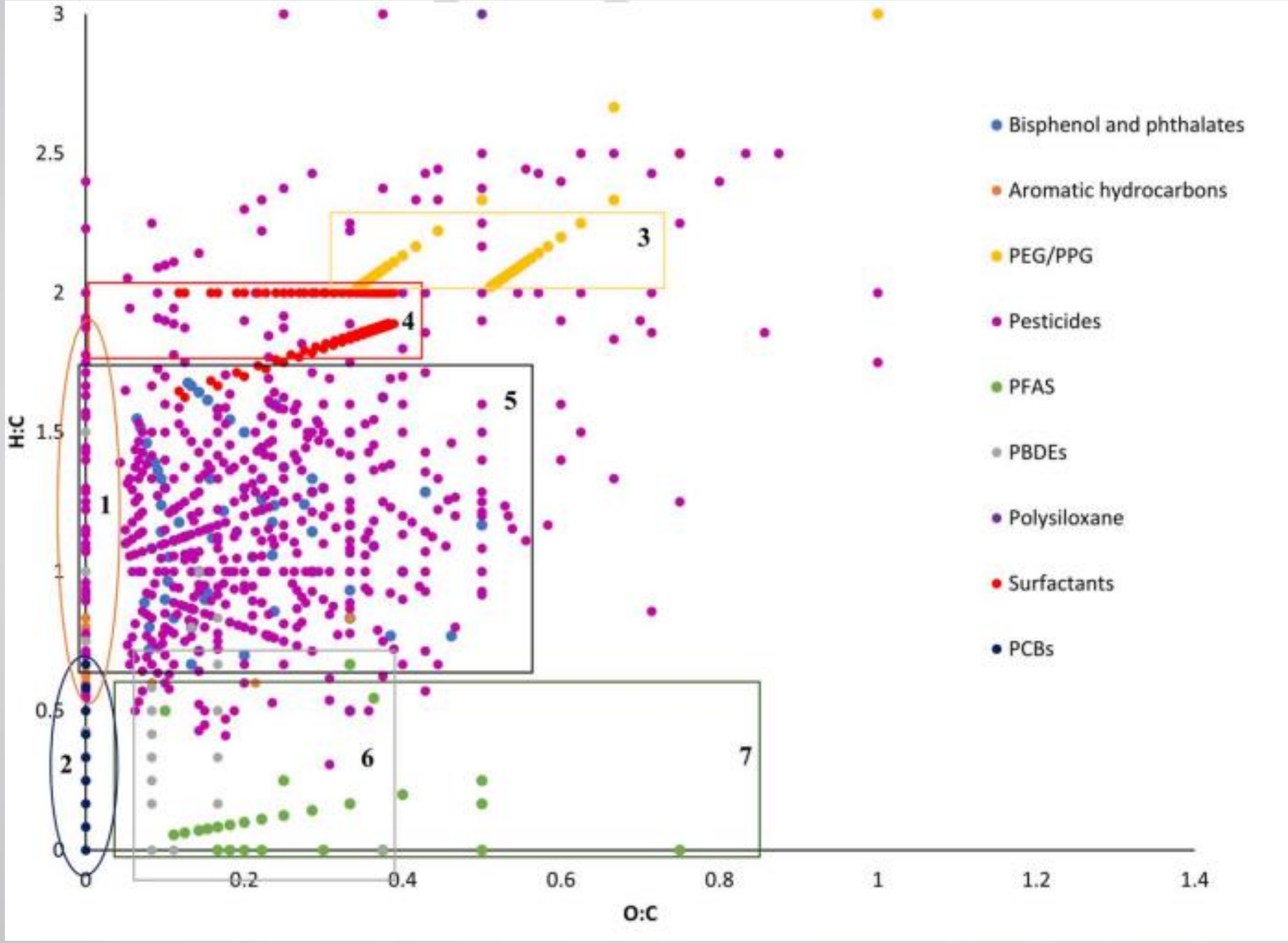
- Online-SPE
  - Water and Urine
  - Automated extraction
  - 20 min run
- Direct Inject
  - Soil, Dust, and Food
  - Manual extraction
  - 12 min run

Water Samples Collection and Analysis

- Tap or bottled water
- Online-SPE
- 10 mL of samples with little sample preparation



Visualization of the data: Van Krevelen Diagram

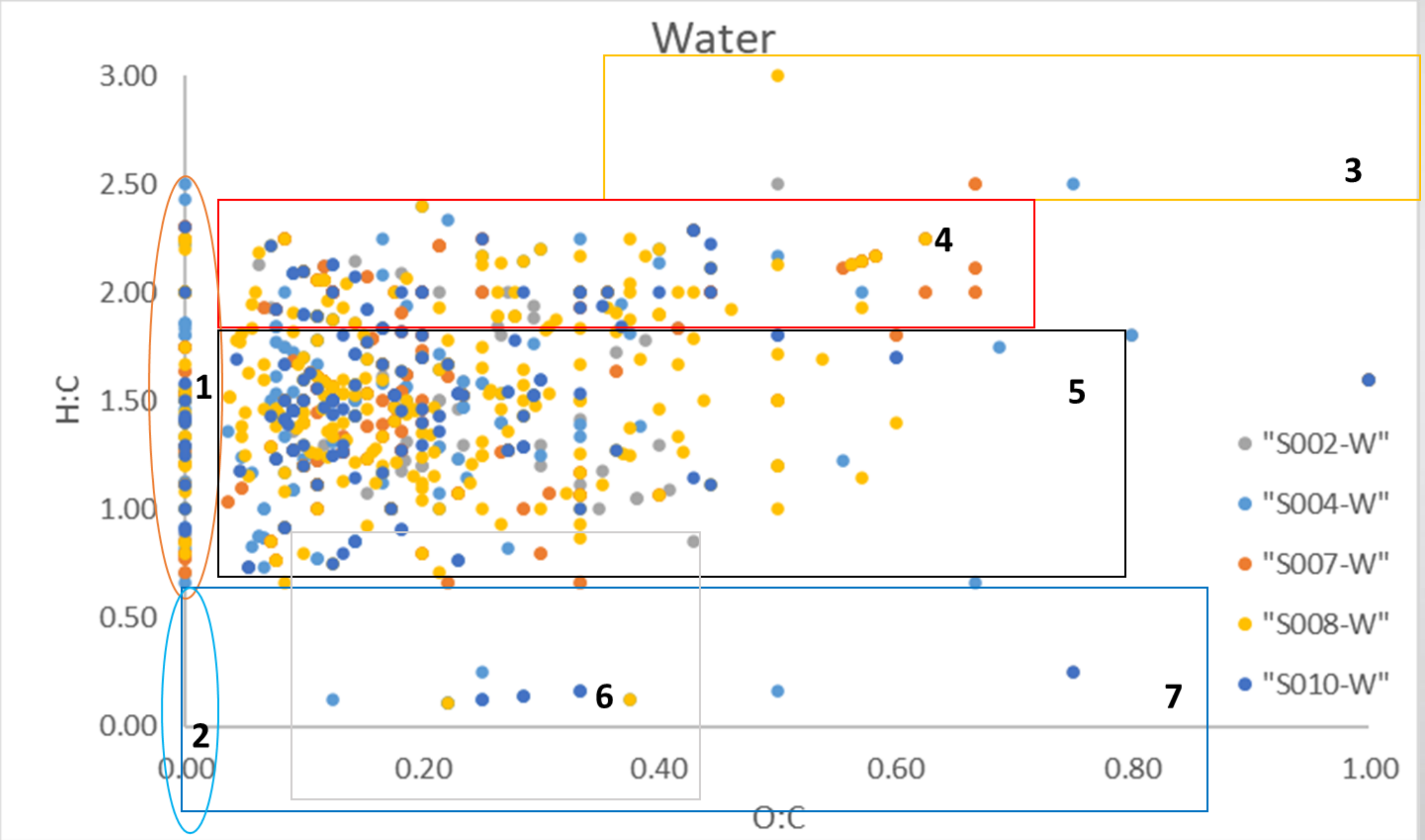


- A VKD is a plot of the H:C against the atomic ratio of oxygen to carbon (O:C) of a specific compound. This further separates compounds based on their degree of saturation (H:C ratio) and by oxygen-containing classes (O:C ratio).

- Theoretical Van Krevelen diagram of selected contaminants of concern taken from the US Environmental Protection Agency's DSSTox library.

Ng, B., Quinete, N. and Gardinali, P. (2022), Differential Organic Contaminant Ionization Source Detection and Identification in Environmental Waters by Nontargeted Analysis. Environ Toxicol Chem, 41: 1154-1164. https://doi.org/10.1002/etc.5268

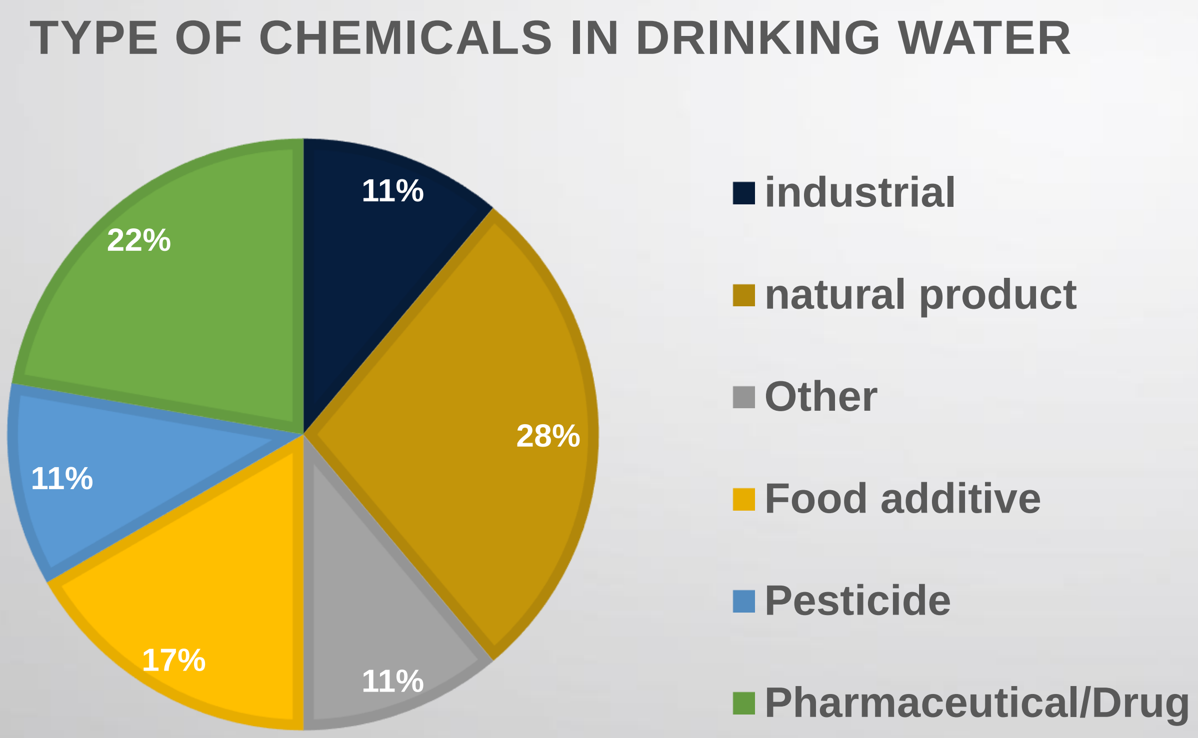
Water samples
Venn Krevelen Plot



1. Aromatic hydrocarbons
2. PCBs
3. PEG/PPG
4. Surfactants
5. Pesticides, bisphenols and phthalates
6. PBDEs
7. PFAS

Water samples

788 features detected in water → 20 unique features detected in common



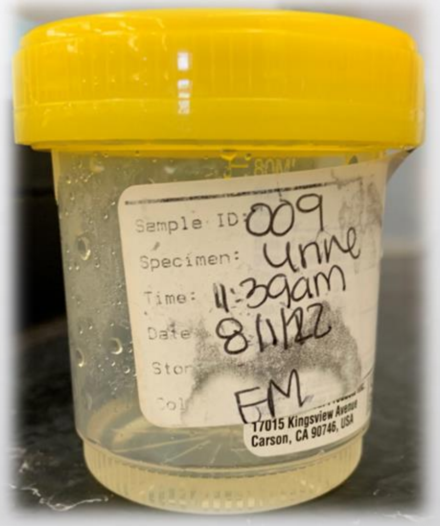
Compound	DF	Peak Area
Jasmone	100	2.63E+09
6-Phenyl-1-hexanol	80	1.18E+09
4-Ethoxy ethylbenzoate	100	8.64E+08
Isophorone	80	8.27E+08
Valerophenone	80	5.5E+08
Meleagrin	70	3.76E+08
Cuminaldehyde	60	1.38E+08
vanillyl nonanoate	50	9.32E+08
3721	50	1.5E+09

Urine Collection and Analysis

- Dilution factor of 2, 5, 10, 20, and 50

- Unhydrolyzed
  - 1:20 dilution with LC grade water
  - Online-SPE

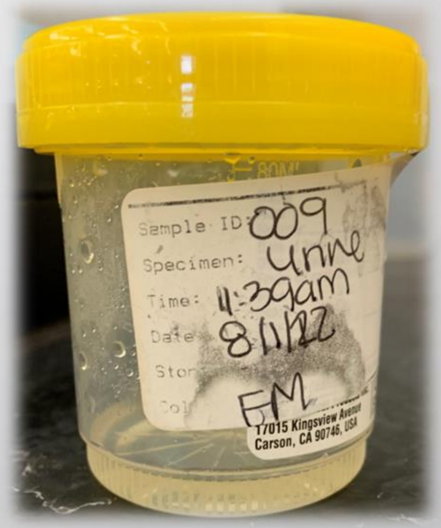
- Hydrolyzed
  - Hydrolysis overnight (37°C)
  - β-glucuronidase/arylsulfatase enzyme
  - 1:20 dilution with LC grade water
  - Online-SPE



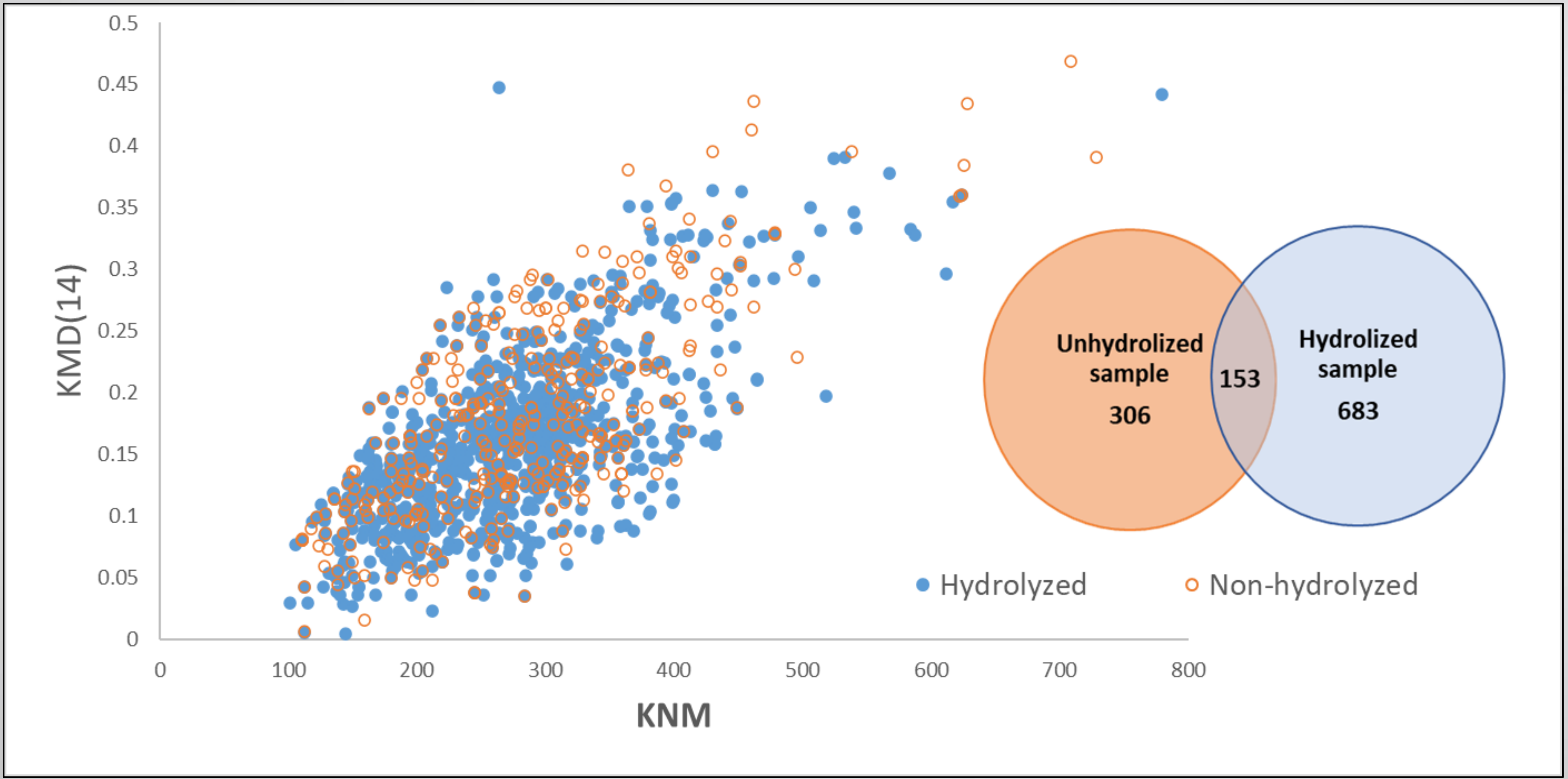
Determination of urine dilution factor

- Spiked QC standards to evaluate the best dilution to minimize matrix effects but still capable of detecting a good amount of compounds
- If the retention time shift is more than 0.5 min or the peak area varies more than 50 % of the average, it would be considered as retention time fail or peak area fail.

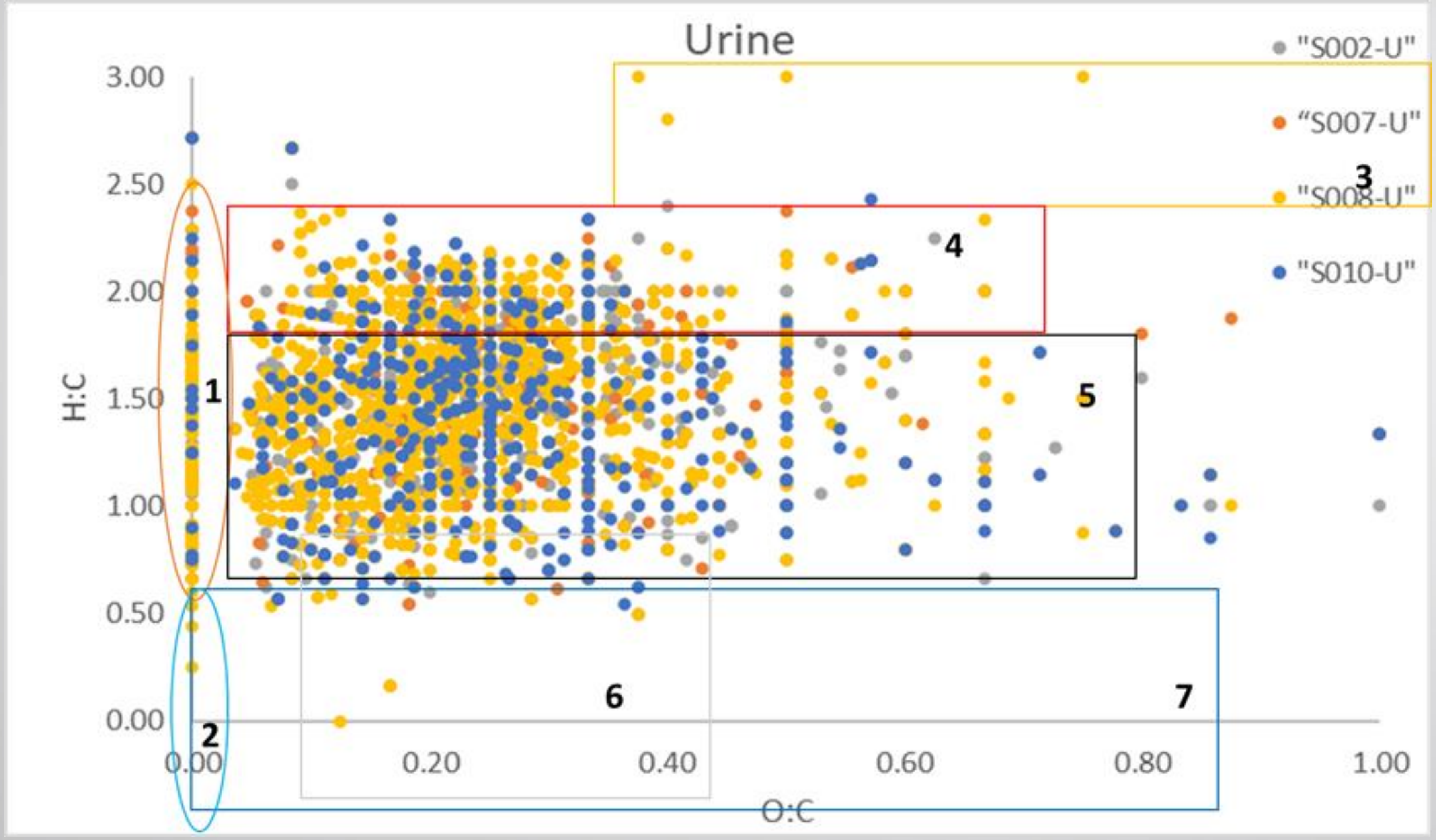
Dilution factor	QC detected	Retention time failed	Peak Area failed
2	12	33.3 %	58.3 %
5	13	23.1 %	46.2 %
10	13	15.4 %	61.5 %
20	15	13.3 %	20.0 %
50	14	21.4%	14.3 %



Comparison Hydrolyzed vs Unhydrolyzed



Urine samples
Venn Krevelen Plot



1. Aromatic hydrocarbons
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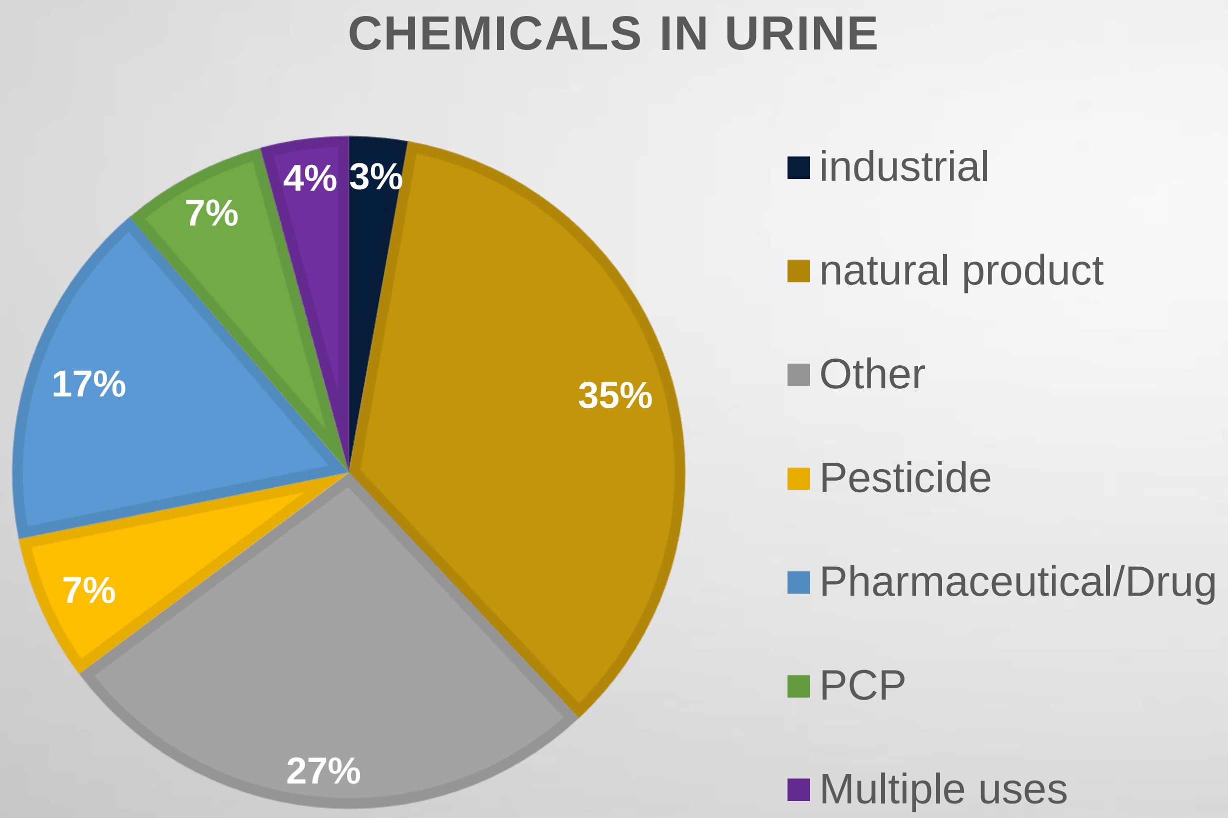
6. PBDEs
7. PFAS

Urine samples

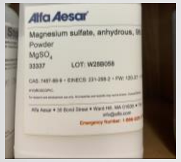
5121 features detected in urine samples → 265 unique features detected in common



Compound	DF (%)	Peak Area
Tetradecanedioic acid	75	5.65E+10
Hippuric acid	88	3.64E+10
Piperanine	75	3.49E+10
S-NONYL-CYSTEINE	63	2.83E+10
7-Methylguanine	63	2.01E+10
Cyclo(Ala-Ile)	63	1.86E+10
N-Phenylacetylglutamine	75	1.23E+10
Capryloylglycine	75	1.03E+10
N2,N2-Dimethyl-guanosine	75	1E+10
Glycocholic acid	100	4.51E+08
Glycoursodeoxycholic acid	100	1.6E+09
Triticonazole	100	2.6E+09

Food extraction: QuEChERS

Sample Homogenization

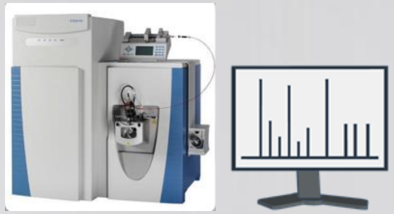


QuEChERS Salts

Addition of Organic Solvent (ACN)

Centrifuge/ Supernatant

PSA and GCB clean up

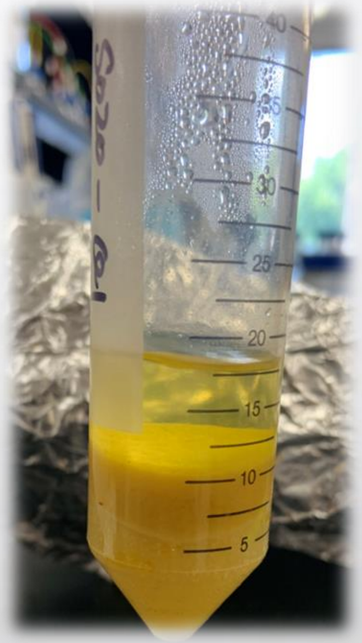


Orbitrap Analysis

Food extraction: QuEChERS

- Original proposal
  - Methanol for organic solvent
  - Cloudy after PSA/GCB clean up

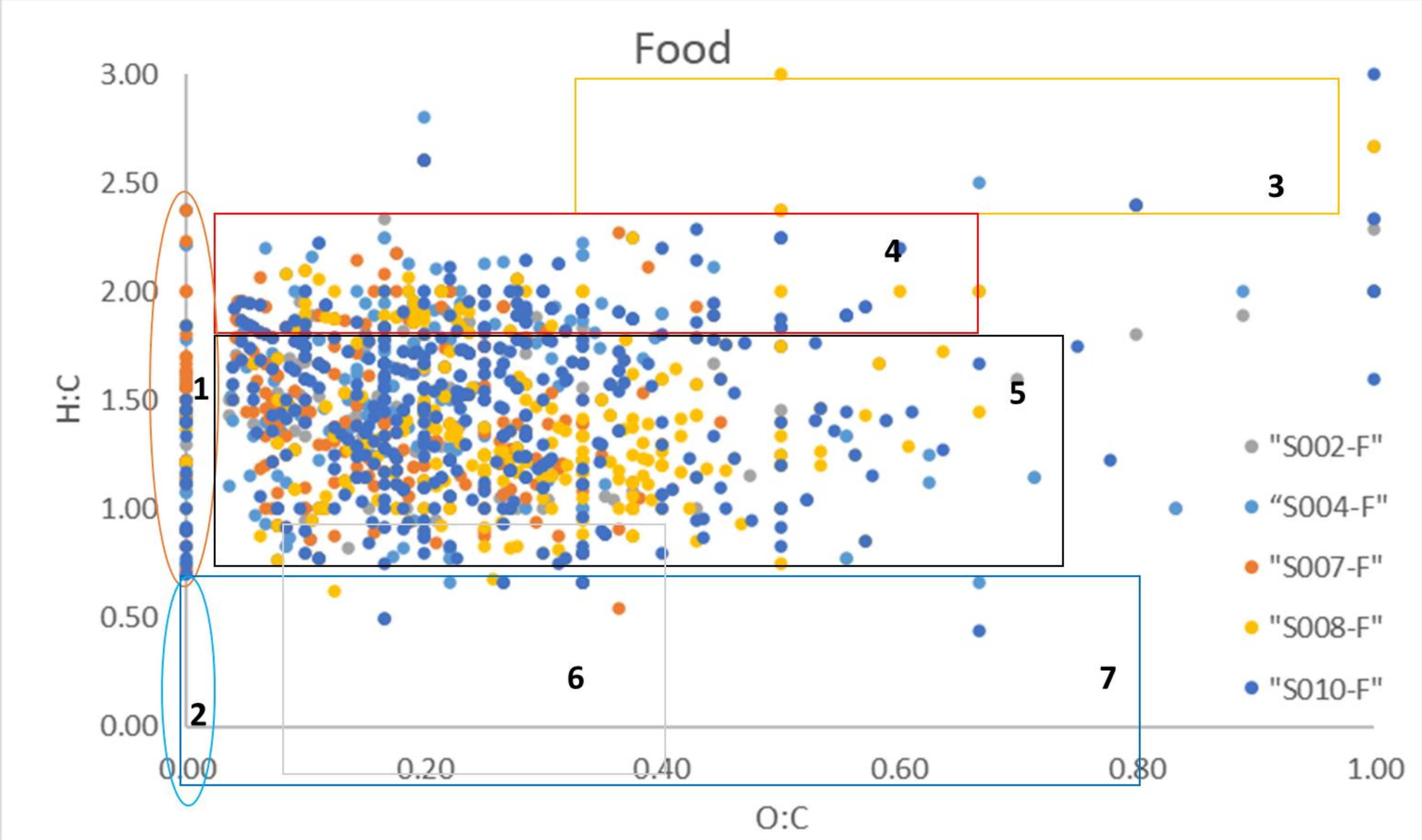
- Acetonitrile
  - Replace Methanol with ACN



Food extraction: QuEChERS

Compounds	log Kow	monitored ions (m/z)	detection mode	ACN	MEOH	ACN/MEOH
sucralose	-1	395.007	-	514975	138609	**3.7**
hydrochlorothiazide	-0.1	295.957	-	30492507	6973708	**4.4**
caffeine	0.16	195.088	+	173896997	40041329	**4.3**
lincomycin	0.29	407.221	+	69941827	226729204	**0.3**
sulfamethoxazole	0.48	254.059	+	510609753	302274447	**1.7**
trimethoprim	0.73	291.145	+	798986842	293952394	**2.7**
norcocaine	1.96	290.139	+	2508331676	651262182	**3.9**
carbamazepine	2.25	237.102	+	1220011570	746093554	**1.6**
diltiazem	2.79	415.169	+	2009629433	150171042	**13.4**
atrazine	2.82	216.101	+	4379749352	2080194051	**2.1**
diphenhydramine	3.11	256.17	+	2955706292	1517110694	**1.9**
diclofenac	4.02	294.009	-	122921945	34465135	**3.6**
fluoxetine	4.65	310.141	+	3852011163	1149551446	**3.4**
gemfibrozil	4.77	249.15	-	37854518	2360833	**16.0**
mefenamic acid	5.28	240.103	-	105742980	32179184	**3.3**
sertraline	5.29	306.081	+	732006025	98904061	**7.4**
clotrimazole	6.26	345.115	+	266510368	82800462	**3.2**

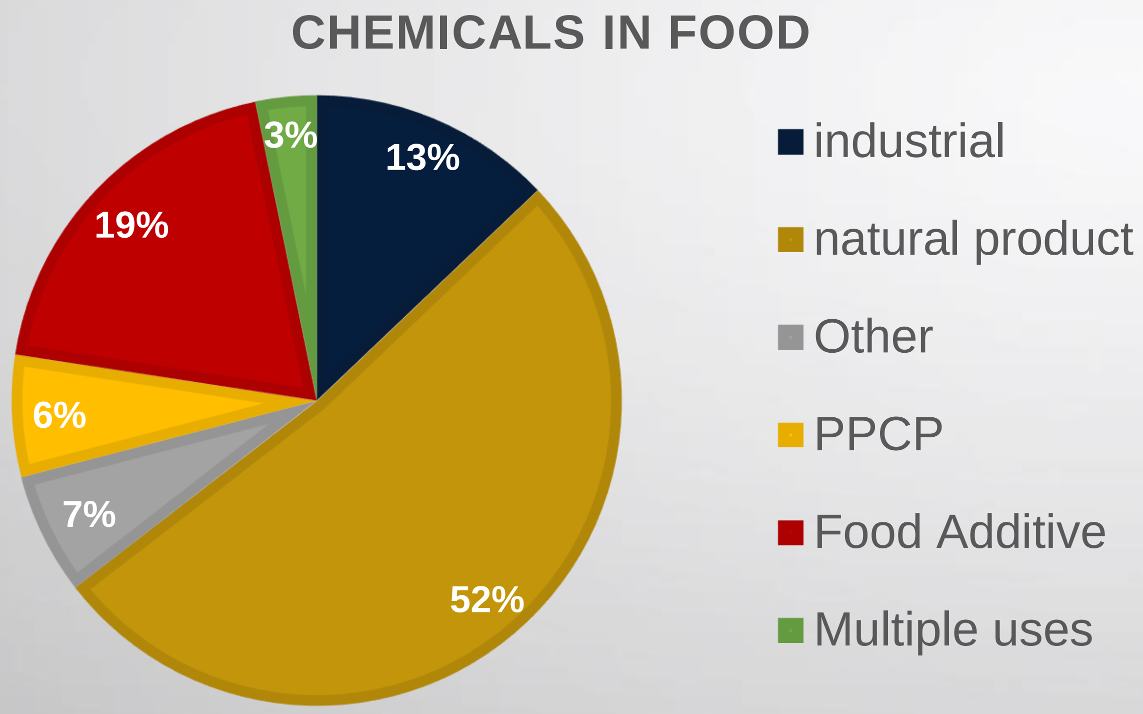
Food samples
Venn Krevelen Plot



1. Aromatic hydrocarbons
2. PCBs
3. PEG/PPG
4. Surfactants
5. Pesticides, bisphenols and phthalates
6. PBDEs
7. PFAS

Food samples

2552 features detected in food samples → 130 unique features detected in common

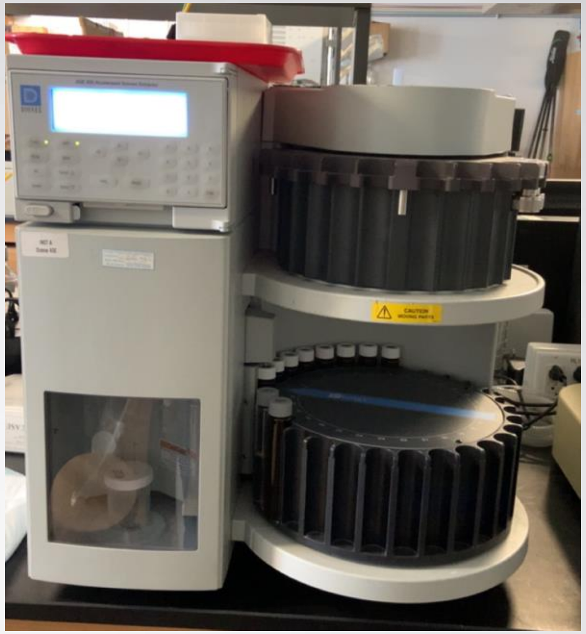


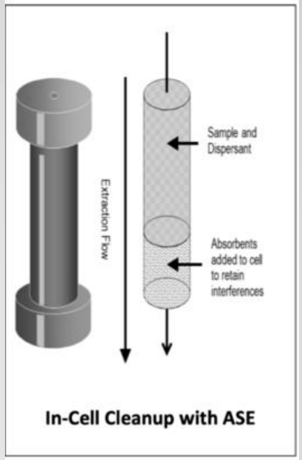
Compound	DF	Peak Area
Piperine	60	7.28E+10
Linoleoyl Ethanolamide	70	3.44E+09
Choline	90	3.12E+09
α-Eleostearic acid	50	1.71E+09
1-(P-TOLYL)-1-CYCLOHEXANECARBONITRILE	50	1.21E+09
3-hydroxy-N-(1-hydroxy-3-methylpentan-2-yl)-5-oxohexanamide	60	1.1E+09
UNII:TYL476W27Y	50	7.71E+08
Makomotine C	50	7.34E+08
2-(Dipentylamino)-1-(1,2,3,4-tetrahydro-9-phenanthrenyl)ethanol	50	7.24E+08
4-Indolecarbaldehyde	70	1.56E+08

Soil/Dust extraction: ASE vs USE

- Accelerated Solvent Extraction (ASE)
  - Heat and solvent
  - Automated and less dry down

- Ultrasonic Extraction (USE)
  - Manual
  - More solvents and more tedious





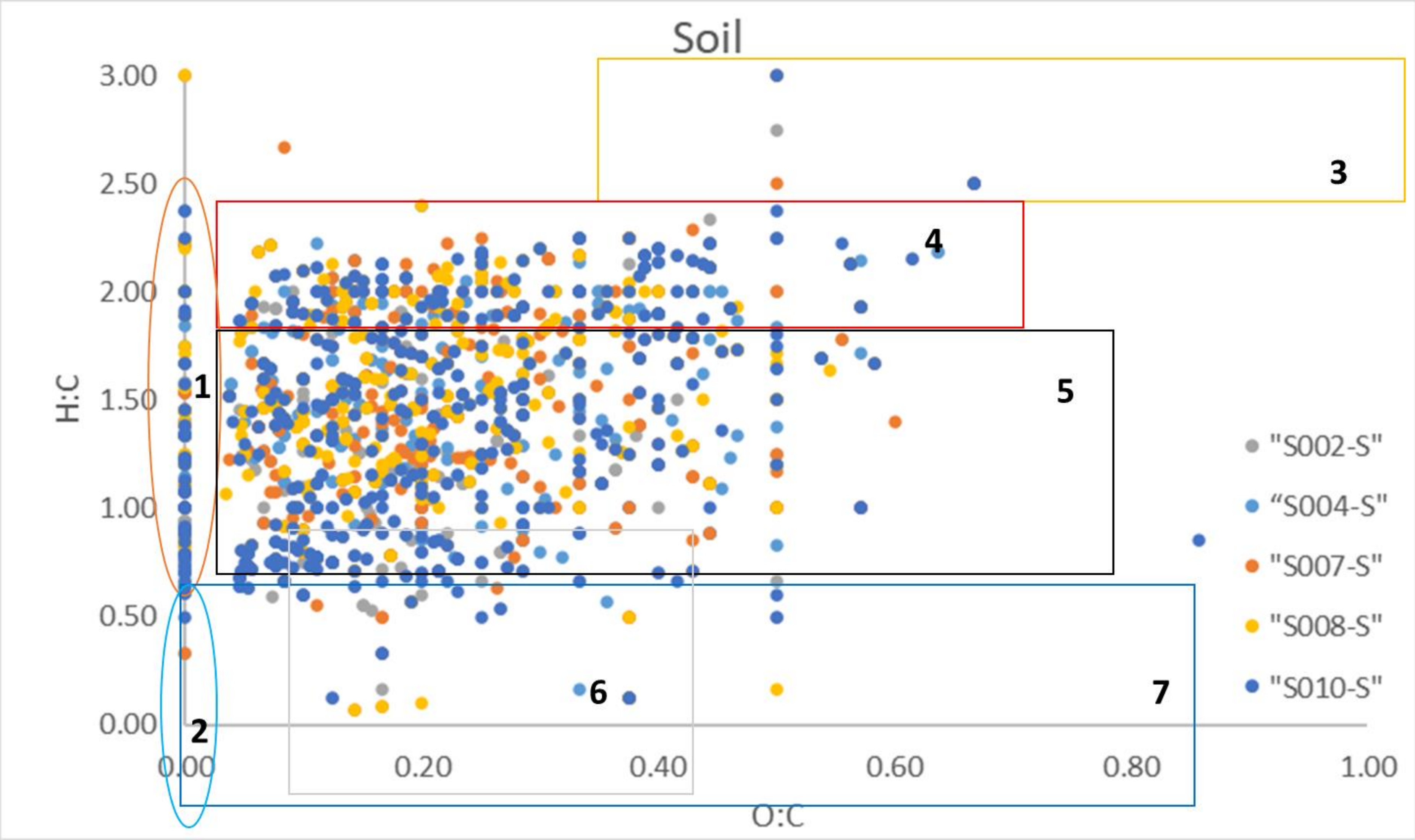
Samples were sieved

150 µm screen

Soil extraction: ASE vs USE

Compounds	log Kow	monitored ions (m/z)	detection mode	USE	ASE	ASE/USE
sucralose	-1	395.007	-	10384	526984	**50.7**
hydrochlorothiazide	-0.1	295.957	-	1203866	14859506	**12.3**
caffeine	0.16	195.088	+	6783448	34914652	**5.1**
lincomycin	0.29	407.221	+	44299853	52728722	**1.2**
sulfamethoxazole	0.48	254.059	+	47537279	68819583	**1.4**
trimethoprim	0.73	291.145	+	106036728	106687512	**1.0**
norcocaine	1.96	290.139	+	17971940	281767662	**15.7**
carbamazepine	2.25	237.102	+	149163592	213054956	**1.4**
diltiazem	2.79	415.169	+	442866	107161515	**242.0**
atrazine	2.82	216.101	+	218078938	637646920	**2.9**
diphenhydramine	3.11	256.17	+	73903594	56458058	**0.8**
diclofenac	4.02	294.009	-	21763	20481873	**941.1**
fluoxetine	4.65	310.141	+	52192443	44356941	**0.8**
gemfibrozil	4.77	249.15	-	20600183	3146426	**0.2**
mefenamic acid	5.28	240.103	-	31454	34239014	**1088.5**
sertraline	5.29	306.081	+	16515867	38614739	**2.3**
clotrimazole	6.26	345.115	+	5526113	5904298	**1.1**

Van Krevelen Plot for Soil samples



1. Aromatic hydrocarbons
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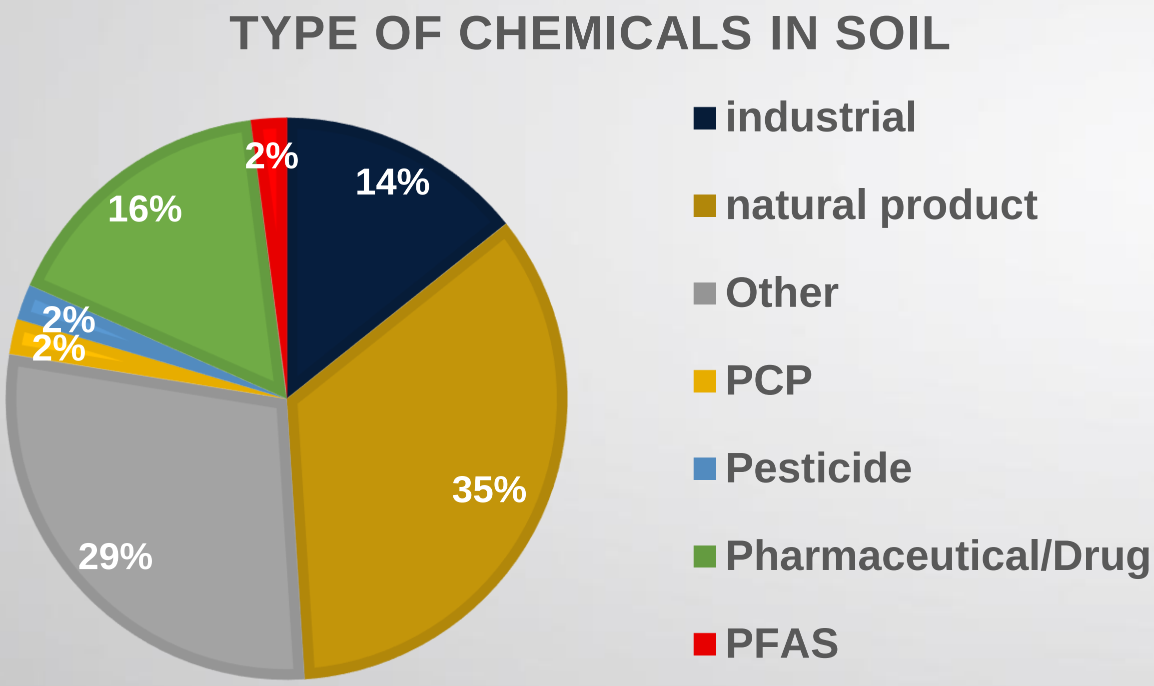
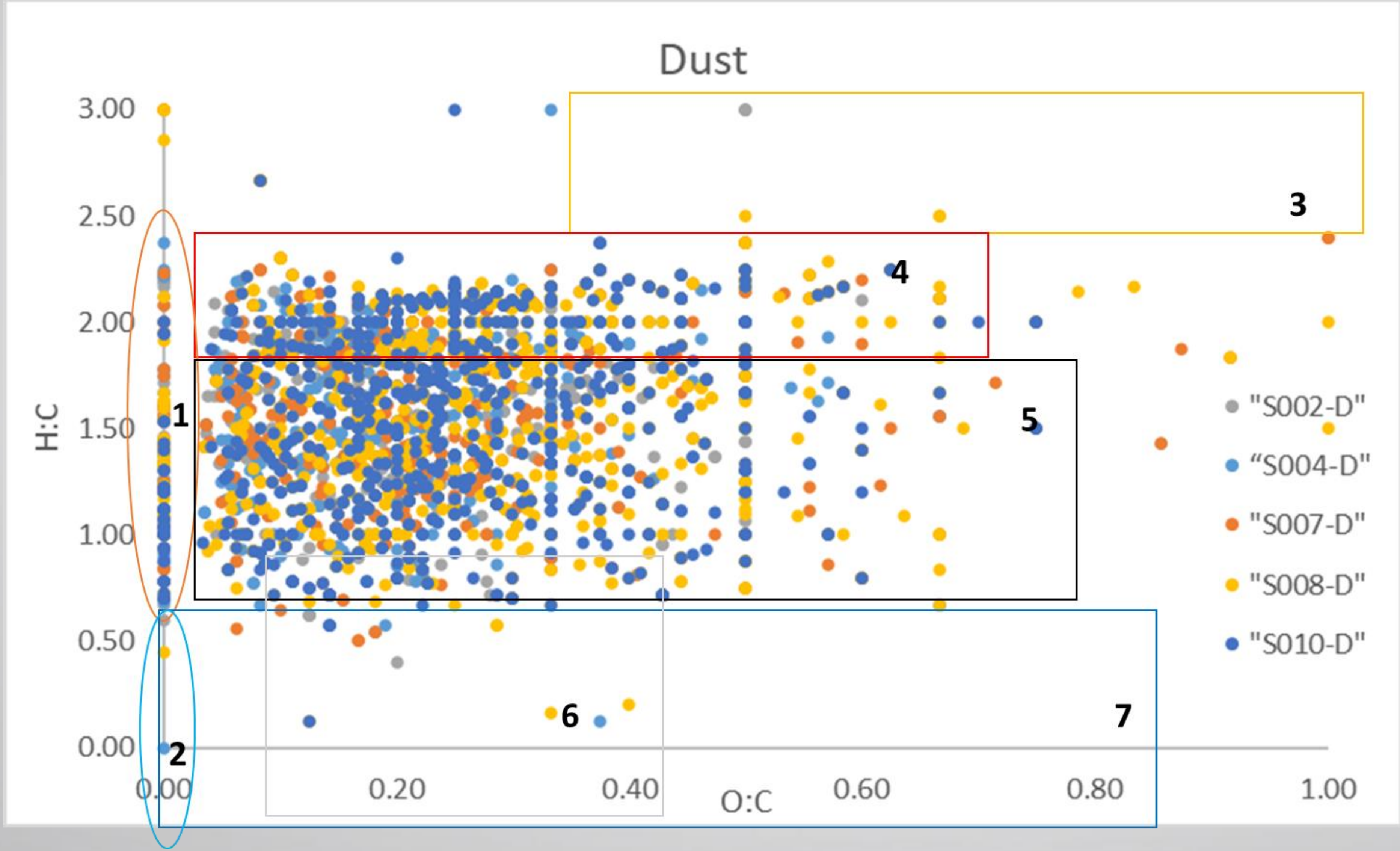
Soil samples

2239 features detected in soil samples → 107 unique features detected in common



Compound	DF	Peak Area
Caprolactam	90	4.84E+10
2,2-Methylenebis(4-ethyl-6-tert-butylphenol)	90	3.49E+09
(9Z)-9-Sulfo-9-octadecenoic acid	100	3E+09
BOC-GLU-OTBU	100	2.03E+09
1,7-Hydroxy-3-methylxanthone	90	1.3E+09
Pentamethylmelamine	90	9.09E+08
[6]-Gingerol	90	8.68E+08
N,N'-Diphenylguanidine	60	8.64E+08
3-O-Butyryl-1,2-O-isopropylidene-alpha-D-glucofuranose	80	7.71E+08
1-Cyclohexyl-2-azetidinecarboxylic acid	80	6.83E+08

Van Krevelen Plot for Dust samples



1. Aromatic hydrocarbons
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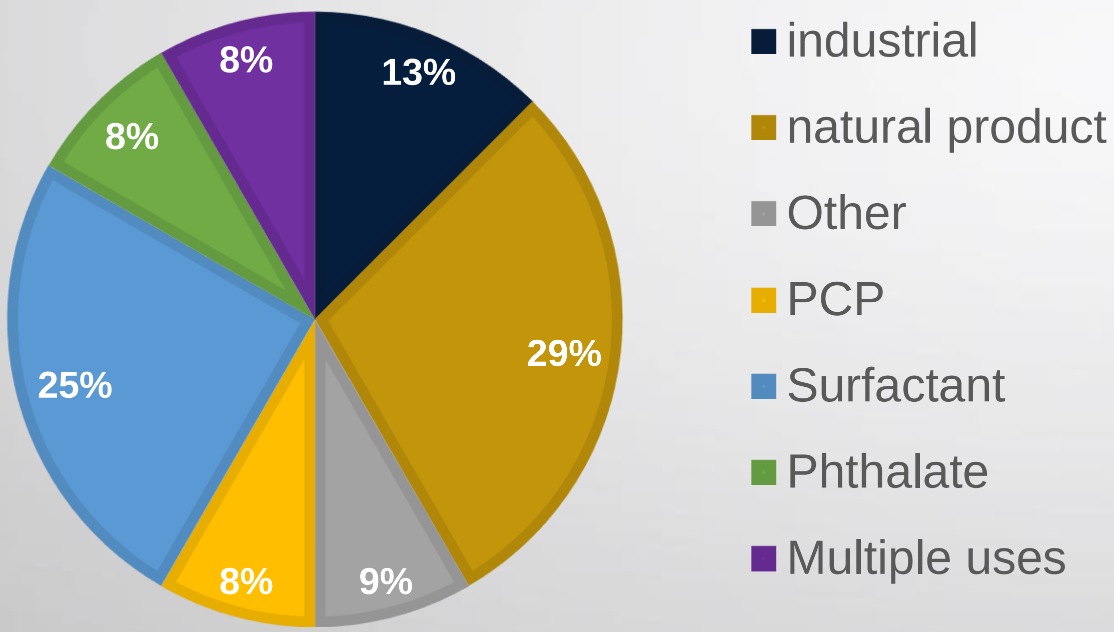
7. PFAS

Dust samples

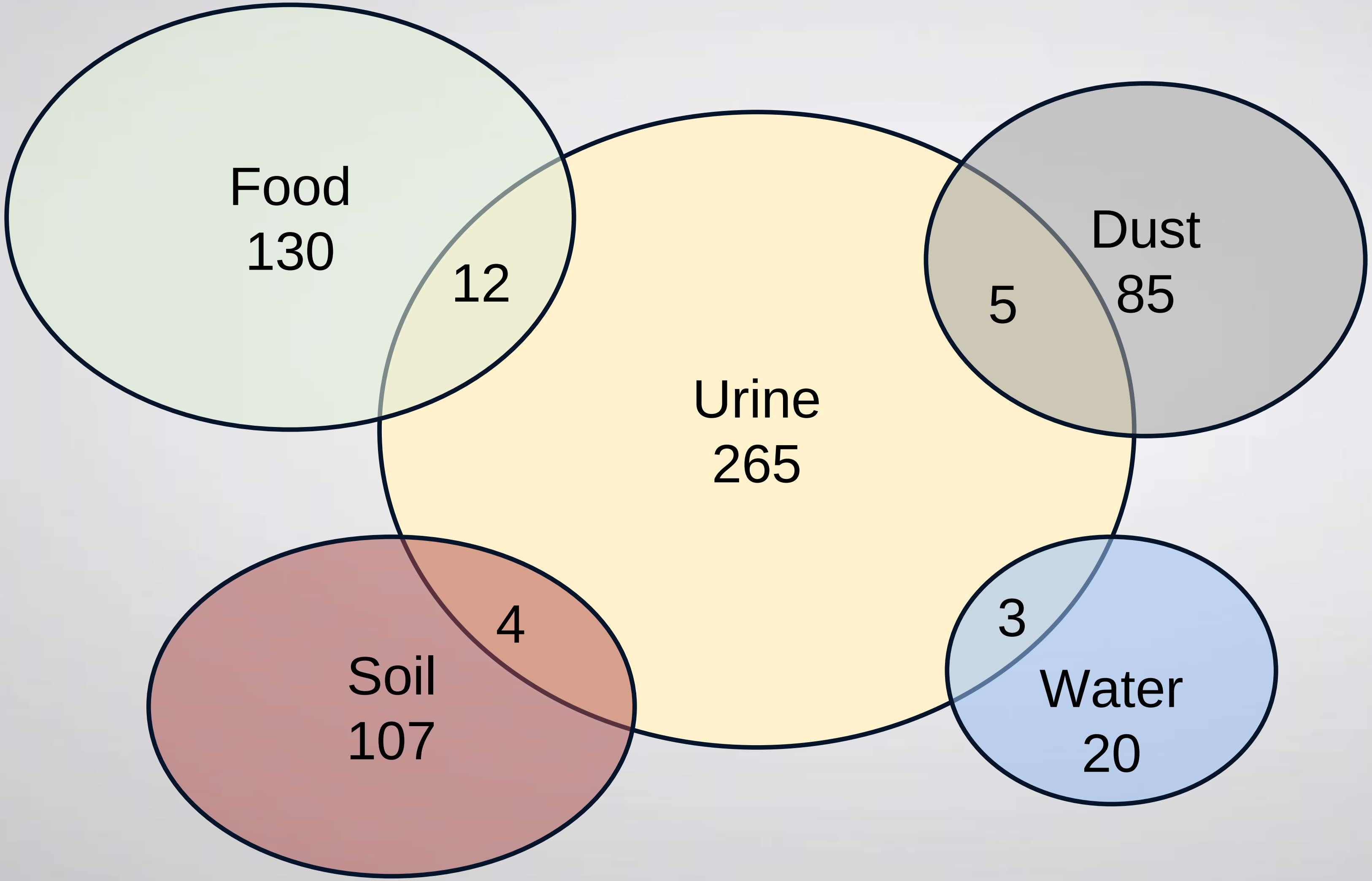
3218 features detected in soil samples → 85 unique features detected in common

CHEMICALS IN DUST



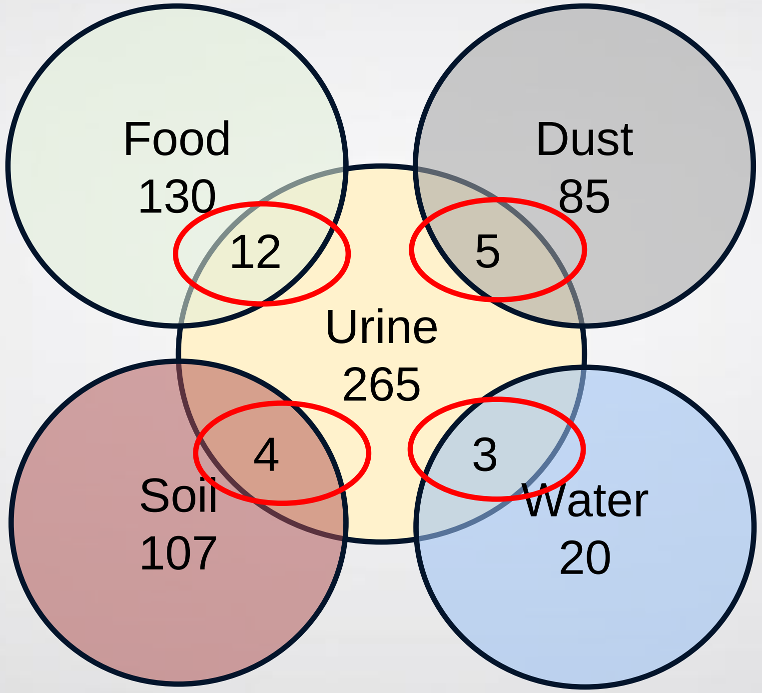
Compound	DF	Peak Area
Dodecyl sulfate	64	3.68E+10
Myristyl sulfate	73	2.39E+10
Bis(2-ethylhexyl) phthalate	73	9.83E+09
Pentadecyl hydrogen sulfate	55	5.36E+09
cetyl sulfate	73	4.1E+09
Linoleic acid	55	2.85E+09
Haplofungin D	82	2.2E+09
Tripropyl citrate	82	1.88E+09
Haplofungin F	82	2.19E+09
Azelaic acid	55	1.09E+09

Combined results – Group 1



Combined results – Group 1

Food vs Urine	Sources
(±)-Abscisic acid	Natural product
3-hydroxy-N-(1-hydroxy-3-methylpentan-2-yl)-5-oxohexanamide	Natural product
4-Indolecarbaldehyde	Industrial
CI-4	Other
Dobutamine	Pharmaceutical
F-36316 C	Natural product
Hexanoylcarnitine	Natural product
Naringenin	Food Additive/natural product
Pactamycin	Pharmaceutical
Phenacetin	Pharmaceutical/PCP
Piperanine	Pharmaceutical
Streptazone F	Natural product



Dust vs Urine	Sources
3,6,9,12,15,18-Hexaoxaicosane-1,20-diol	Industrial
3-[(3-Hydroxydecanoyl)oxy]decanoic acid	Natural product
Piperanine	Pharmaceutical
Tetraacetylethylenediamine	PCP (Bleaching agent, Surfactant, Cosmetics)
Uric acid	Natural product

Soil vs Urine	Sources
1-(Tripropoxymethoxy)propane	Other
Caprolactam	Industrial
Dibutyl ethylmalonate	Natural product
Oseltamivir	Pharmaceutical

Water vs Urine	Sources
Cuminaldehyde	Natural product/Food Additive
Isophorone	Industrial
Naphthaleneacetamide	Pesticide

Conclusions

- Recruitment of 73 children to date
- Extractions and Analysis
  - QuEChERS, ASE, and Urine Hydrolysis
- A total of 242 samples were collected and 180 were analyzed
- Initial results promising
  - Overlap of unique compounds in dust and soil and urine was observed
- Further Analysis to be conducted

Future Work

- Intensify recruitment with the training of more research assistants
- Continue collection of samples and analysis
- Post-processing of analyzed samples
- Submission of the first manuscript
- Creation of an online database
- Statistical Analysis to identify specific tracers and models to be used for soil and dust ingestion rate.

FIU

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Thank you for your Attention!

QUESTIONS ?

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