Implementing a chemical space mapping tool to improve interpretation of nontargeted analyses of biosolids: A proof-of-concept study

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[3]U.S. Geological Survey, California Water Science Center, Sacramento, California, USA

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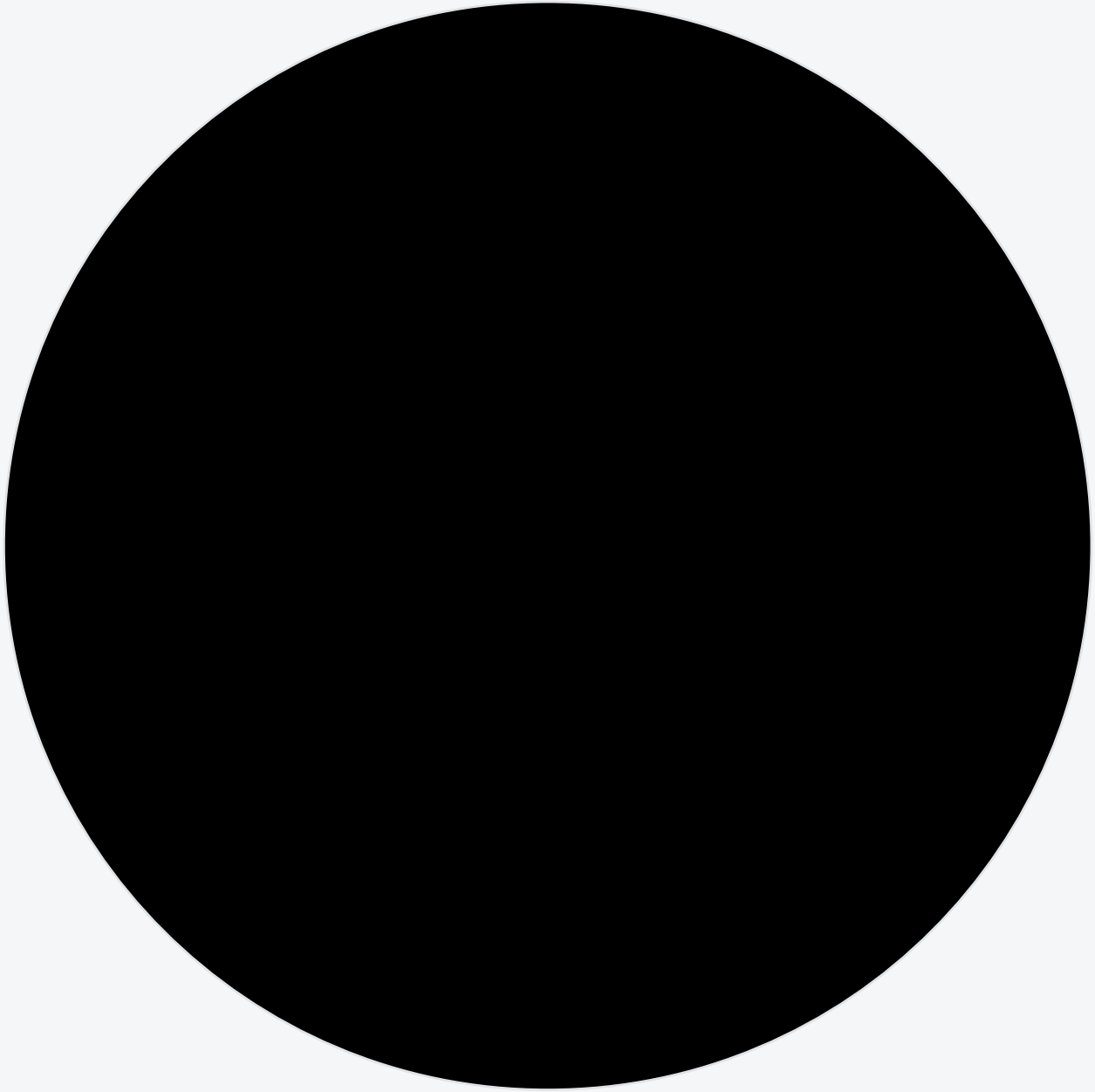

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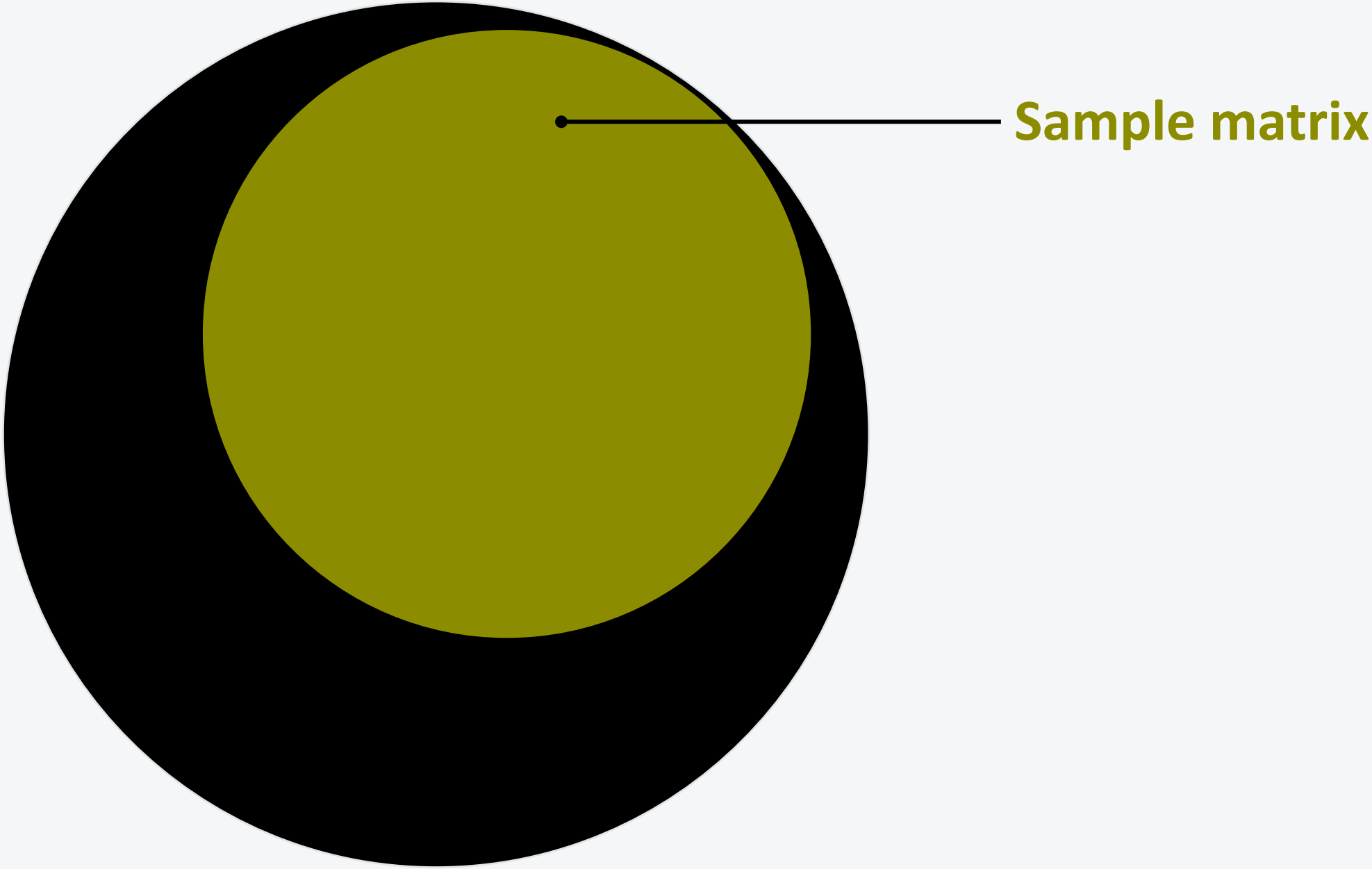

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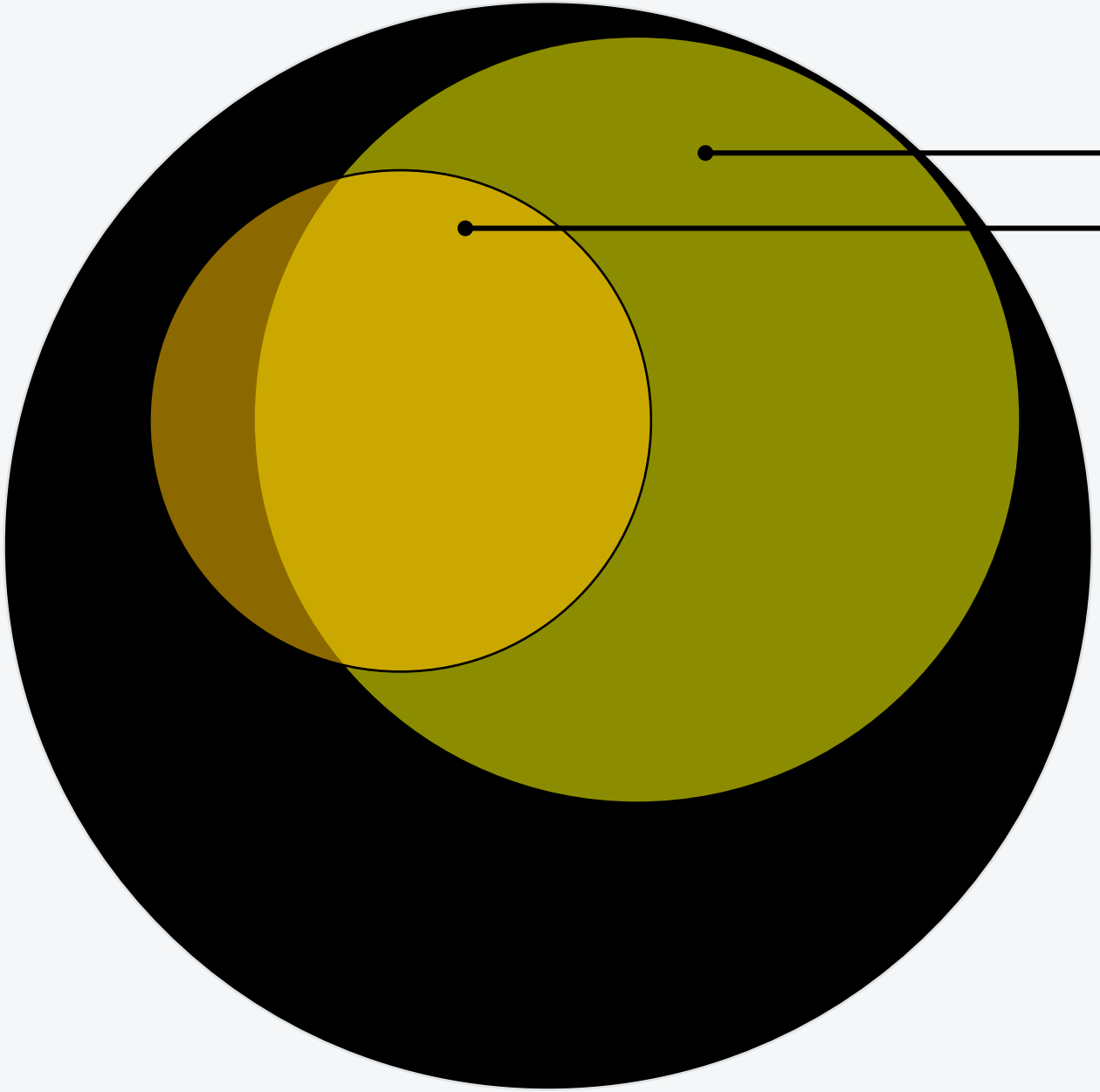

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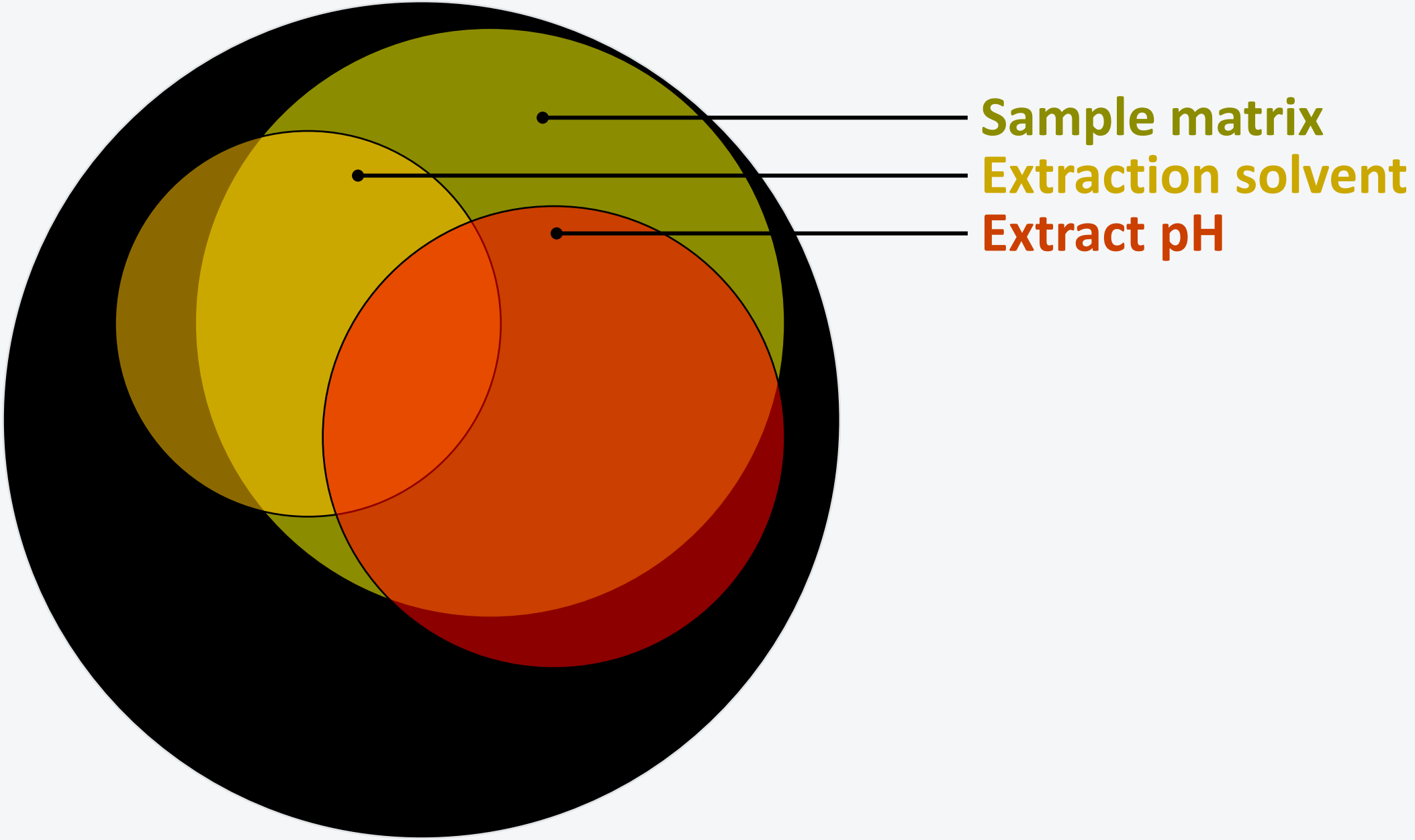

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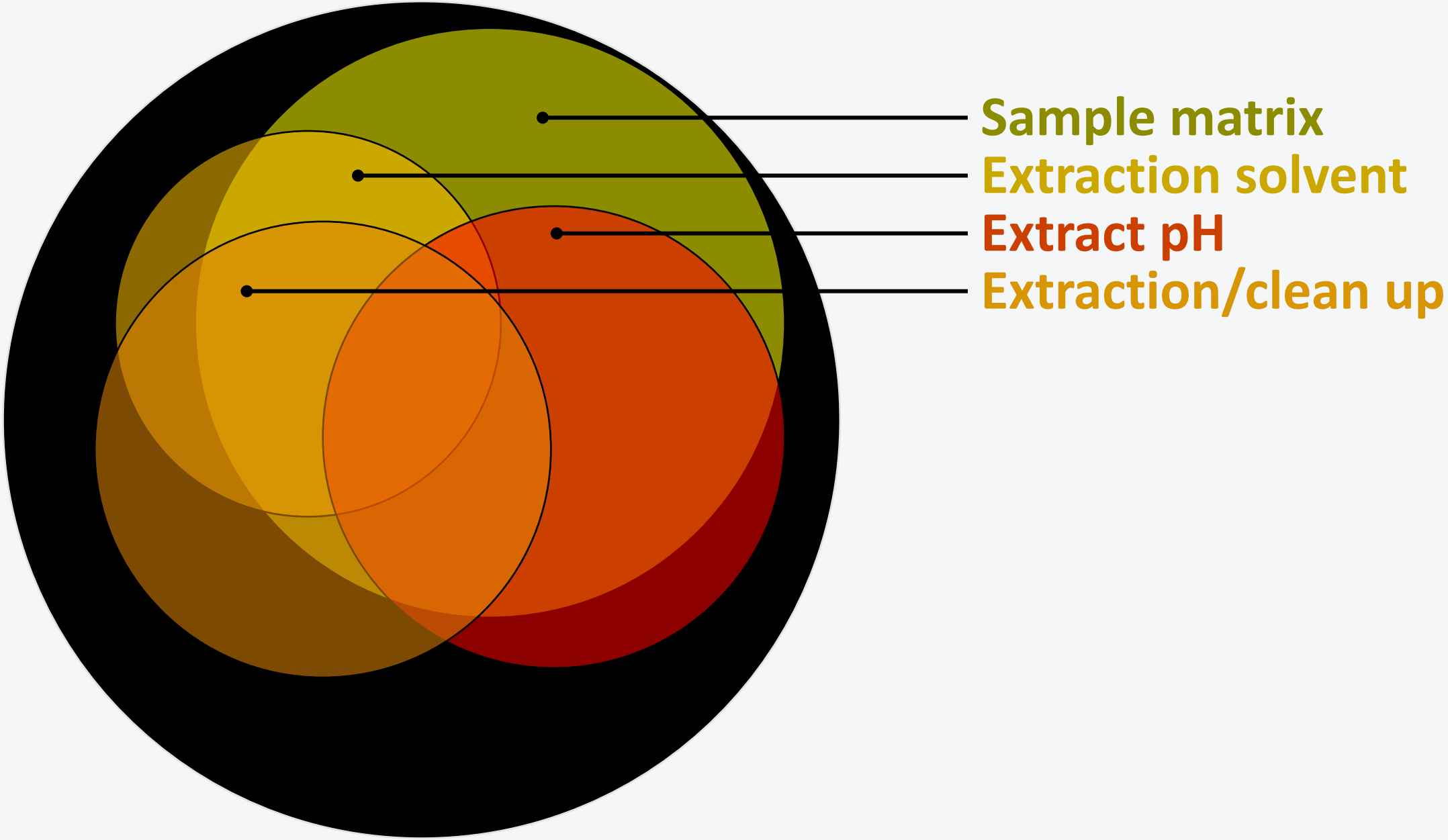

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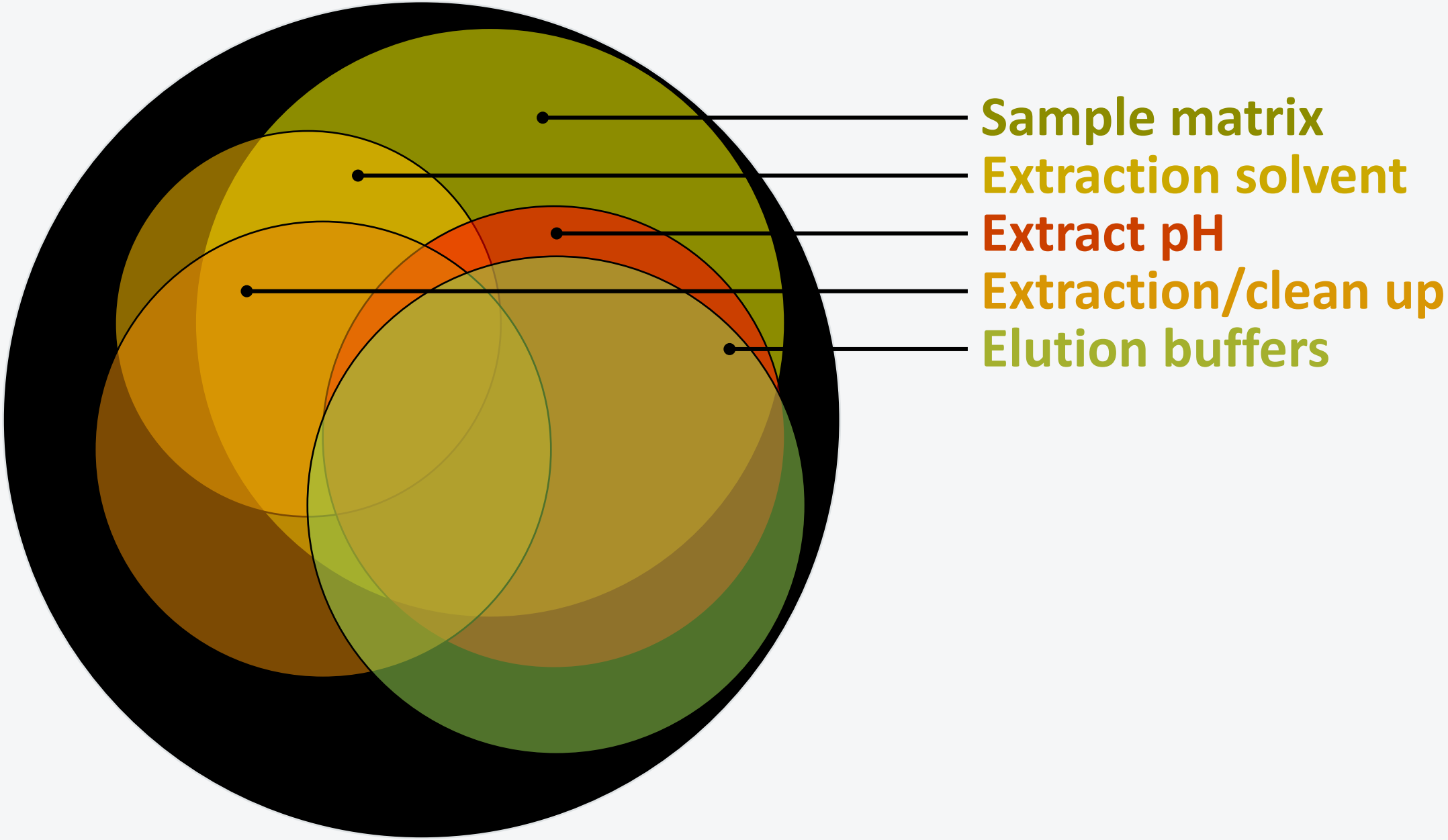

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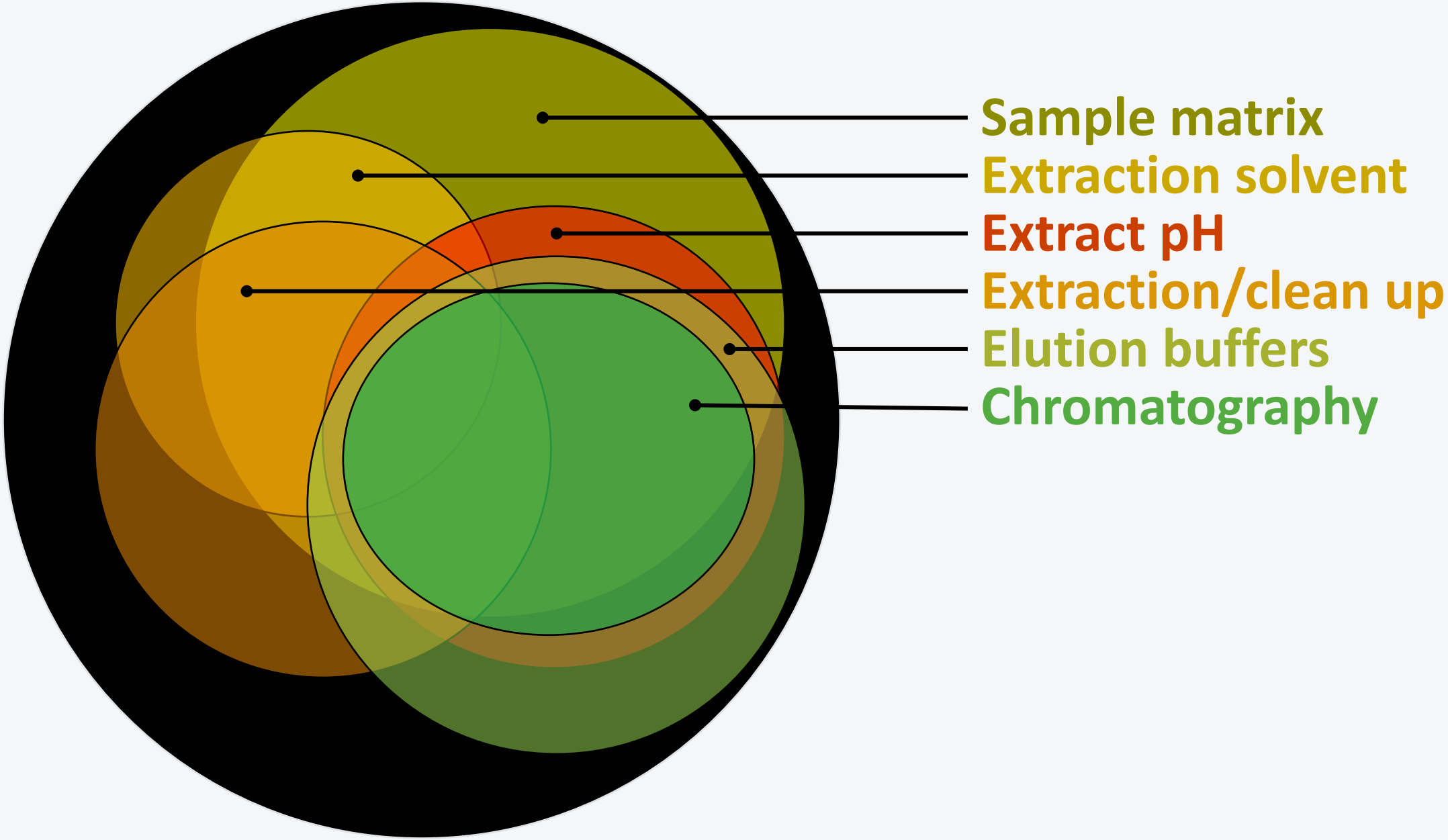

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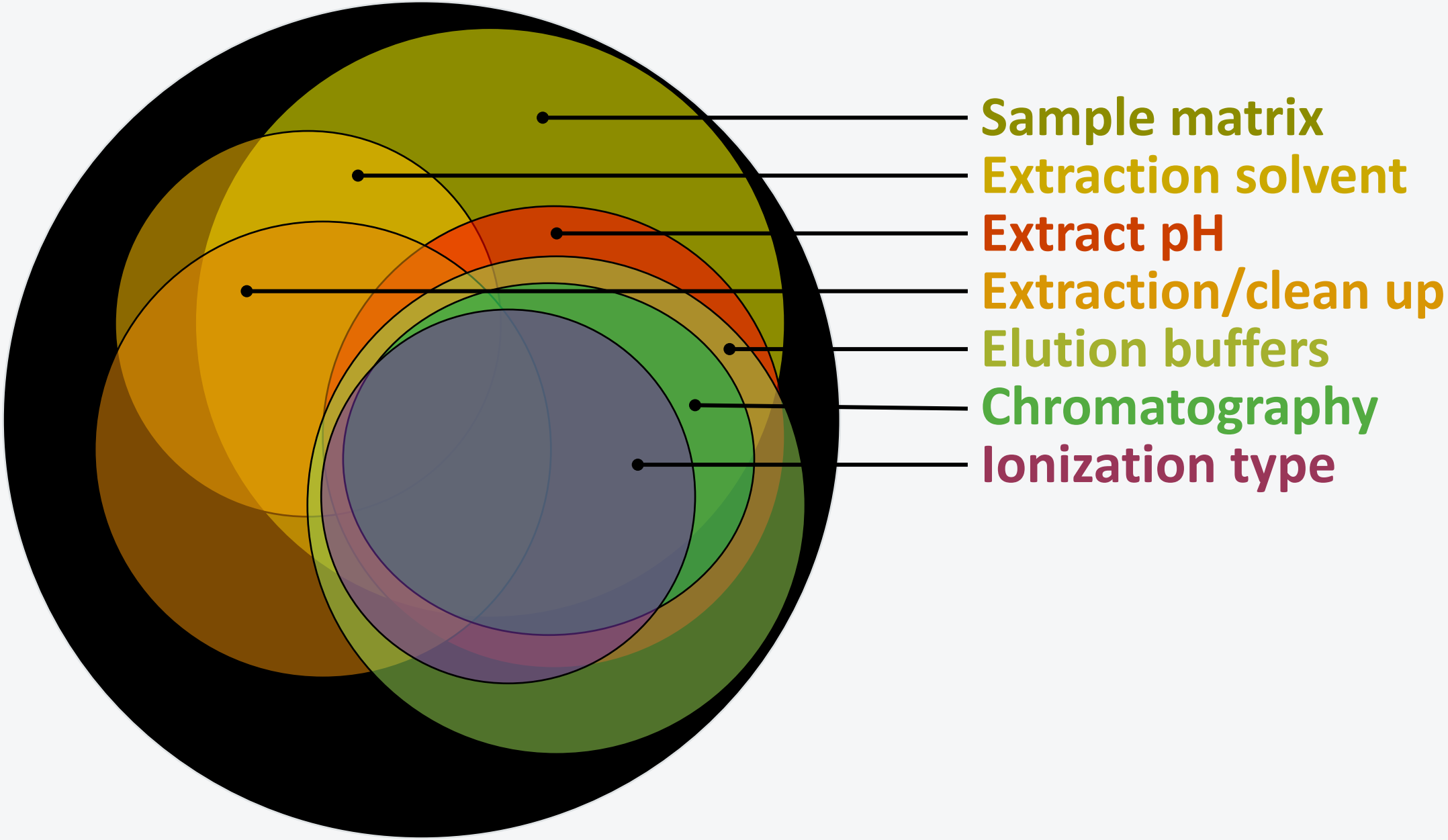

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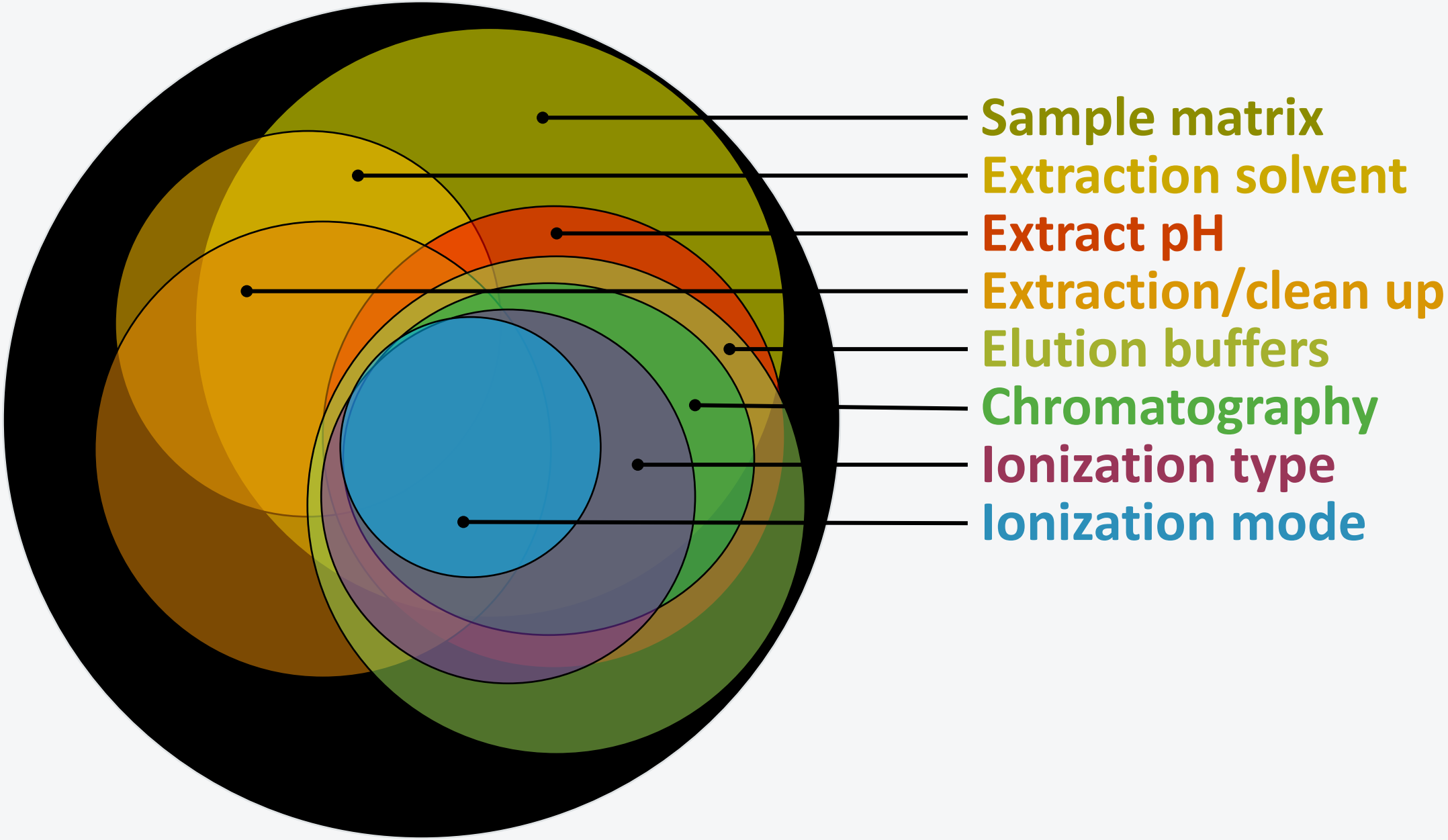

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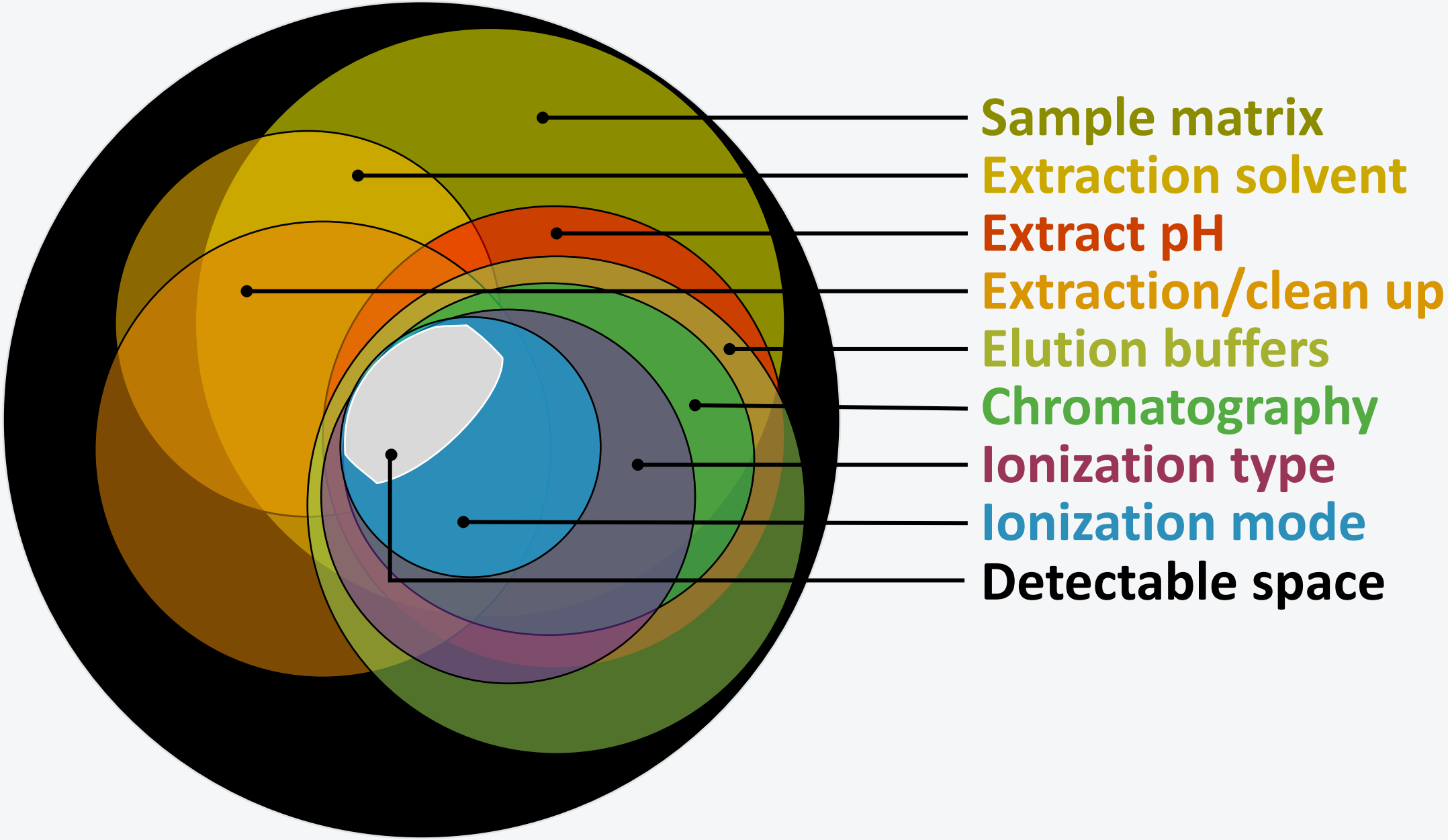

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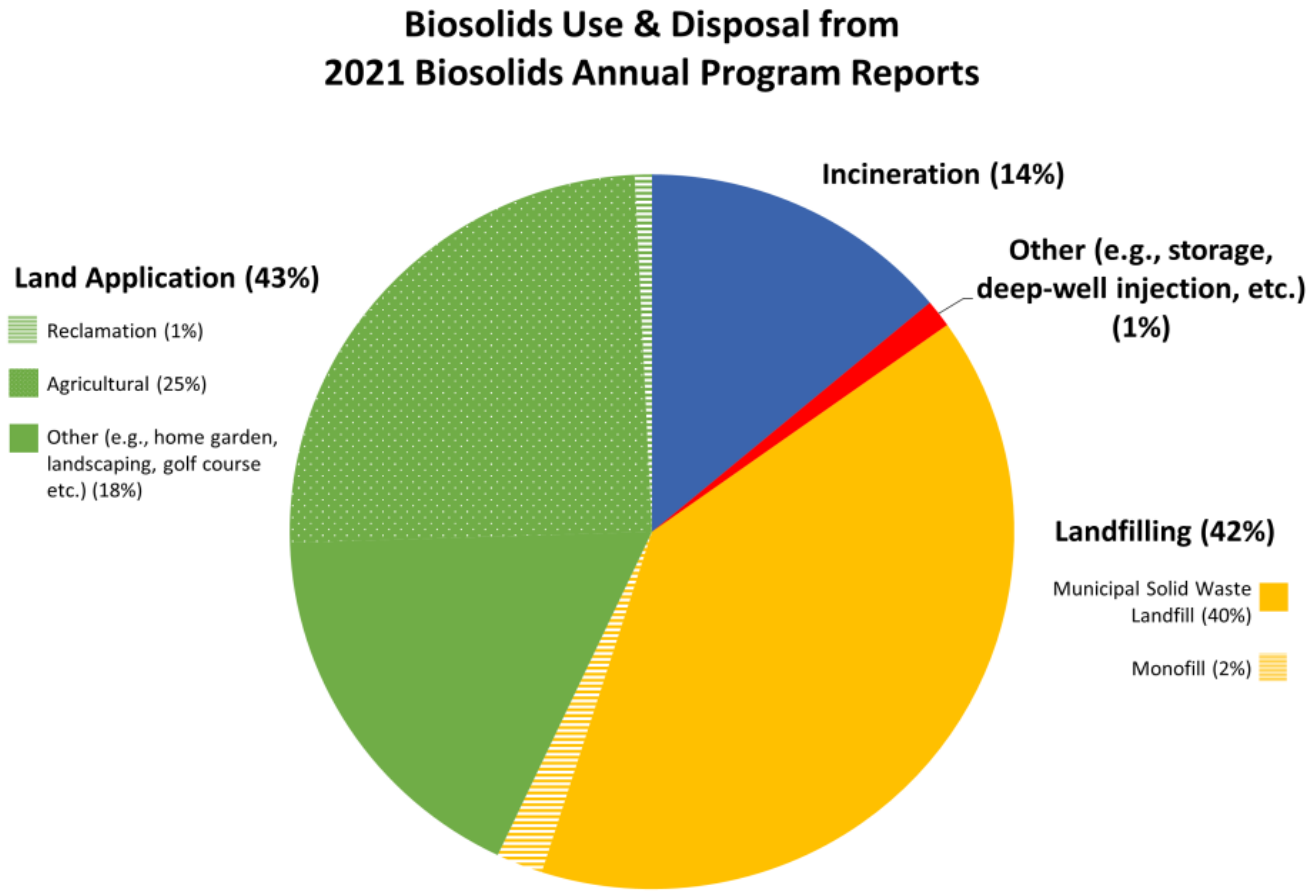
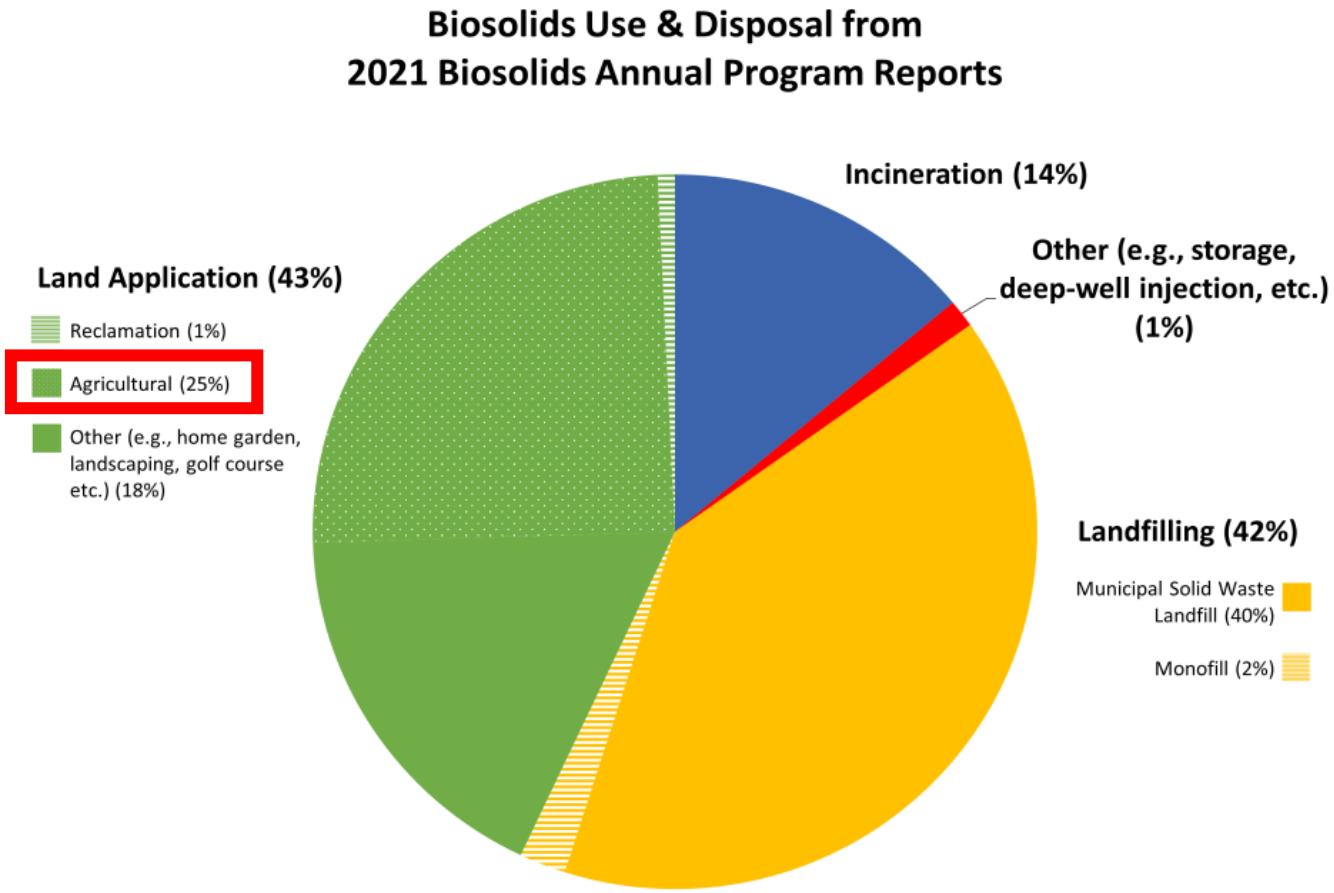


Basic Information about Biosolids: Biosolid Uses. https://www.epa.gov/biosolids/basic-information-about-biosolids#uses. Accessed 2023 Feb 16



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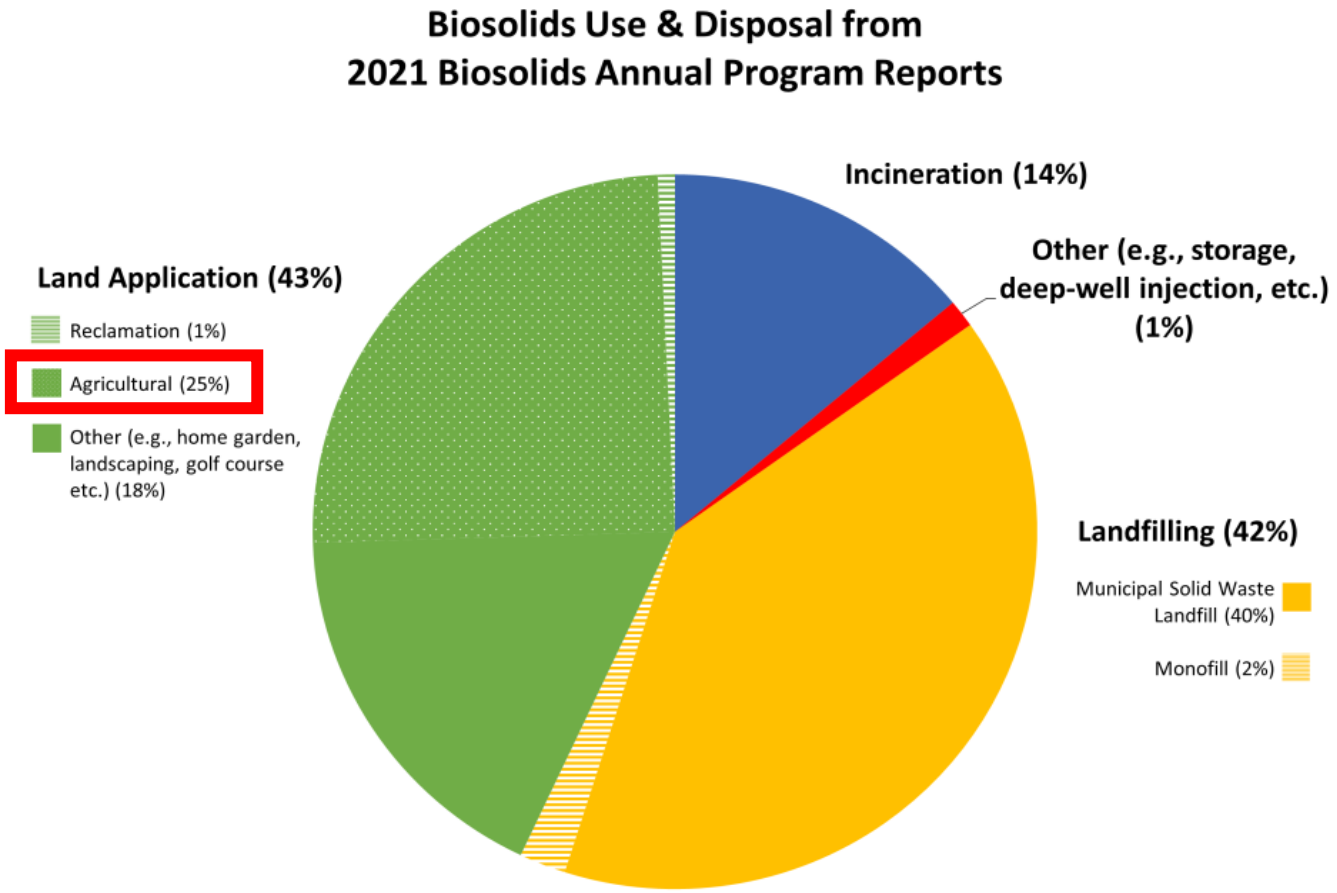

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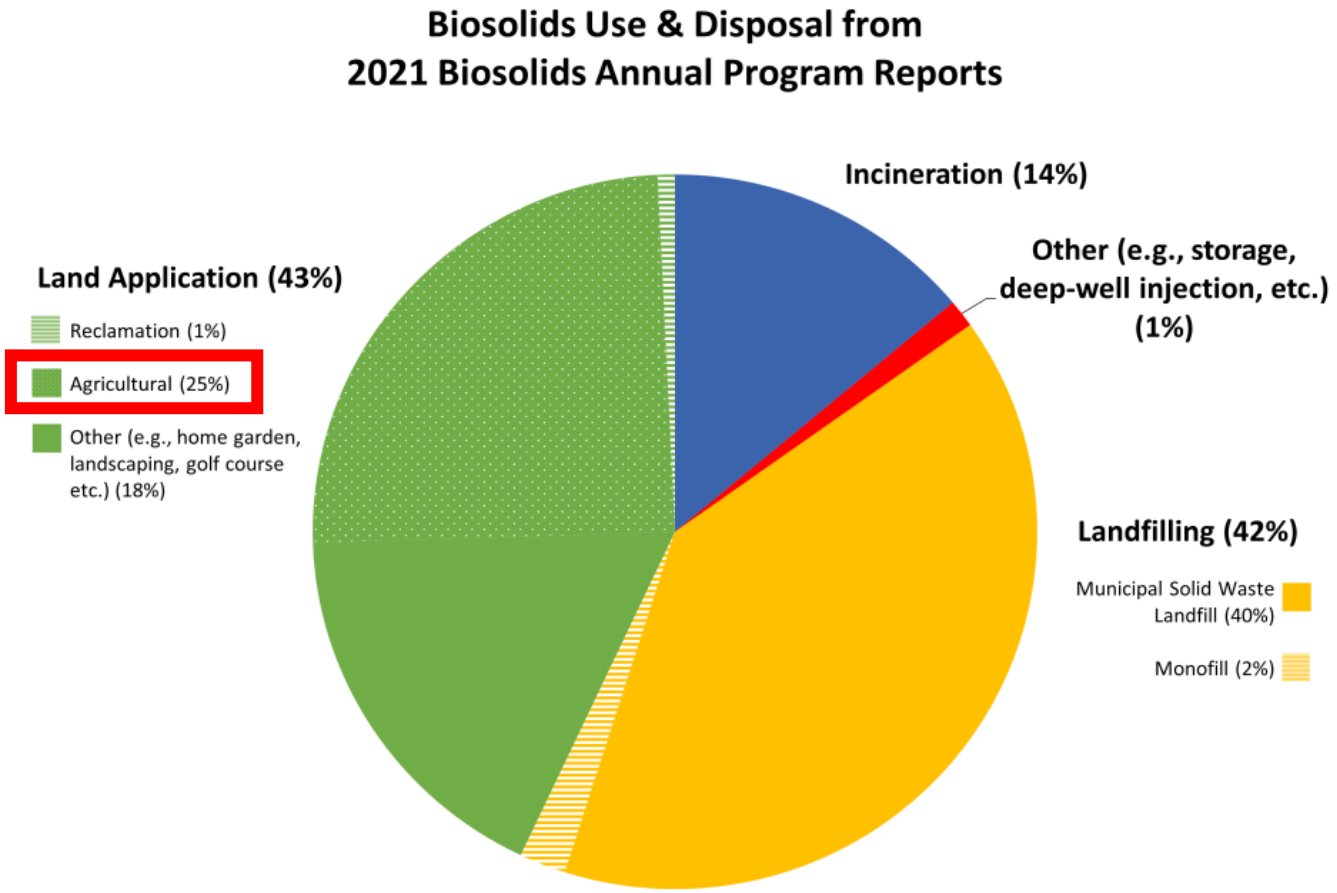
Biosolid land application can be beneficial:

- ✓ Improves soil qualities
- ✓ Supplies nutrients
- ✓ Diverts from landfilling & incineration



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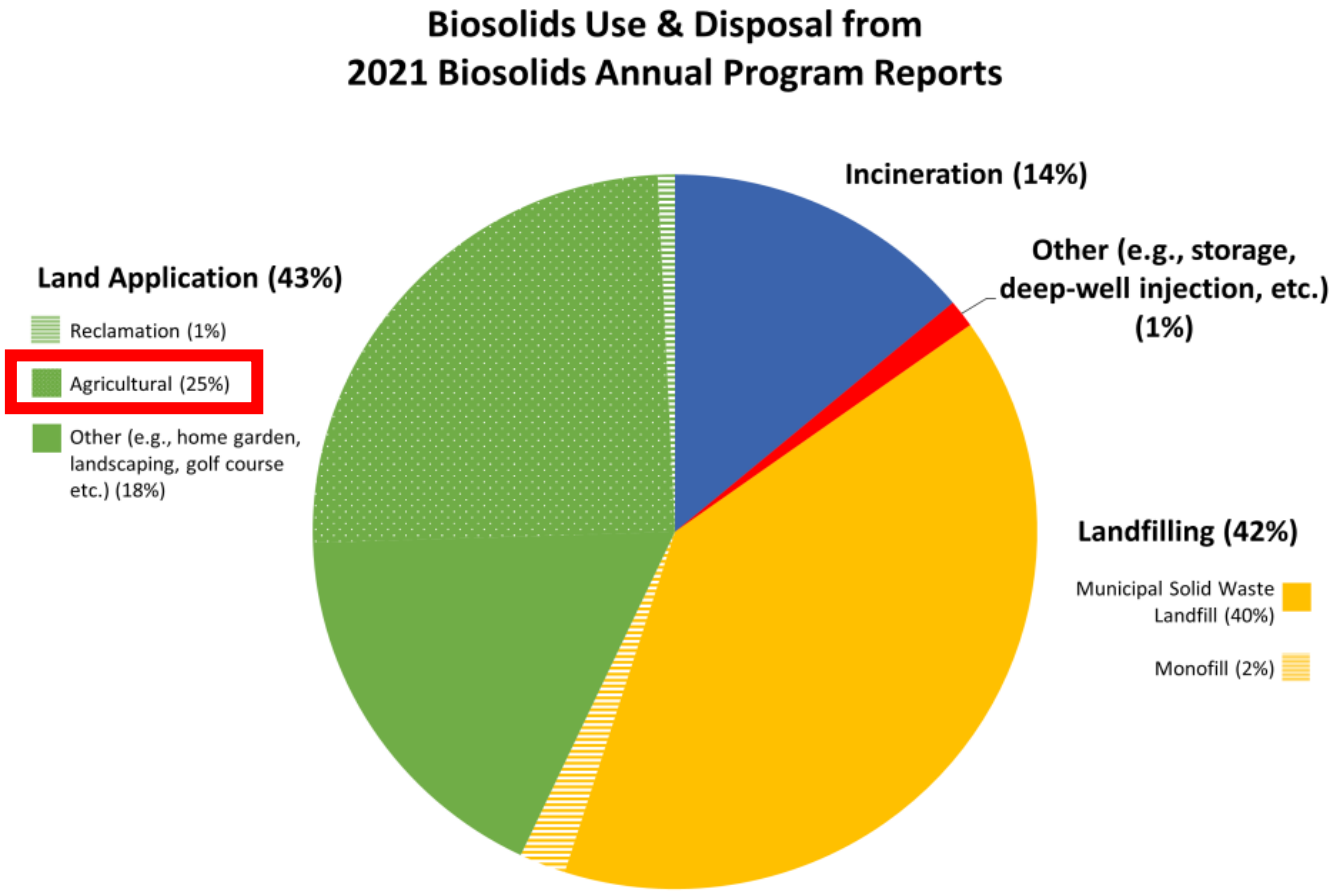
Current federal regulation (40 CFR Part 503) of biosolid quality includes:

- ➢ Limits on 10 heavy metals
- ➢ Requirements for pathogen & vector attraction reduction



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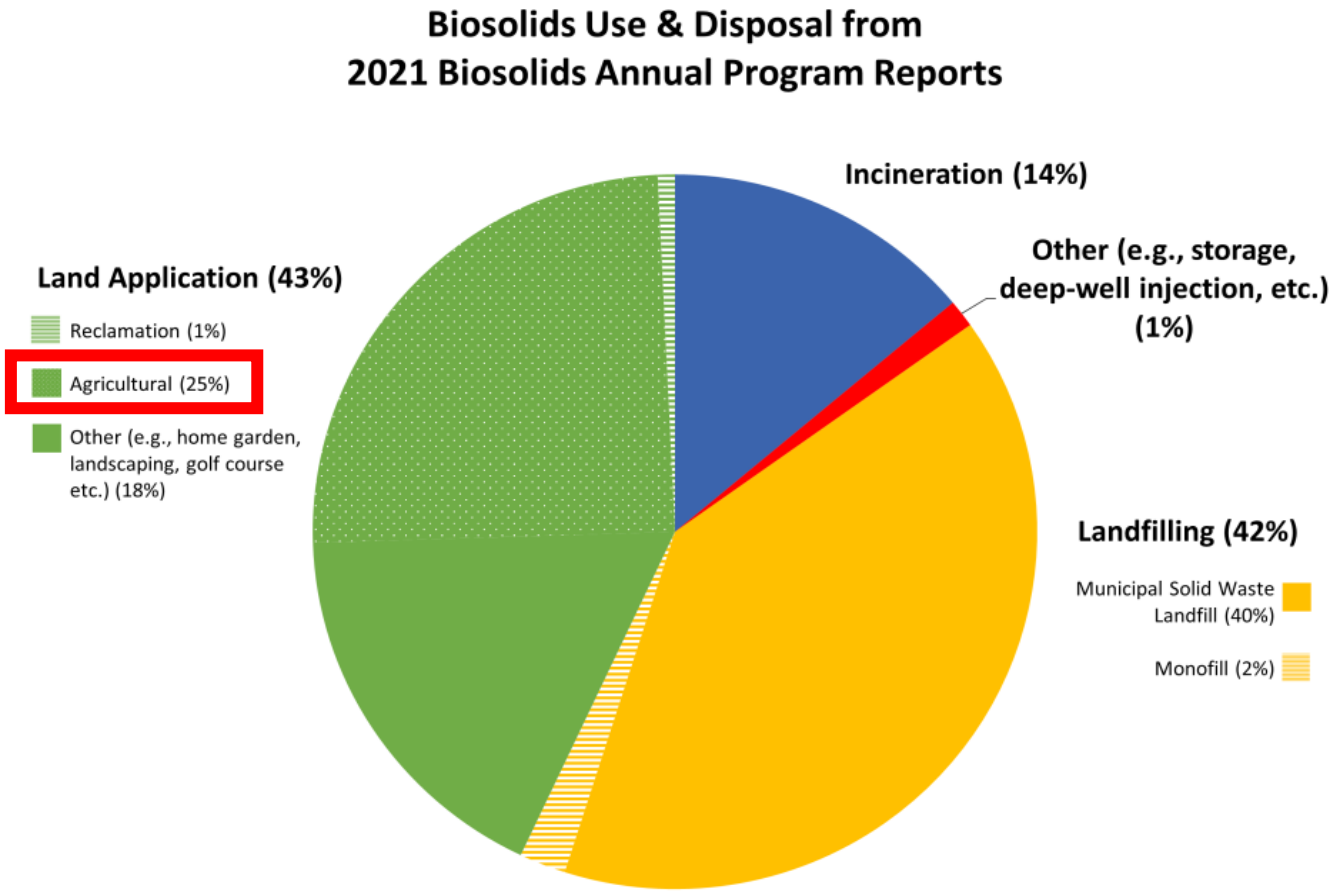
Currently, no regulations pertaining to organic contaminants exist



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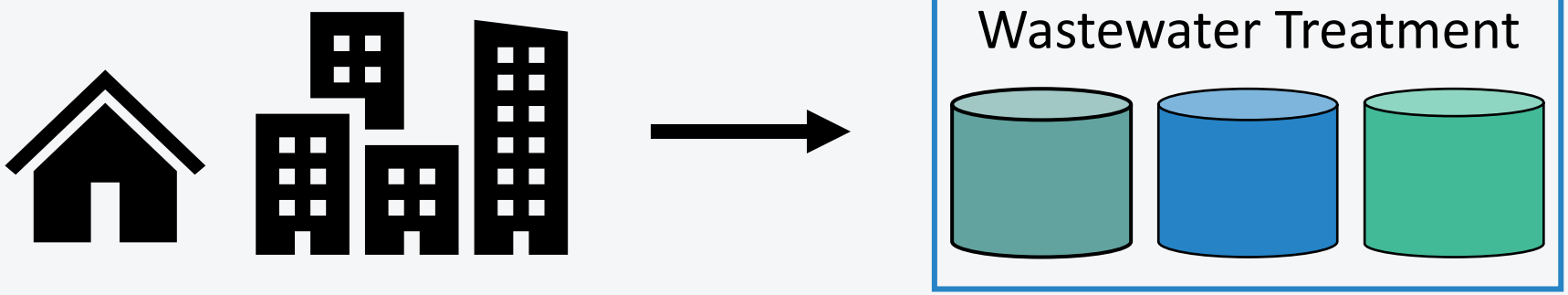
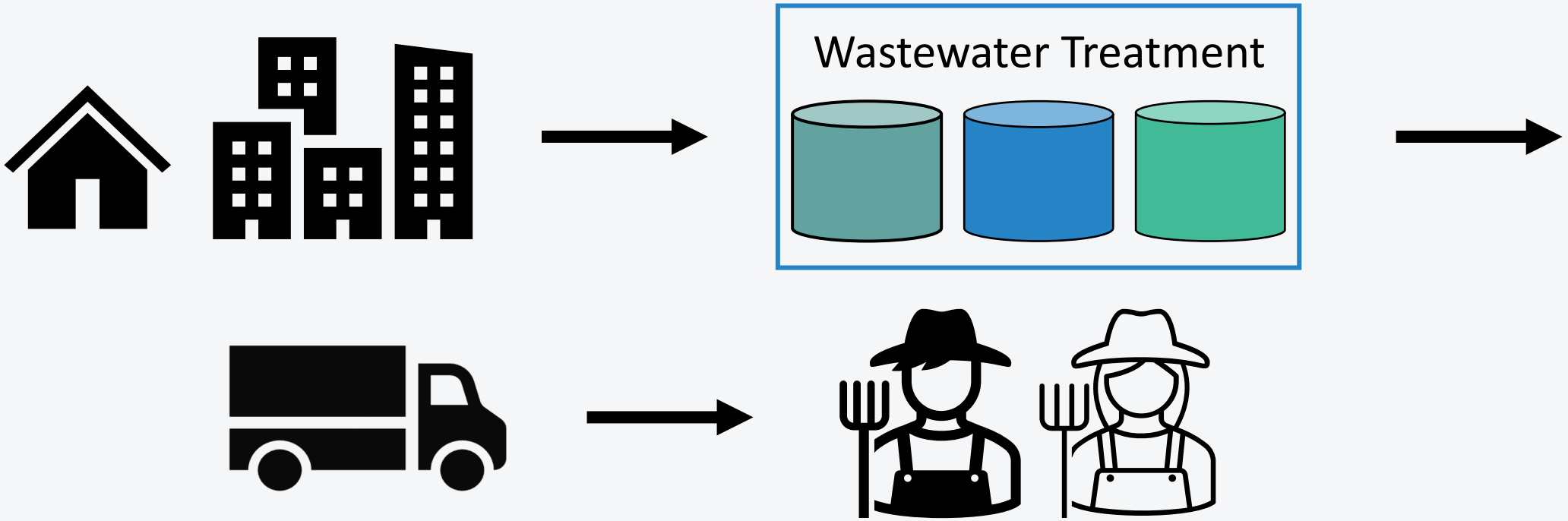
Characterizing biosolid-associated organic contaminants (BOCs) necessary for risk assessments


Wastewater Treatment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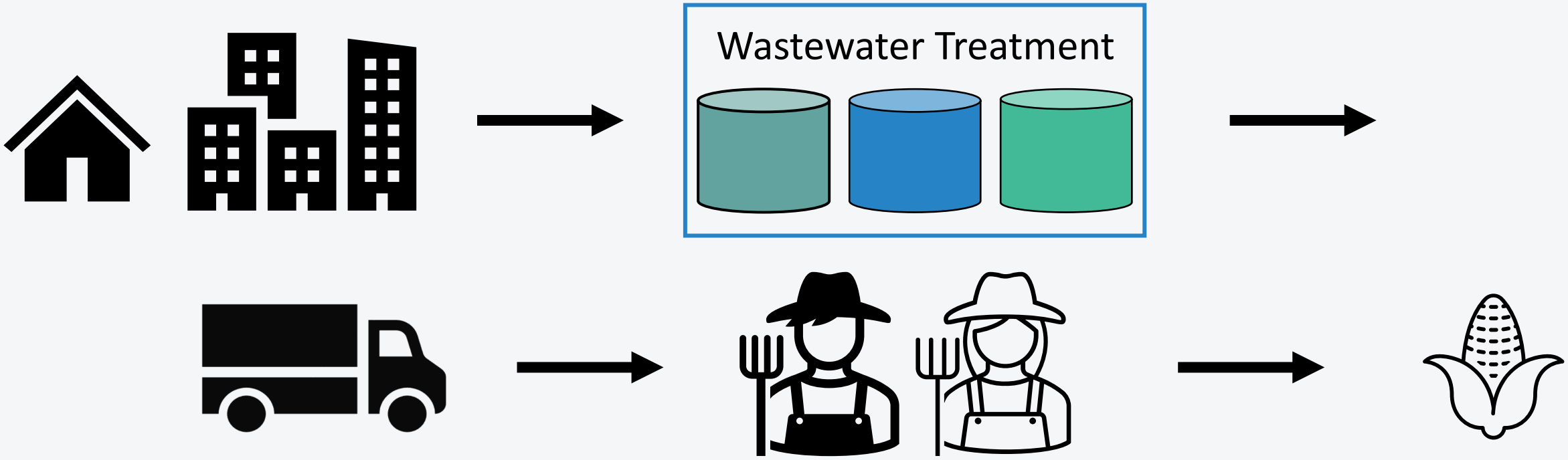
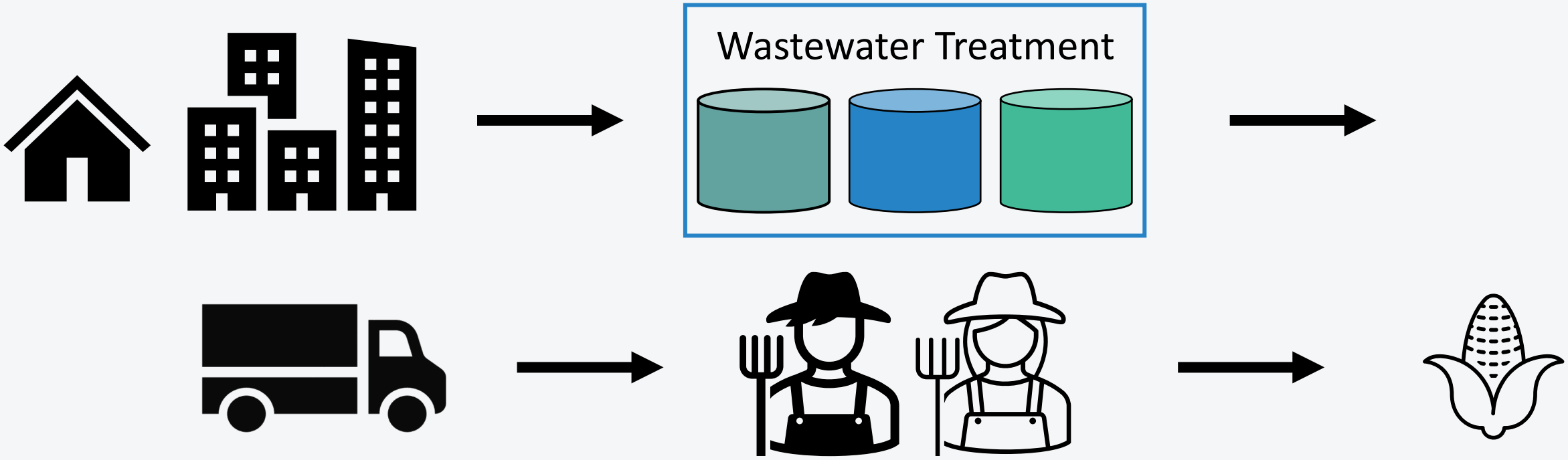
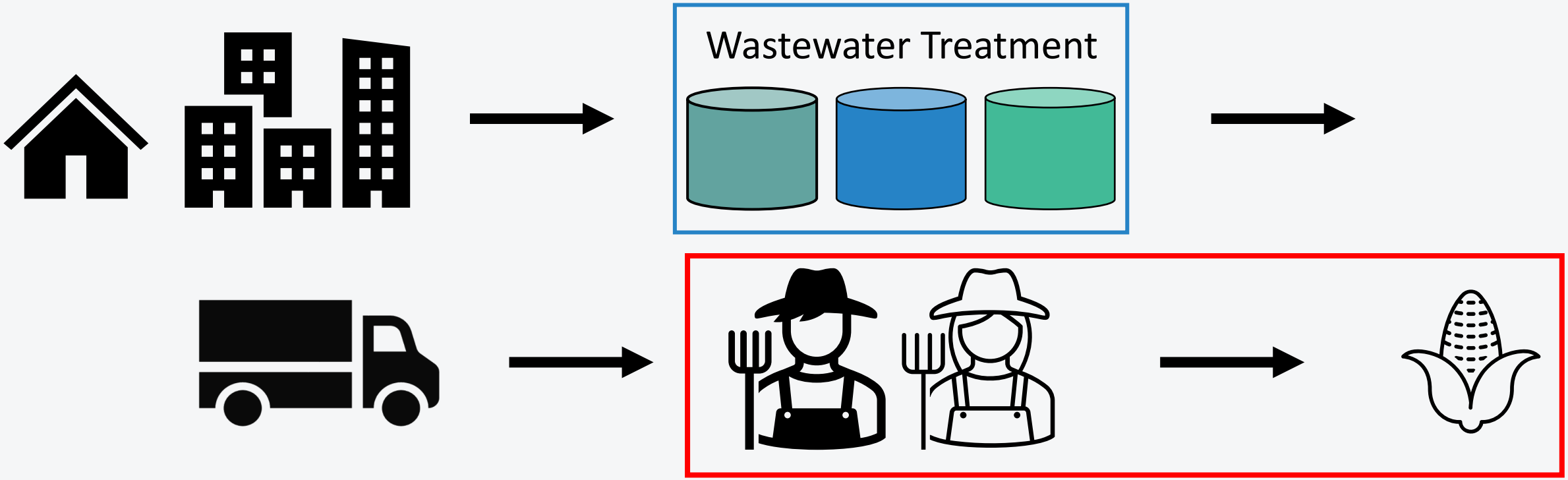
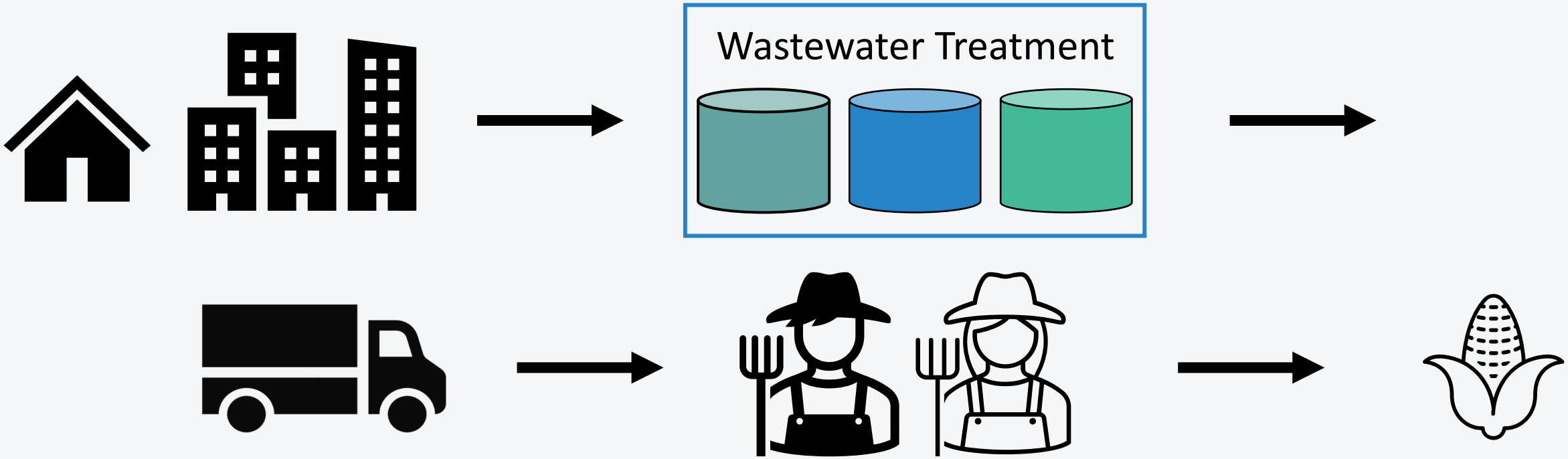

Wastewater Treatment



- Characterize the occurrence, fate, and transport of biosolids-associated organic compounds (BOCs) after agricultural land application?
- Develop and apply a health risk-driven prioritization framework for BOCs



- Characterize the occurrence, fate, and transport of biosolids-associated organic compounds (BOCs) after agricultural land application?
- Develop and apply a health risk-driven prioritization framework for BOCs
  - Quantify BOC exposures for both agricultural workers and public that consume foods grown on biosolid-amended soils



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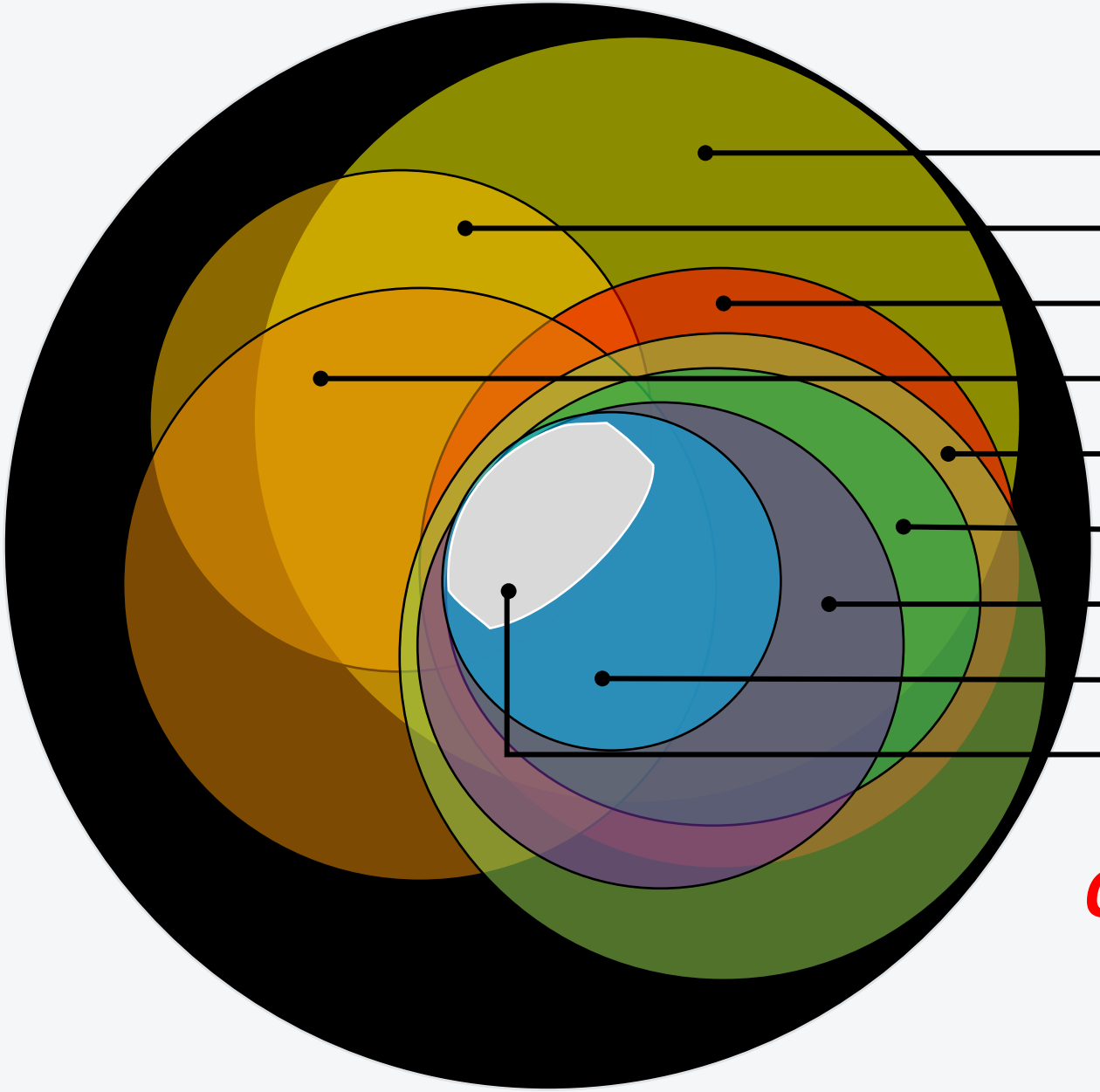
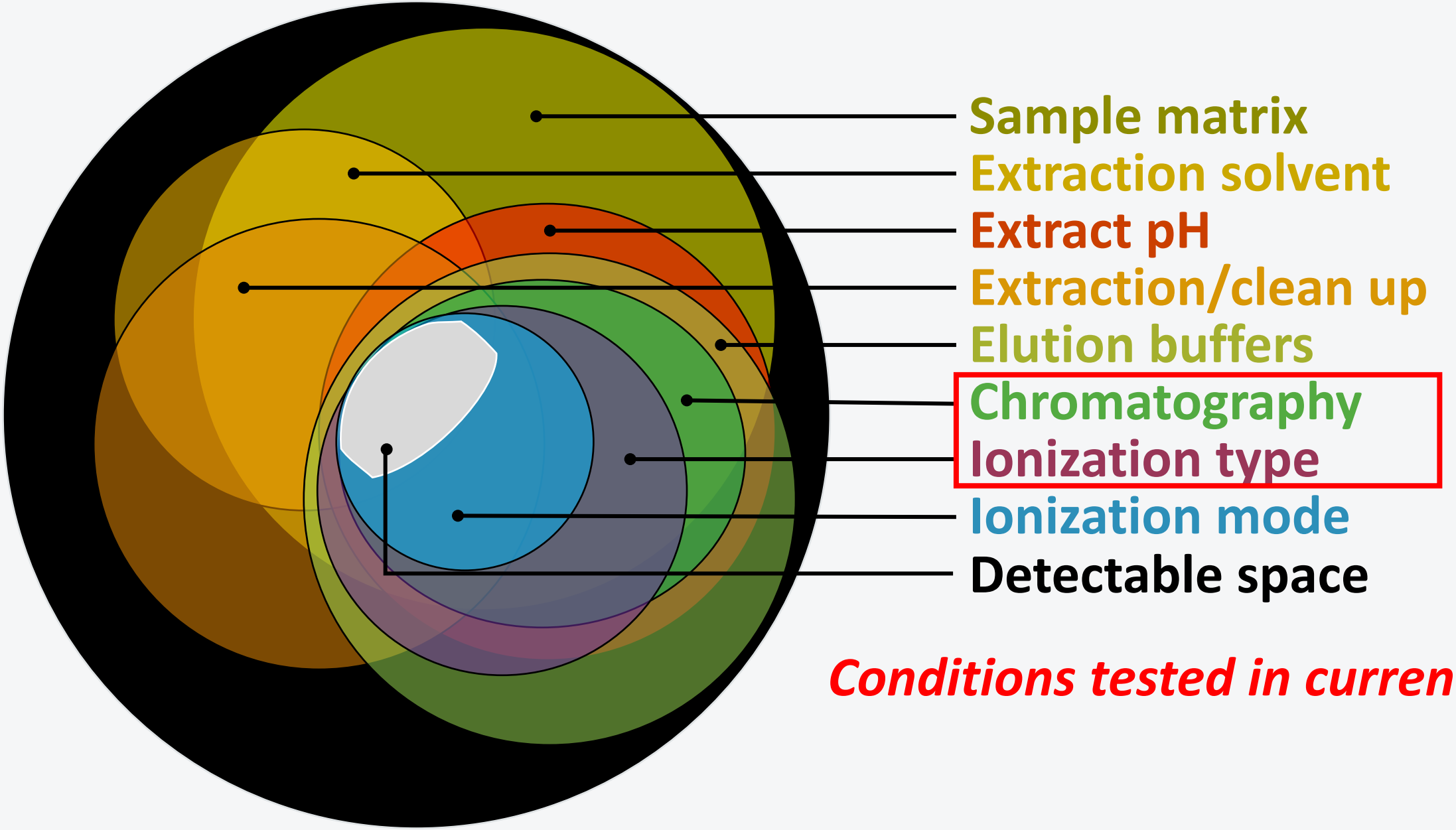
An understanding of the detectable chemical space can improve interpretation of our analyses



Sample matrix
Extraction solvent
Extract pH
Extraction/clean up
Elution buffers
Chromatography
Ionization type
Ionization mode
Detectable space

QuEChERS (remain constant)

Adapted from Black et al., Anal. Bioanal. Chem. 2022



Conditions tested in current study

QuEChERS extraction with dSPE on 5 biosolid samples from across the United States

→

LC-HRMS:
- Full Scan/dd-MS^2
- Polarity switching

→

Analyze extracts with 8 different conditions:
- MeOH or MeCN, w/ or w/o FA
- ESI or APCI

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Submit to workflow:
- Align RTs
- Detect & group compounds
- Predict formulas

Filter results based on:
- Extraction blank comparison
- Duplicate injection reproducibility
- MS^2 spectrum collected

Manually review results

Export results & repeat for each condition

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- MS^2 spectrum collected

Manually review results

Export results & repeat for each condition

Adjust database parameters:
- Match m/z scan range
- Water Solubility & log K_{ow}
- Ionization amenability

Search Molecular Formula & generate suspect list

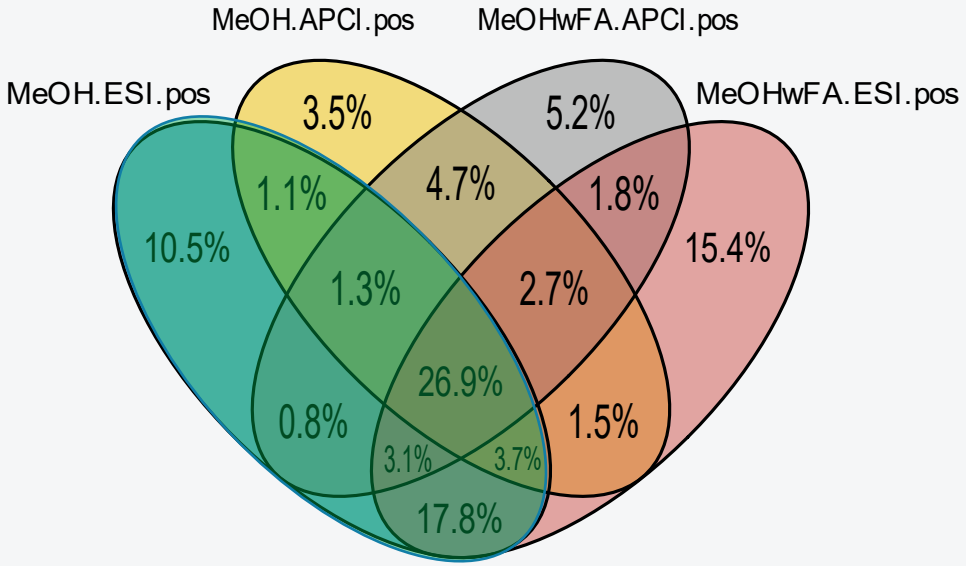
Submit MS^2 spectra to *in-silico* fragmentation searches, narrow hits based on suspect list

Percent of unique molecular formulas detected in each instrumental condition

Positive	MeOH	MeOH + 0.1% FA	MeCN	MeCN + 0.1% FA
ESI				
APCI				

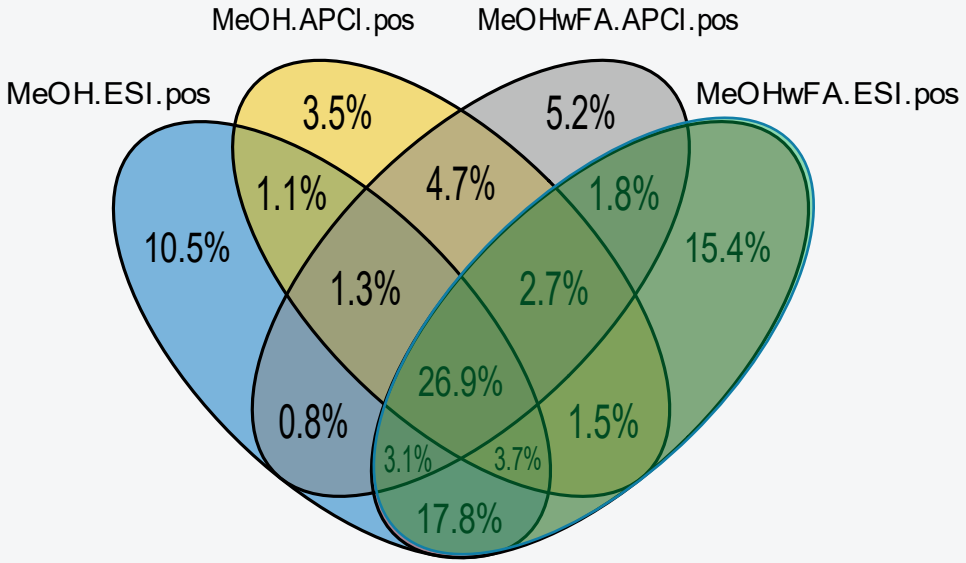
Percent of unique molecular formulas detected in each instrumental condition

Positive	MeOH	MeOH + 0.1% FA	MeCN	MeCN + 0.1% FA
ESI	**65.2%**			
APCI				



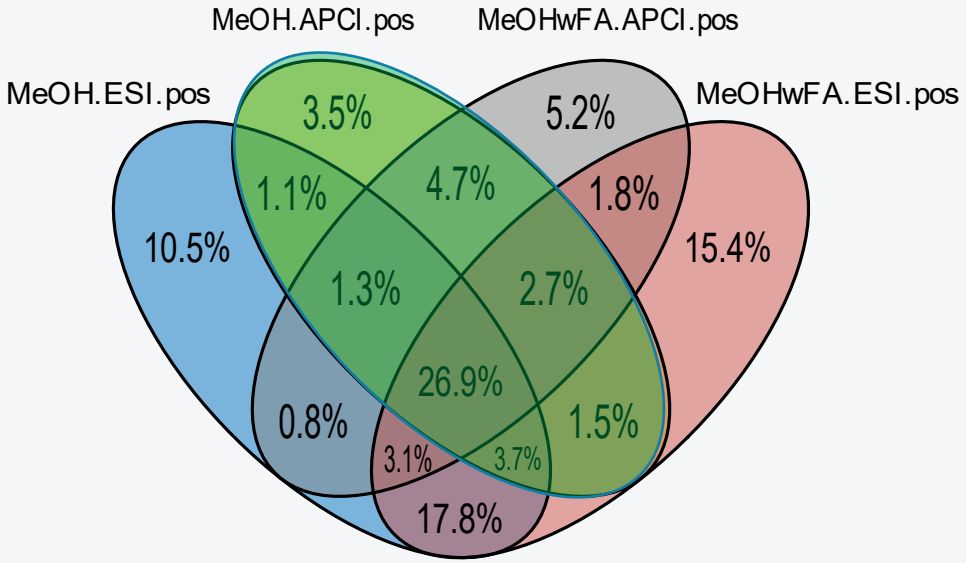
Percent of unique molecular formulas detected in each instrumental condition

Positive	MeOH	MeOH + 0.1% FA	MeCN	MeCN + 0.1% FA
ESI	**65.2%**	**72.9%**		
APCI				



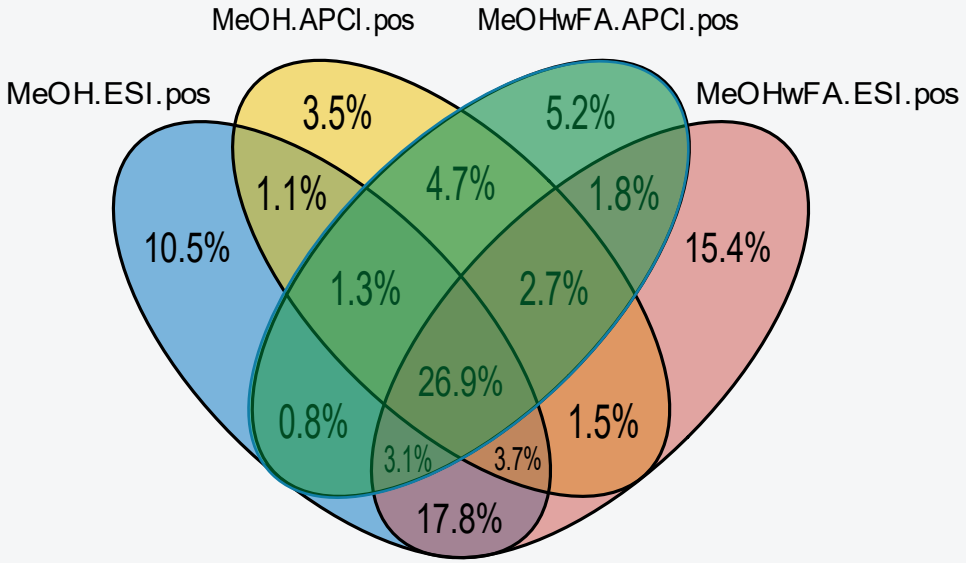
Percent of unique molecular formulas detected in each instrumental condition

Positive	MeOH	MeOH + 0.1% FA	MeCN	MeCN + 0.1% FA
ESI	**65.2%**	**72.9%**		
APCI	**45.4%**			



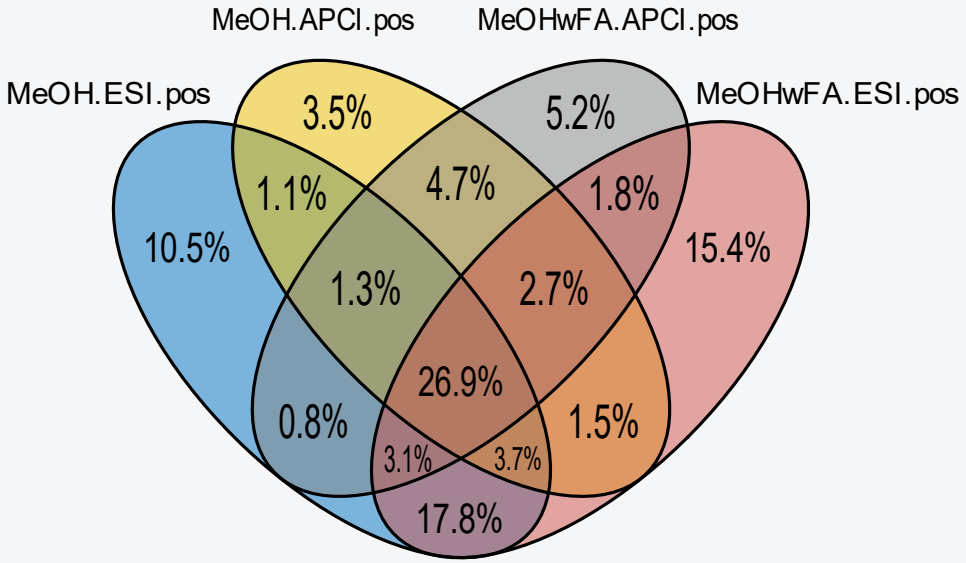
Percent of unique molecular formulas detected in each instrumental condition

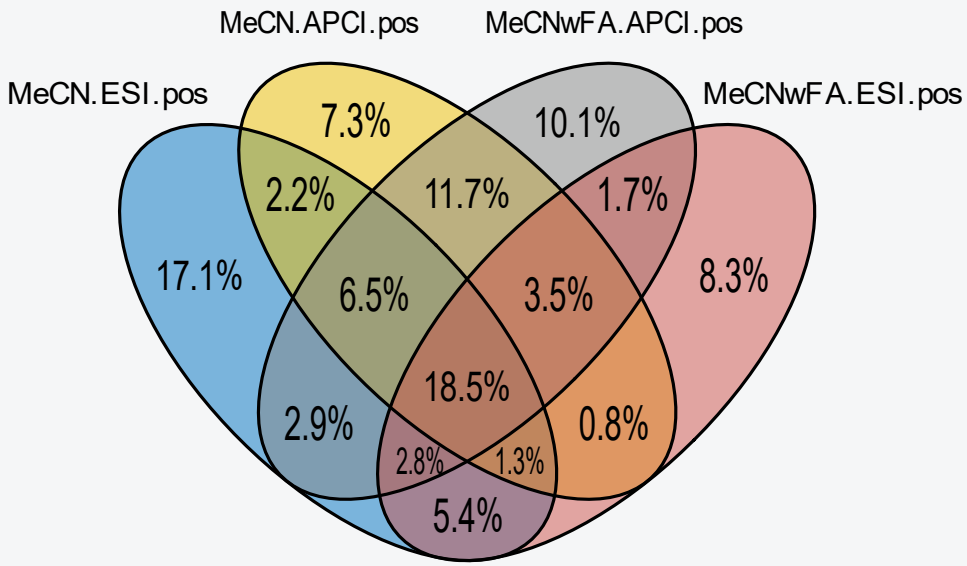
Positive	MeOH	MeOH + 0.1% FA	MeCN	MeCN + 0.1% FA
ESI	**65.2%**	**72.9%**		
APCI	**45.4%**	**46.5%**		



Percent of unique molecular formulas detected in each instrumental condition

Positive	MeOH	MeOH + 0.1% FA	MeCN	MeCN + 0.1% FA
ESI	**65.2%**	**72.9%**	**56.7%**	**42.3%**
APCI	**45.4%**	**46.5%**	**51.8%**	**57.7%**



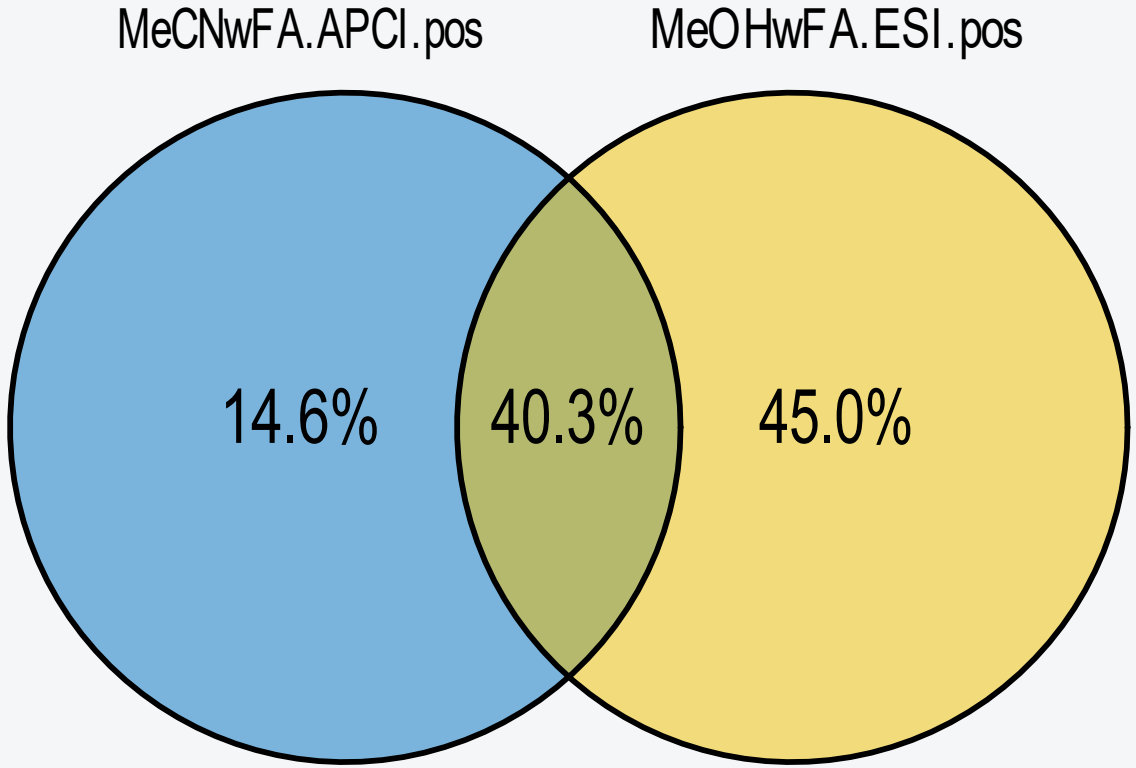


Percent of unique molecular formulas detected in each instrumental condition

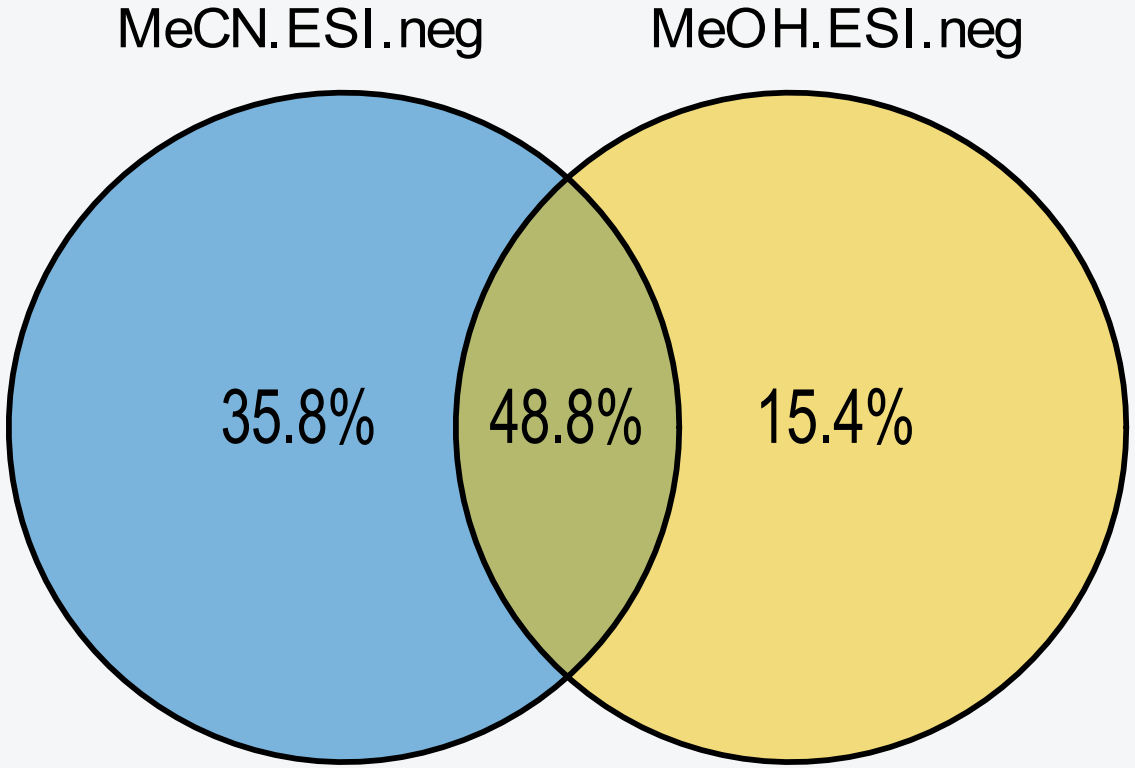
Positive	MeOH	MeOH + 0.1% FA	MeCN	MeCN + 0.1% FA
ESI	**65.2%**	**72.9%**	**56.7%**	**42.3%**
APCI	**45.4%**	**46.5%**	**51.8%**	**57.7%**

Percent of unique molecular formulas detected in each instrumental condition

Negative	MeOH	MeOH + 0.1% FA	MeCN	MeCN + 0.1% FA
ESI	**70.6%**	**23.9%**	**67.0%**	**28.6%**
APCI	**40.9%**	**29.7%**	**50.2%**	**26.5%**



~85% of unique molecular formulas detected in positive mode with MeOH + 0.1% formic acid & ESI when compared to MeCN + 0.1% FA & ESI



~85% of unique molecular formulas detected in negative mode with MeCN & ESI when compared to MeOH & ESI

ChemSpace Mapping Tool: “functional prototype”

ChemSpace Tool

Categories

Reset Checkboxes

- TSCA Active
- Consumer Products
- Drugs
- Illicit Drugs
- Smoking&Vaping
- Pesticides
- Surfactants
- PFAS
- Food Products
- Hazardous Chemicals
- Dyes
- Plastics
- Water
- Halocarbons
- Phenols
- Exposome
- Metabolites
- Biosolids
- Biosolids QCMix

Download

LCMS ESI+ Amenability

- (•) No filter
- ( ) Amenable only
- ( ) Unamenable only

LCMS ESI- Amenability

- (•) No filter
- ( ) Amenable only
- ( ) Unamenable only

Molecular Formula Search

Molecular Formula...

Database | Physicochemical Properties | Descriptor Reduction

Show 15 entries

	DTXSID	Preferred Name	Molecular Formula	Average Mass	Water Solubility (log(mol/L))	Vapor Pressure (log(mmHg))	Boiling Point (C)	Log(Kow)	Log(Koa)	LC-MS ESI- Amenability	LC-MS ESI+ Amenability
1	DTXSID7021605	Hexanedioic acid	C6H10O4	146.14	-0.82	-6.5	337	0.08	7.59	Amenable	Unamenable
2	DTXSID2020688	Hexachlorocyclopentadiene	C5Cl6	272.76	-5.18	-1.22	239	5.04	6.91	Unamenable	Unamenable
3	DTXSID8021515	Butanoic acid	C4H8O2	88.11	-0.18	0.21	164	0.79	4.23	Unamenable	Unamenable
4	DTXSID6063199	2,4,5-T Triethanolamine salt	C14H20Cl3NO6	404.67	-3.68	-4.43	350	-0.11	9.06	Amenable	Unamenable
5	DTXSID7020425	Dichlone	C10H4Cl2O2	227.04	-5.7	-5.95	290	2.86	7.38	Unamenable	Unamenable
6	DTXSID90866339	Butan-2-yl (2,4,5-trichlorophenoxy)acetate	C12H13Cl3O3	311.58	-2.89	-4.48	363	4.35	8.53	Unamenable	Unamenable
7	DTXSID3039240	Antimony potassium tartrate trihydrate	C8H10K2O15Sb2	667.87	0.47	-7.19	202	-2.23	6.61	Amenable	Amenable
8	DTXSID3020964	Nitrobenzene	C6H5NO2	123.11	-1.79	-0.61	211	1.85	3.87	Unamenable	Unamenable
9	DTXSID7021100	Parathion	C10H14NO5PS	291.26	-4.36	-5.17	375	3.83	9.33	Unamenable	Amenable
10	DTXSID5024057	Dimethylamine	C2H7N	45.08	1.56	3.18	7	-0.38	2.02	Unamenable	Amenable
11	DTXSID1027007	Propanoic anhydride	C6H10O3	130.14	-0.77	0.13	170	1.07	3.92	Unamenable	Unamenable
12	DTXSID5021881	Ethylenediamine	C2H8N2	60.1	1.22	1.08	117	-2.04	6.7	Unamenable	Amenable
13	DTXSID1020647	Furfural	C5H4O2	96.08	-0.1	0.35	153	0.41	3.53	Unamenable	Unamenable
14	DTXSID8041329	2,4,5-T 2-Ethylhexyl	C16H21Cl3O3	367.69	-5.5	-5.91	359	5.15	9.89	Unamenable	Unamenable
15	DTXSID7021106	Pentachlorophenol	C6HCl5O	266.32	-3.82	-3.96	309	5.12	8.46	Unamenable	Unamenable

Showing 1 to 15 of 94,505 entries

Previous 1 2 3 4 5 ... 6,301 Next

Filtered Dataset Size

94505

Tweak Property Ranges

Average Mass: 1 to 2000

Water Solubility (log(mol/L)): -14 to 3

Vapor Pressure (log(mmHg)): -14 to 10

Boiling Point (C): -170 to 550

Octanol Water Partition Coefficient (log(Kow)): -6 to 10.5

Octanol Air Partition Coefficient (log(Koa)): -1 to 12.5

Hydrogen Bond Donor Count: 0 to 27

Hydrogen Bond Acceptor Count: 0 to 52

Topological Polar Surface Area: 0 to 872.52

Number of Rotatable Bonds: 0 to 82

ChemSpace Mapping Tool: “functional prototype”

	DTXSID	Preferred Name	Molecular Formula	Average Mass	Water Solubility (log(mol/L))	Vapor Pressure (log(mmHg))	Boiling Point (C)	Log(Kow)	Log(Koa)	LC-MS ESI- Amenability	LC-MS ESI+ Amenability
1	DTXSID7021605	Hexanedioic acid	C6H10O4	146.14	-0.82	-6.5	337	0.08	7.59	Amenable	Unamenable
2	DTXSID2020688	Hexachlorocyclopentadiene	C5Cl6	272.76	-5.18	-1.22	239	5.04	6.91	Unamenable	Unamenable
3	DTXSID8021515	Butanoic acid	C4H8O2	88.11	-0.18	0.21	164	0.79	4.23	Unamenable	Unamenable
4	DTXSID6063199	2,4,5-T Triethanolamine salt	C14H20Cl3NO6	404.67	-3.68	-4.43	350	-0.11	9.06	Amenable	Unamenable
5	DTXSID7020425	Dichlone	C10H4Cl2O2	227.04	-5.7	-5.95	290	2.86	7.38	Unamenable	Unamenable
6	DTXSID90866339	Butan-2-yl (2,4,5-trichlorophenoxy)acetate	C12H13Cl3O3	311.58	-2.89	-4.48	363	4.35	8.53	Unamenable	Unamenable
7	DTXSID3039240	Antimony potassium tartrate trihydrate	C8H10K2O15Sb2	667.87	0.47	-7.19	202	-2.23	6.61	Amenable	Amenable
8	DTXSID3020964	Nitrobenzene	C6H5NO2	123.11	-1.79	-0.61	211	1.85	3.87	Unamenable	Unamenable
9	DTXSID7021100	Parathion	C10H14NO5PS	291.26	-4.36	-5.17	375	3.83	9.33	Unamenable	Amenable
10	DTXSID5024057	Dimethylamine	C2H7N	45.08	1.56	3.18	7	-0.38	2.02	Unamenable	Amenable
11	DTXSID1027007	Propanoic anhydride	C6H10O3	130.14	-0.77	0.13	170	1.07	3.92	Unamenable	Unamenable
12	DTXSID5021881	Ethylenediamine	C2H8N2	60.1	1.22	1.08	117	-2.04	6.7	Unamenable	Amenable
13	DTXSID1020647	Furfural	C5H4O2	96.08	-0.1	0.35	153	0.41	3.53	Unamenable	Unamenable
14	DTXSID8041329	2,4,5-T 2-Ethylhexyl	C16H21Cl3O3	367.69	-5.5	-5.91	359	5.15	9.89	Unamenable	Unamenable
15	DTXSID7021106	Pentachlorophenol	C6HCl5O	266.32	-3.82	-3.96	309	5.12	8.46	Unamenable	Unamenable

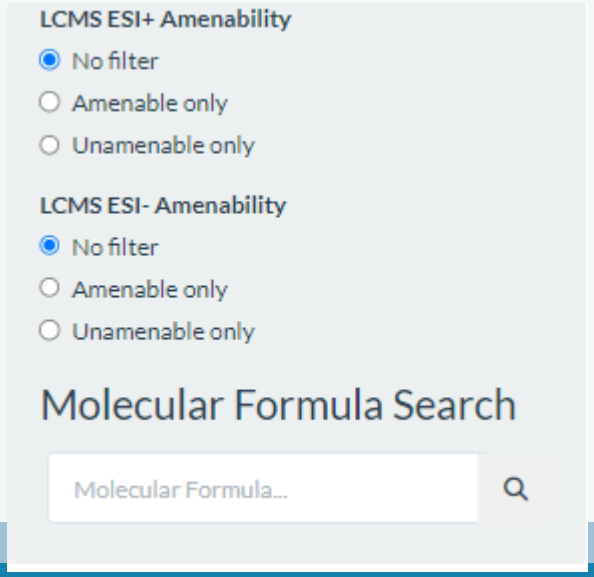
Showing 1 to 15 of 94,505 entries

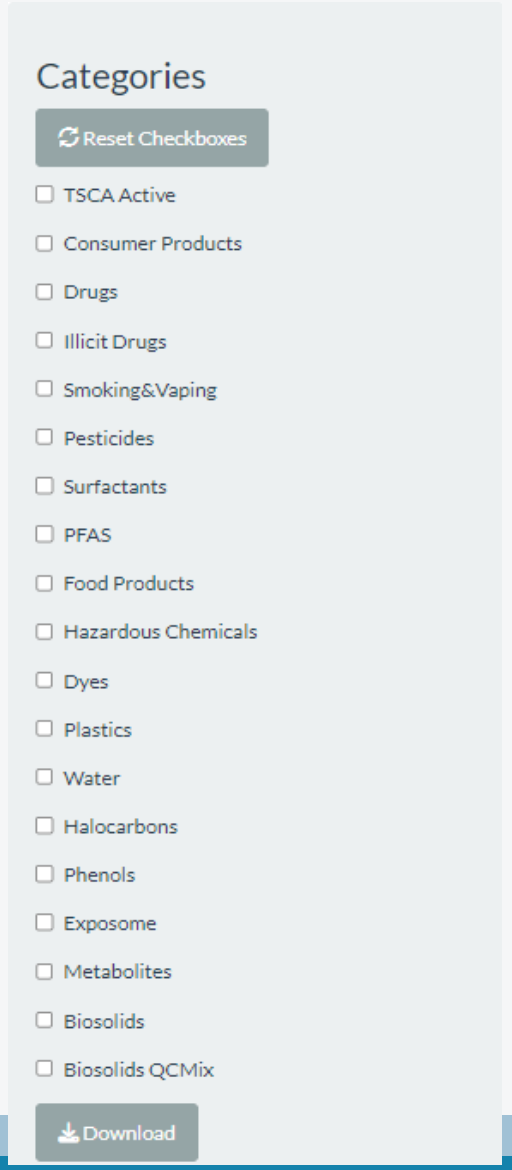
Previous 1 2 3 4 5 ... 6,301 Next

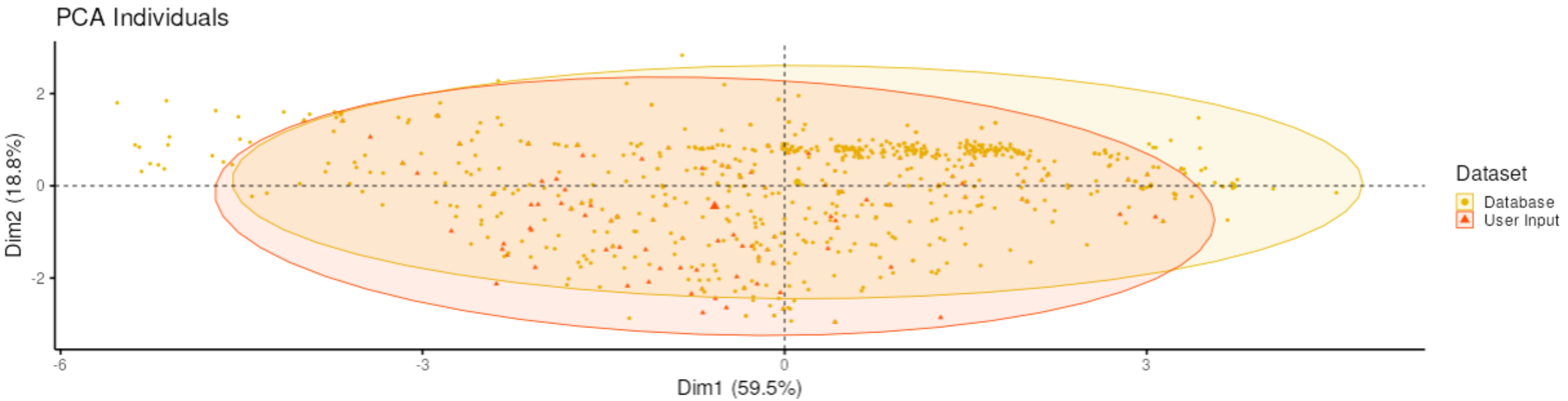
ChemSpace Mapping Tool: “functional prototype”

Various filters available for narrowing down the dataset based on:

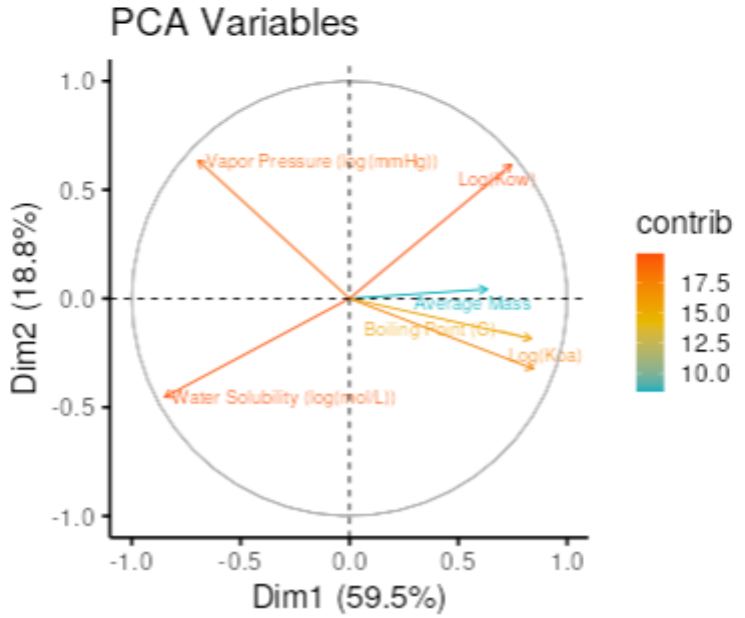
- Physical/chemical properties
- Compound categories
- Ionization amenability







Generate various built-in visualizations to compare the dataset to input chemicals



N = 93,835

- Complete dataset, no filtering

N = 93,835

- Complete dataset, no filtering

N = 79,342

- Average Mass: 100-1000
- Water Solubility(log(mol/L): -10–0
- Octanol/Water Partition (logK_{ow}): 0–8

} Based on Biosolids List

N = 93,835

- Complete dataset, no filtering

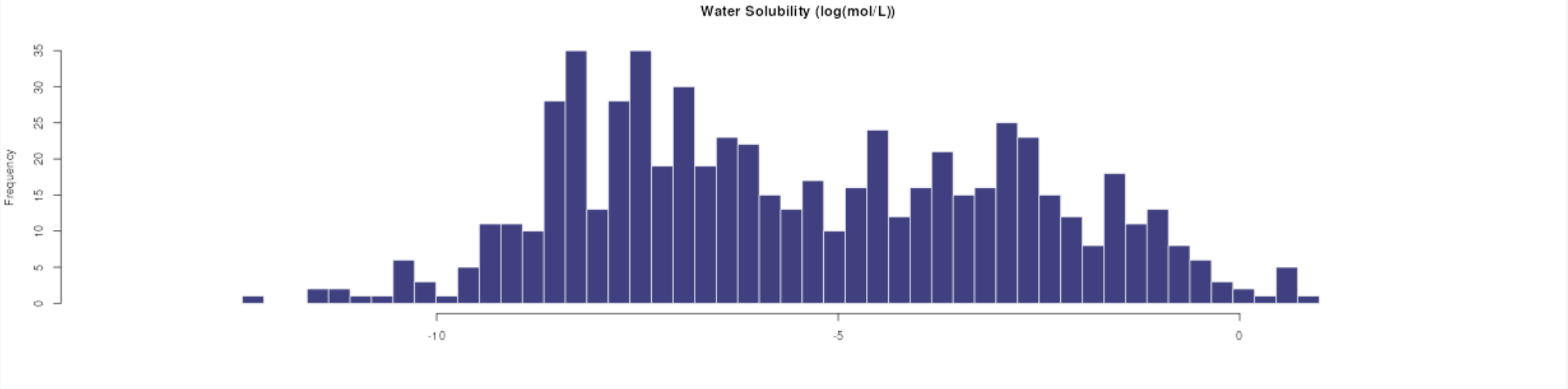
N = 79,342

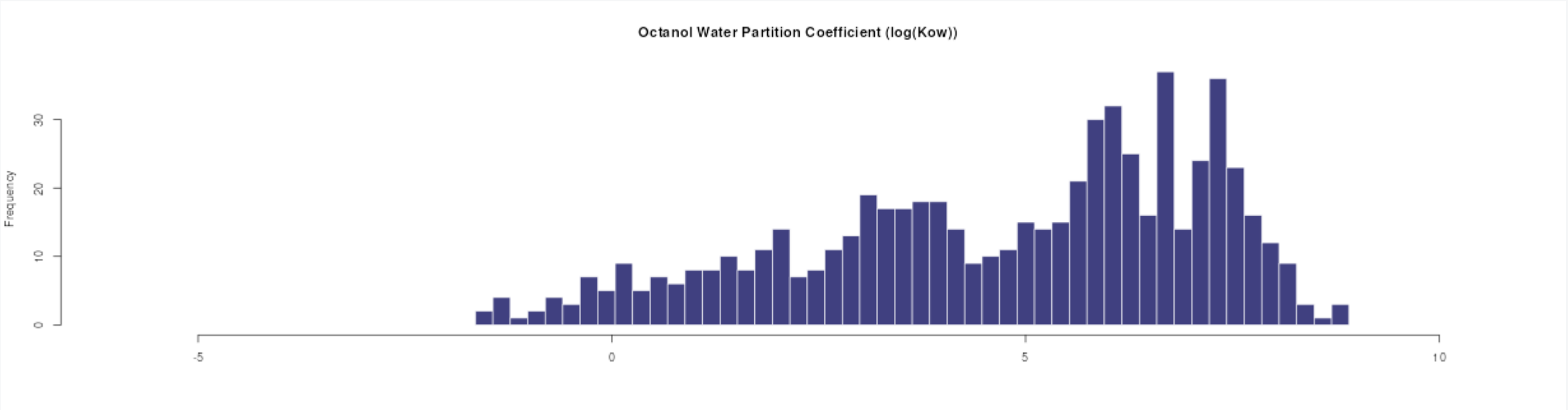
- Average Mass: 100-1000
- Water Solubility(log(mol/L): -10–0
- Octanol/Water Partition (logK_{ow}): 0–8

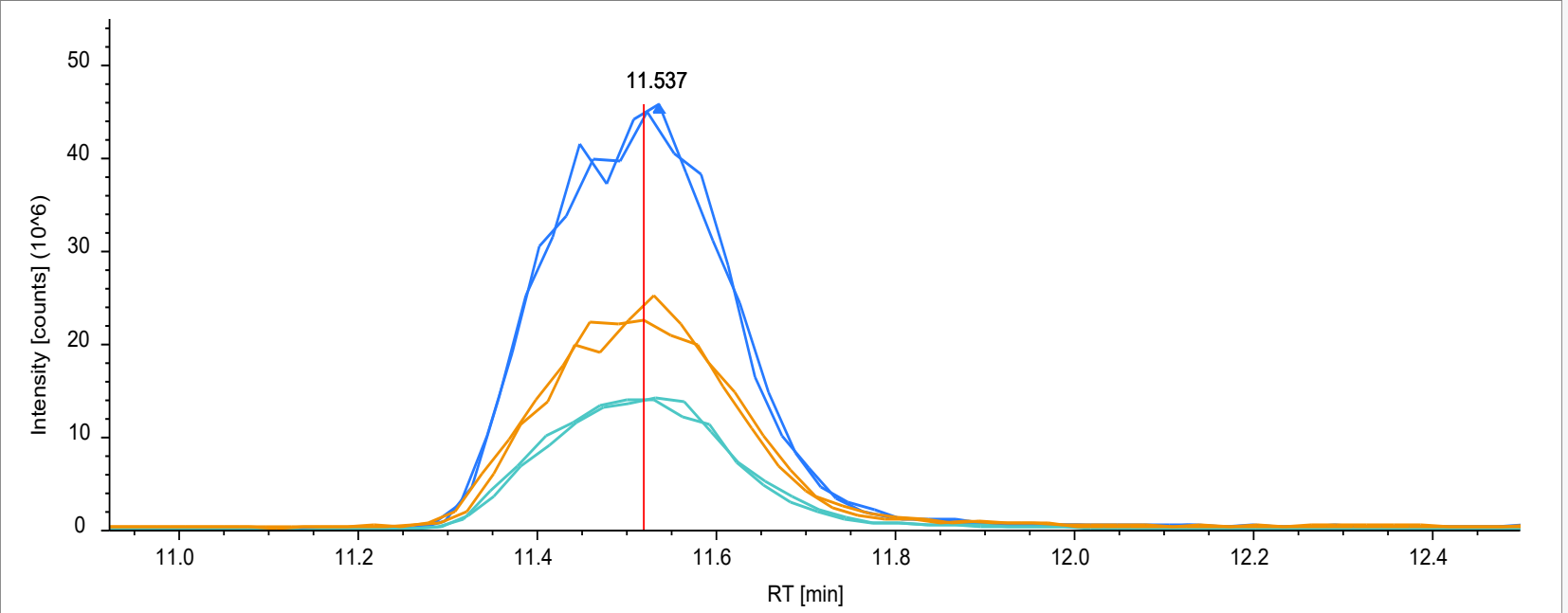
} Based on Biosolids List

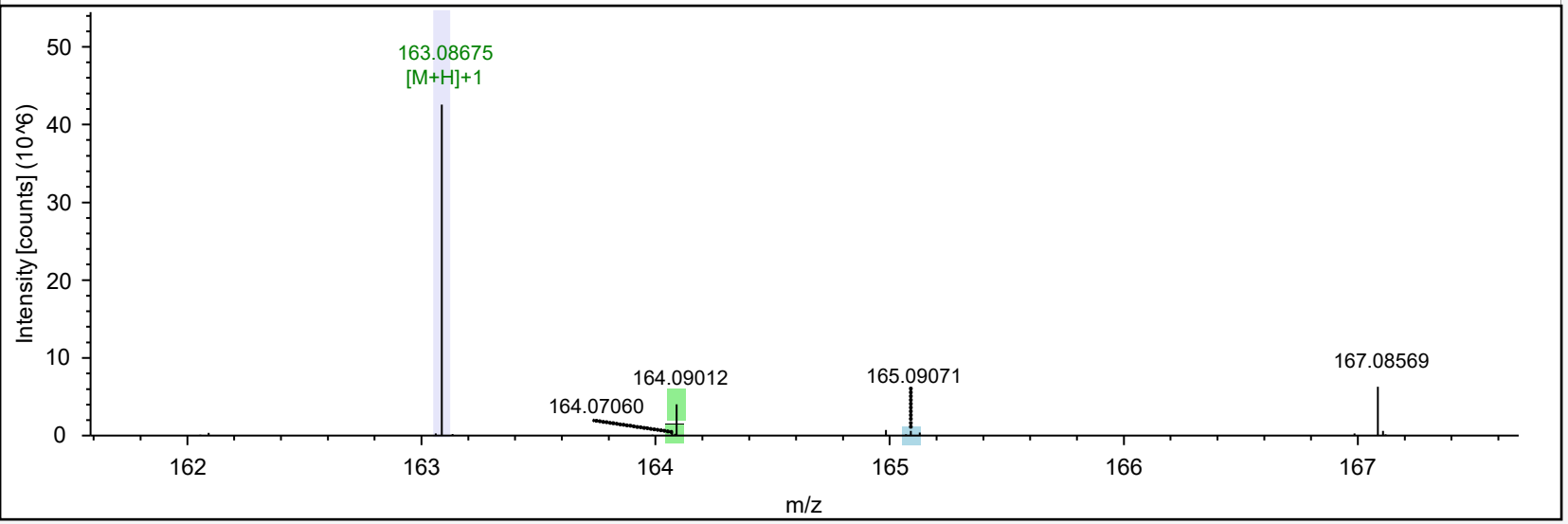
N = 29,647

- LCMS ESI+ Amenable only


Water Solubility (log(mol/L))
Frequency
0
5
10
15
20
25
30
35
-10
-5
0


Octanol Water Partition Coefficient (log(Kow))
Frequency
0
10
20
30
-5
0
5
10

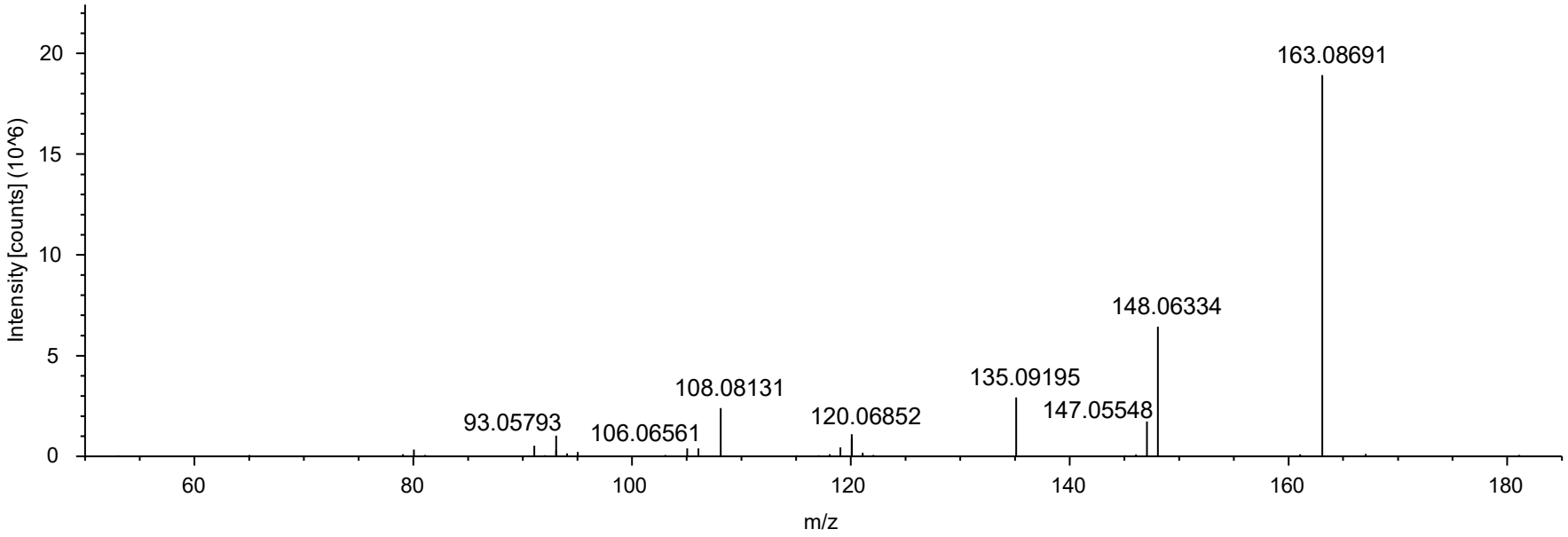




Compound Match	Formula	Molecular Weight	ΔMass [Da]	ΔMass [ppm]	RDBE	H/C	Rank	# Matched Iso.	# Missed Iso.	# Matched Frag.	SFit [%]
	C9 H10 N2 O	162.07931	0.00011	0.70	6.0	1.1	1	3	0	20	89

A compound was detected in 3 biosolid samples in ESI+ mode using MeOH + 0.1% FA

Based on the MS1 spectrum a single formula was predicted by Compound Discover with a mass accuracy of 0.70 ppm: **$C_9H_{10}N_2O$**



We have an MS^2 spectrum but no database matches

There are 4 formula matches in our filtered dataset

	DTXSID	Preferred Name	Molecular Formula	Average Mass	Water Solubility (log(mol/L))	Vapor Pressure (log(mmHg))	Boiling Point (C)	Log(Kow)	Log(Koa)	LC-MS ESI- Amenability	LC-MS ESI+ Amenability
1	DTXSID70143296	1-Ethyl-2-benzimidazolinone	C9H10N2O	162.19	-2.43	-4.8	291	1.69	9.03	Unamenable	Amenable
2	DTXSID00862867	Aminorex	C9H10N2O	162.19	-1.98	-3.11	338	0.23	7.52	Unamenable	Amenable
3	DTXSID10174226	1,3-Dihydro-5,6-dimethyl-2H-benzimidazol-2-one	C9H10N2O	162.19	-2.78	-5.46	317	0.72	8.18	Unamenable	Amenable
4	DTXSID30970080	2-Methyl-1,2-dihydroquinazolin-4-ol	C9H10N2O	162.19	-1.92	-5.48	363	1.82	7.84	Unamenable	Amenable

Submitting the spectrum to SIRIUS CSI:FingerID and searching the NORMAN database returned 7 hits…

rank	CSI:Finger	InChIkey2D	name	smiles
1	-114.014	ORWJLFLEIZBRBR	5,6-Dimethyl-2-hydroxybenzimidazole	CC1=CC2=C(C=C1C)NC(=O)N2
2	-141.761	CXUCKELNYMZTRT	Tocris-1041	CCN1C2=CC=CC=C2NC1=O
3	-193.613	OWNQIZJEBGJLSW	2-(4-methoxyanilino)acetonitrile	COC1=CC=C(C=C1)NCC#N
4	-225.161	XZHWEHOSQYNGOL	zlchem 1089	CC(C1=NC2=CC=CC=C2N1)O
5	-259.006	DDGGFHPNJTXXNE	1-prop-2-enylpyridin-1-ium-3-carboxamide	C=CC[N+]1=CC=CC(=C1)C(=O)N
6	-278.454	CMCWWLVWPDLCRM	Fenidon	C1CN(NC1=O)C2=CC=CC=C2
7	-387.443	SYAKTDIEAPMBAL	Apiquel	C1C(OC(=N1)N)C2=CC=CC=C2

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rank	CSI:Finger	InChIkey2D	name	smiles
1	-114.014	ORWJLFLEIZBRBR	5,6-Dimethyl-2-hydroxybenzimidazole	CC1=CC2=C(C=C1C)NC(=O)N2
2	-141.761	CXUCKELNYMZTRT	Tocris-1041	CCN1C2=CC=CC=C2NC1=O
3	-193.613	OWNQIZJEBGJLSW	2-(4-methoxyanilino)acetonitrile	COC1=CC=C(C=C1)NCC#N
4	-225.161	XZHWEHOSQYNGOL	zlchem 1089	CC(C1=NC2=CC=CC=C2N1)O
5	-259.006	DDGGFHPNJTXXNE	1-prop-2-enylpyridin-1-ium-3-carboxamide	C=CC[N+]1=CC=CC(=C1)C(=O)N
6	-278.454	CMCWWLVWPDLCRM	Fenidon	C1CN(NC1=O)C2=CC=CC=C2
7	-387.443	SYAKTDIEAPMBAL	Apiquel	C1C(OC(=N1)N)C2=CC=CC=C2

…and 3 compounds are in common with our suspect list generated from the ChemSpace Mapping Tool

DTXSID	Preferred Name	INCHIKEY	SMILES
DTXSID70143296	1-Ethyl-2-benzimidazolinone	CXUCKELNYMZTRT-UHFFFAOYSA-N	CCN1C(=O)NC2=CC=CC=C12
DTXSID00862867	Aminorex	SYAKTDIEAPMBAL-UHFFFAOYSA-N	NC1=NCC(O1)C1=CC=CC=C1
DTXSID10174226	1,3-Dihydro-5,6-dimethyl-2H-benzimidazol-2-one	ORWJLFLEIZBRBR-UHFFFAOYSA-N	CC1=CC2=C(NC(=O)N2)C=C1C
DTXSID30970080	2-Methyl-1,2-dihydroquinazolin-4-ol	PPEWCSSFVFQSCD-UHFFFAOYSA-N	CC1NC2=CC=CC=C2C(O)=N1

Future of the Chemical Space Mapping Tool

- Updated ionization amenability predictions
  - Model refinement with new, additional amenability data
- Improved visualizations
- Take advantage of MS-READY structures
- Additional scrutiny and improved categorization of chemicals

Next steps for biosolids analysis

- Have received ~20 biosolid samples from across the U.S. & Canada

- Will analyze via QuEChERS extraction and two chromatographic & ionization conditions:
  - ESI+ with MeOH + 0.1% formic acid
  - ESI- with MeCN

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Keeve Nachman
Matthew N. Newmeyer
Noor Hamdan
Kate Burgener
Dominc Sanchez

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Prasse Lab Homepage

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