Potential Human and Aquatic Toxicity of Petroleum Biodegradation Metabolites in Groundwater at Fuel Release Sites

Battelle Chlorinated Remediation Conference
Palm Springs, CA
April 12, 2018

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Evaluating Complex Mixtures is Challenging

- Biodegradation produces complex mixtures composed of hundreds to thousands of polar metabolites representing many different chemical structures.
- The complex mixture is continually changing over time.
- USEPA and other agencies recommend three approaches:
  - Toxicity testing of the whole mixture
  - Toxicity testing of similar mixtures
  - Evaluating the toxicity of the components of the mixture

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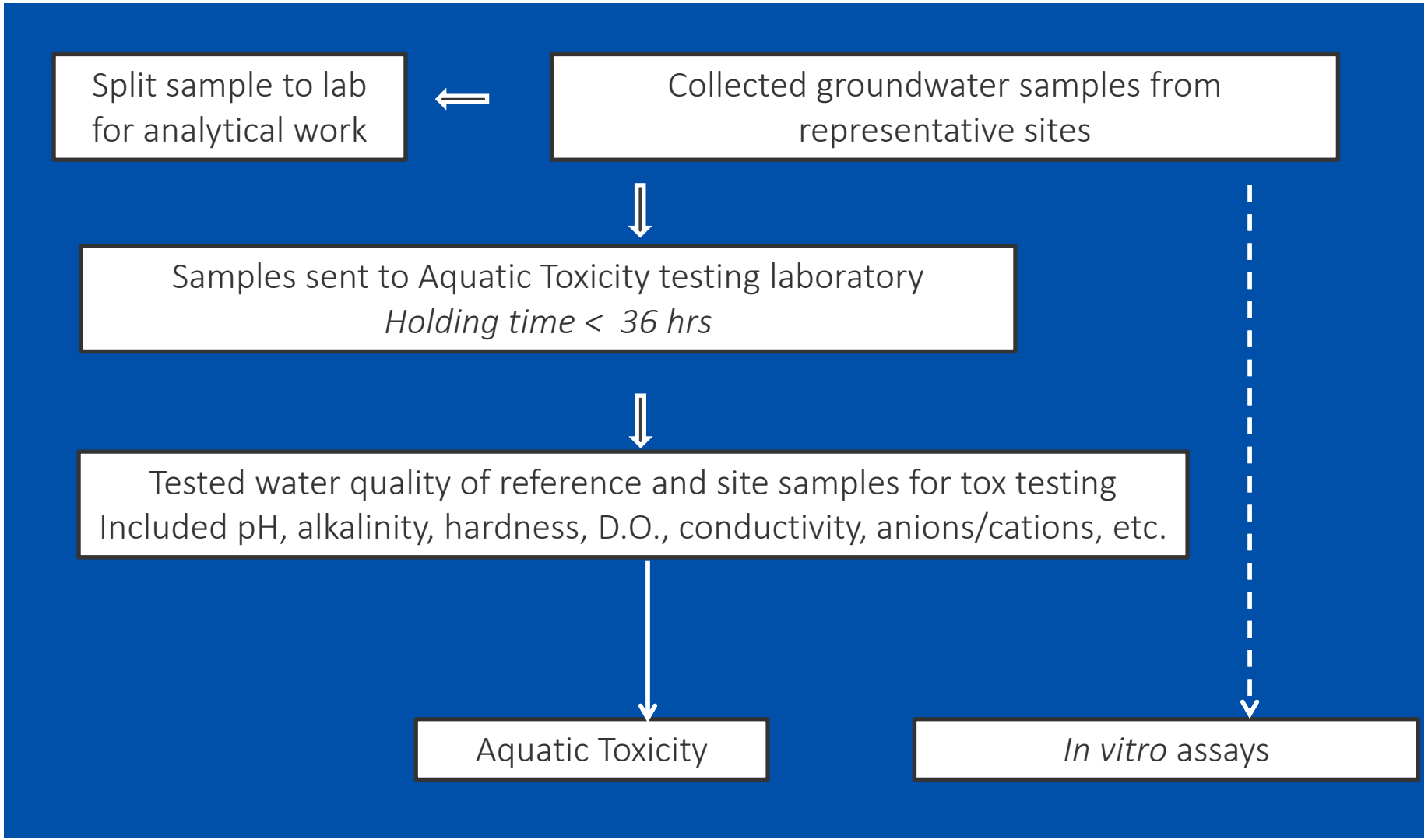
Potential Toxicity of Polar Metabolites Whole Mixtures



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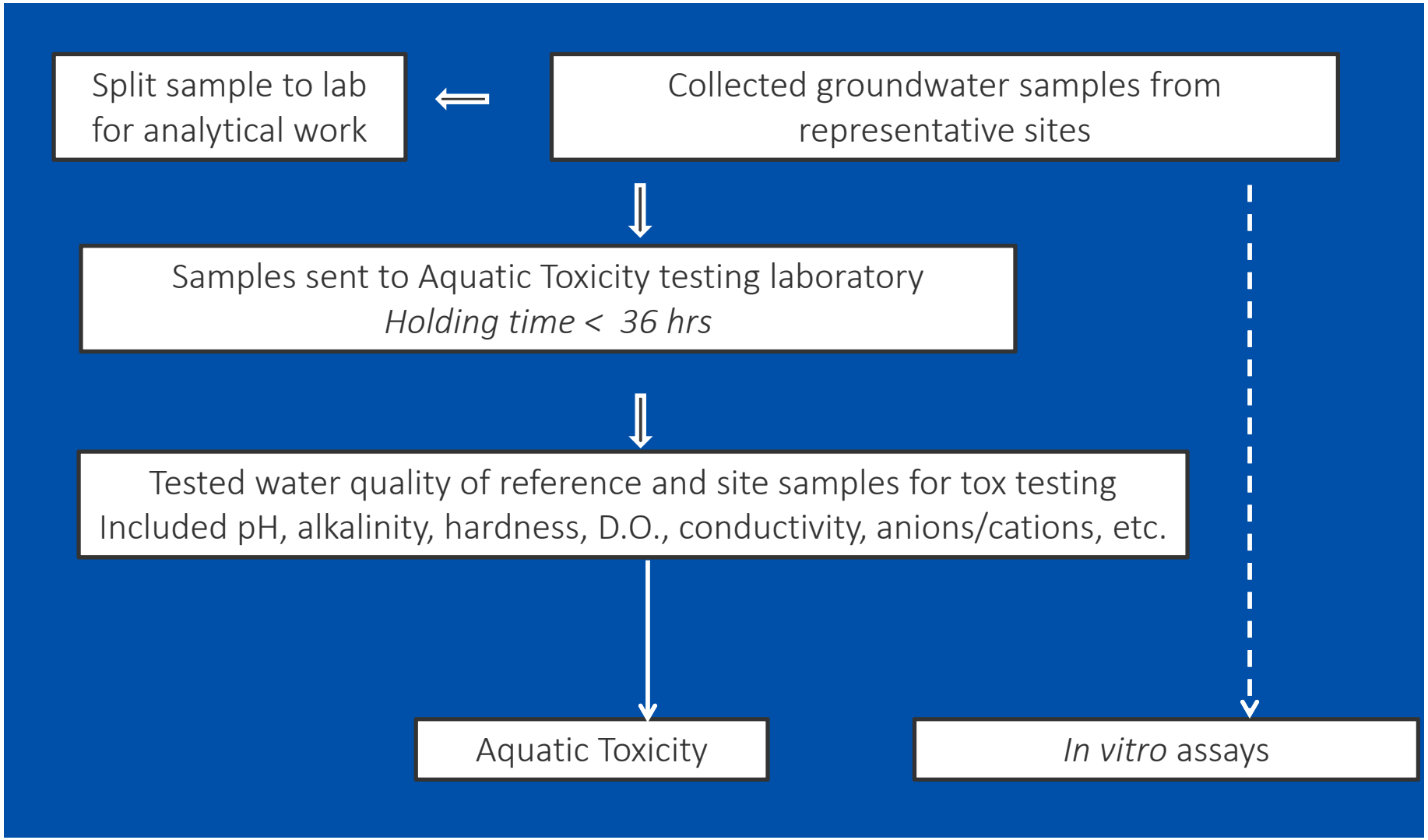
Potential Aquatic Toxicity of Polar Metabolites Whole Mixtures



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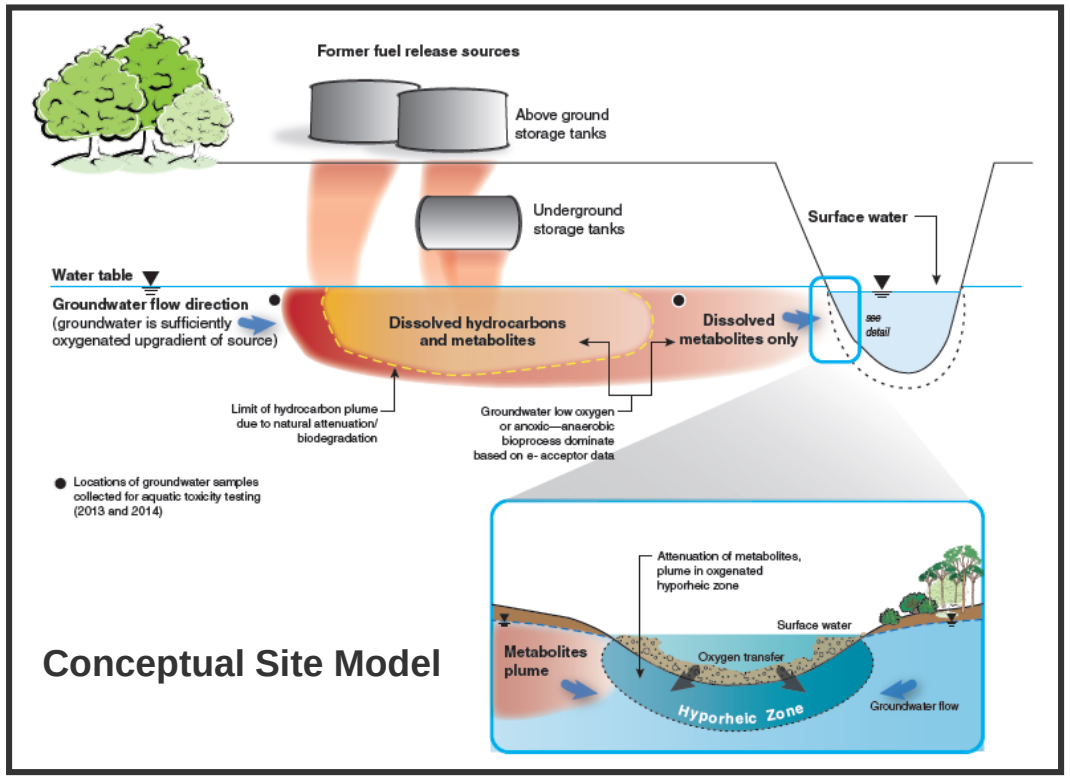
Aquatic Toxicity Testing Approach
Whole Effluent Toxicity Tests

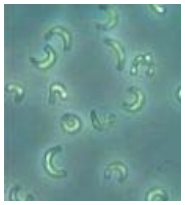
US EPA freshwater 3-species short-term chronic toxicity testing of undiluted groundwater samples



7-day fish test (survival & growth) EPA method 1003

7-day daphnia test (survival & reproduction) EPA method 1002

96-hr green algae test (growth) EPA method 1000



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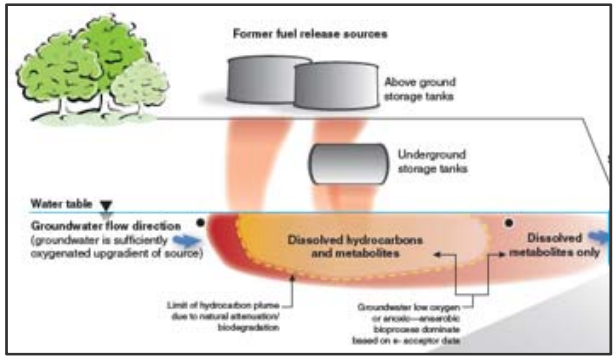
Aquatic Toxicity
Groundwater Sample Collection

- 14 fuel terminal sites evaluated, all in upland settings
  - 9 “representative” sites: upgradient sample (*free of polar metabolites and hydrocarbons*) vs. downgradient sample (*containing polar metabolites but no hydrocarbons*)
  - 5 “remaining” sites:
    - 3 sites with no polar metabolites upgradient or downgradient
    - 1 site with polar metabolites both upgradient and downgradient
    - 1 site with polar metabolites both cross- and downgradient
- Sampling in Q2 2013, Q3 2013, Q3 2014
  - 2 sites sampled all three quarters



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Aquatic Toxicity Test Results
8 of 9 Representative Fuel Terminal Sites

Algae
- Inconsistent results
- Decreased growth not related to polars
- Confounded by "plating"

Daphnia
- No survival decreases
- Reproduction decreased in all samples regardless of presence of polars

Fish
- No survival or growth decreases

Aquatic Toxicity Summary for "Representative" Sites

Site	TPHd as polars (µg/L)	Chronic Toxicity Present Relative to Lab Control?				
		Algae	Daphnia		Fish	
		Growth	Survival	Reproduction	Survival	Growth
A	<100	YES	no	YES	no	no
A	610	YES	no	YES	no	no
A repeat	<100	YES	no	YES	no	no
A repeat	250	YES	no	YES	no	no
A repeat	<97	YES	no	YES	no	no
A repeat	230	YES	no	YES	no	no
B	<100	no	no	YES	no	no
B	1,800	no	no	YES	no	no
C	<100	no	no	YES	no	no
C	640	YES	no	YES	no	no
D	<97	no	no	YES	no	no
D	120	no	no	YES	no	no
E	<95	no	no	YES	no	no
E	480	no	no	YES	no	no
F	<98	no	no	YES	no	no
F	410	YES	no	YES	no	no
G	<94	no	no	YES	no	no
G	120	YES	no	YES	no	no
H	<99	no	no	YES	no	no
H	400	no	no	YES	no	no

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Aquatic Toxicity Test Results - Site I

- Only site with any toxicity observed in fish
  - other sites with similar (or more) levels of polars had negative results
- Inconsistent results in algae and daphnia
- GRI model predicted potential toxicity in fish and daphnia based on ionic profile of groundwater

Aquatic Toxicity Summary for Site I						
Site #	TPHd as polars (µg/L)	Chronic Toxicity Present Relative to Lab Control?				
		Algae	Daphnia		Fish	
		Growth	Survival	Reproduction	Survival	Growth
I	<100	no	no	YES	no	no
I	680	no	no	YES	YES	YES
I repeat	<100	no	YES	YES	no	no
I repeat	540	YES	YES	YES	YES	YES
I repeat	<97	YES	YES	-	no	no
I repeat	780	YES	YES	-	YES	YES

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Potential Aquatic Toxicity of Polar Metabolites Summary

- No difference in aquatic toxicity profile between upgradient and downgradient samples relative to lab control at most sites
  - Daphnid and Fish survival not impacted by presence of polars
  - Fish growth not impacted by presence of polars
- When toxicity was observed, background water quality appears to be a major factor
  - Decreased algal growth and daphnid reproduction seen in samples with and without polars.
  - Survival and growth in fish decreased only at one site at which the ionic composition of the groundwater may have caused the observed toxicity

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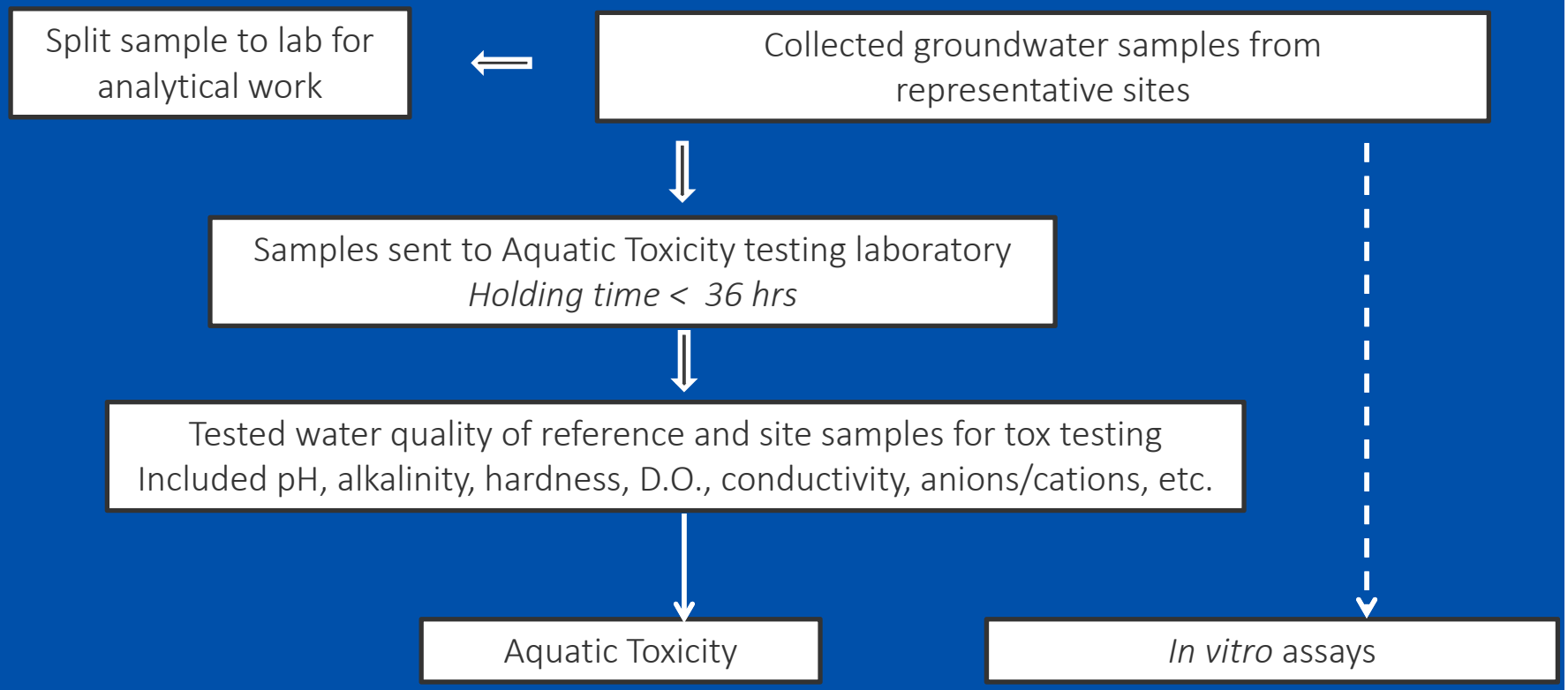
Potential Human Toxicity of Polar Metabolites Whole Mixtures

Traditional approaches involving animal testing not feasible



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In Vitro Assays
Whole Mixture

- No single regulatory accepted "screening test" available for comprehensive evaluation of chronic human health toxicity endpoints


Image:
laboratory set up (Cyprotex)

- Selected two assays to address different chronic toxicity endpoints
  - Endocrine Endpoint Test: Estrogenic Effects (Estrogen Receptor Transcriptional Activation, HeLa-9903)
  - Genetic Toxicity Test: DNA Damage (γH2AX Assay, HepG2)
- Challenges
  - *In Vitro* assay systems are not specifically designed for testing chemicals in environmental media; requires pre-testing to validate
  - Must work within the operating parameters of the assay system

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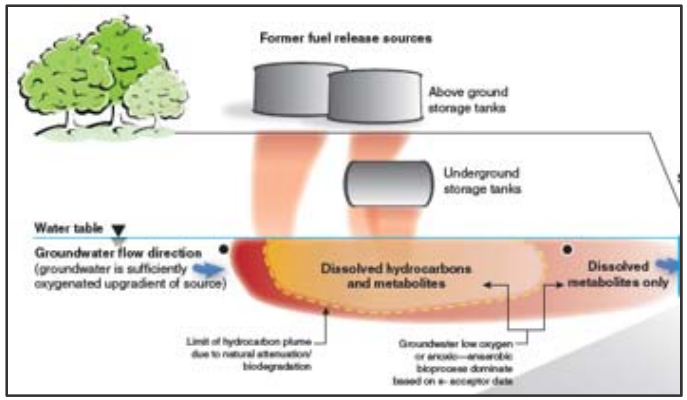
In Vitro Assays
Groundwater Sample Collection

- 12 fuel terminal sites evaluated, all in upland settings
  - 7 “representative” sites: upgradient sample (*free of polar metabolites and hydrocarbons*) vs. downgradient samples (*containing polar metabolites but no hydrocarbons*)
  - 5 “remaining” sites:
    - 4 sites with no polar metabolites upgradient or downgradient
    - 1 site with polar metabolites both upgradient and downgradient
- Sampling conducted Q1 and Q3 2015
  - 7 sites sampled both quarters



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In Vitro Assay Results
Seven "Representative" Fuel Terminal Sites

Estrogenic Effects:

- Negative results in ERTA assay in all samples in absence of co-exposure
- Activity seen in one sample spiked with a weak estrogenic agonist

DNA Damage:

- Negative results in all γH2AX tests
- Positive control included with all assays; activity noted as expected.

In vitro Test Results for "Representative" Sites				
Site	TPHd as polars (µg/L)	Estrogenic Effects (ERTA)		DNA Damage (γH2AX)+
		Activity	Spiked*Activity	Activity
J	<96	no	no	no
	120	no	negative[1]	no
J repeat	<94	no	no	no
	<94	no	no	no
	190	no	no	negative[1]
K	<96	no	no	no
	1500	no	no	no
K repeat	<95	no	no	no
	1900	no	no	no
L	<96	no	no	no
	780	no	no	no
L repeat	<94	no	no	no
	760	no	no	no
M	<99	no	no	no
	1200	no	negative[1]	no
M repeat	<95	no	no	no
	980	no	no	no
N	<96	no	no	no
	590	no	no	no
N repeat	<95	no	no	no
	590	no	no	no
O	<99	no	no	no
	350	no	no	no
O repeat	<100	no	no	no
	130	no	no	no
P	<96	no	no	no
	660	no	no	no
P repeat	<96	no	no	no
	1000	no	**Yes**	no

+Positive control camptothecin run with each assay
*Sample spiked with weak agonist 4-cumylphenol;
[1] Activity noted only at levels that produced cell toxicity.

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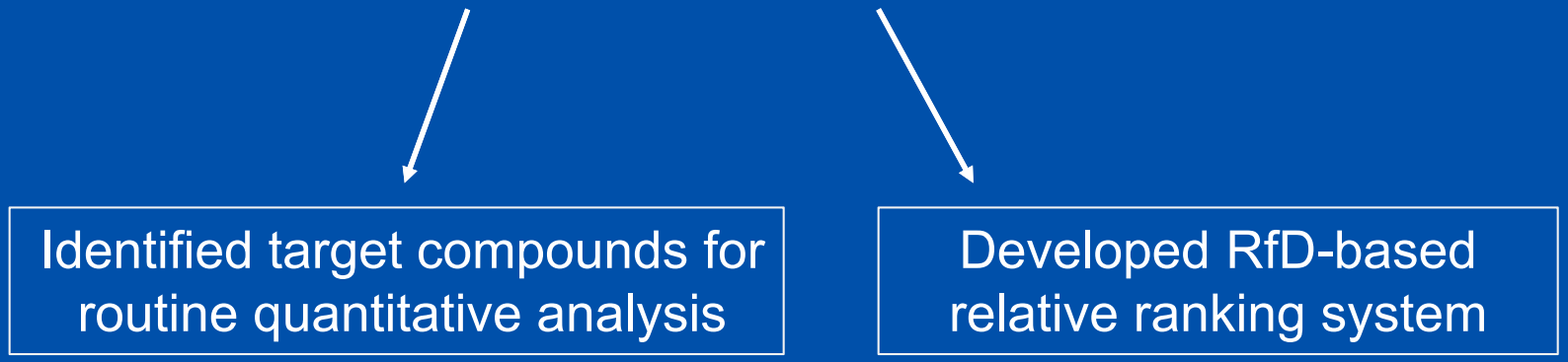
Potential Human Toxicity of Polar Metabolites Component Approaches

Identified expected structural families and classes for potential polar metabolites

Reviewed existing regulatory databases and toxicology data for chemicals in these groups



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Expected Structural Families and Classes

Polar Family	Specific Structural Class	Expected Chronic Oral Toxicity to Humans
Alcohols (and diols)	(n- and alkyl) alcohols	?
	(alkyl) cyclic alcohols	?
	(alkyl) polycyclic alcohols	?
	(alkyl) aromatic alcohols	?
	(alkyl) polyaromatic alcohols	?
Acids (and esters)	(n- and alkyl) acids/esters	?
	(alkyl) cyclic acids/esters	?
	(alkyl) polycyclic acids/esters	?
	(alkyl) aromatic acids/esters	?
	(alkyl) polyaromatic acids/esters	?
Ketones	(n- and alkyl) ketones	?
	(alkyl) cyclic ketones	?
	(alkyl) polycyclic ketones	?
	(alkyl) aromatic ketones	?
	(alkyl) polyaromatic ketones	?
Aldehydes	(n- and alkyl) aldehydes	?
	(alkyl) cyclic aldehydes	?
	(alkyl) polycyclic aldehydes	?
	(alkyl) aromatic aldehydes	?
	(alkyl) polyaromatic aldehydes	?
Phenols	(alkyl) phenols	?
	Phenol	?

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Target List for Routine Quantitative Analysis

- Identified target compounds for routine quantitative analysis
  - Initial target list included 57 compounds and expanded to 76 in 2012
  - Compounds selected primarily based on their potential toxicity, chemical structure, the availability of standards, boiling point, and extractability
  - 19 compounds had regulatory RfDs at the time of selection
- Target compounds quantitatively analyzed using standard EPA 8270C (semi-volatile organics by GC-MS)

Numbers of Target Analytes by Chemical Family					
Polar Chemical Family	Alcohols	Acids/Esters	Ketones	Aldehydes	Phenols
Number of Analytes	16	13	19	10	19

- Target analytes were rarely detected, at reporting limits ~ 10 ug/L
- None of the detected target analytes had agency derived RfDs

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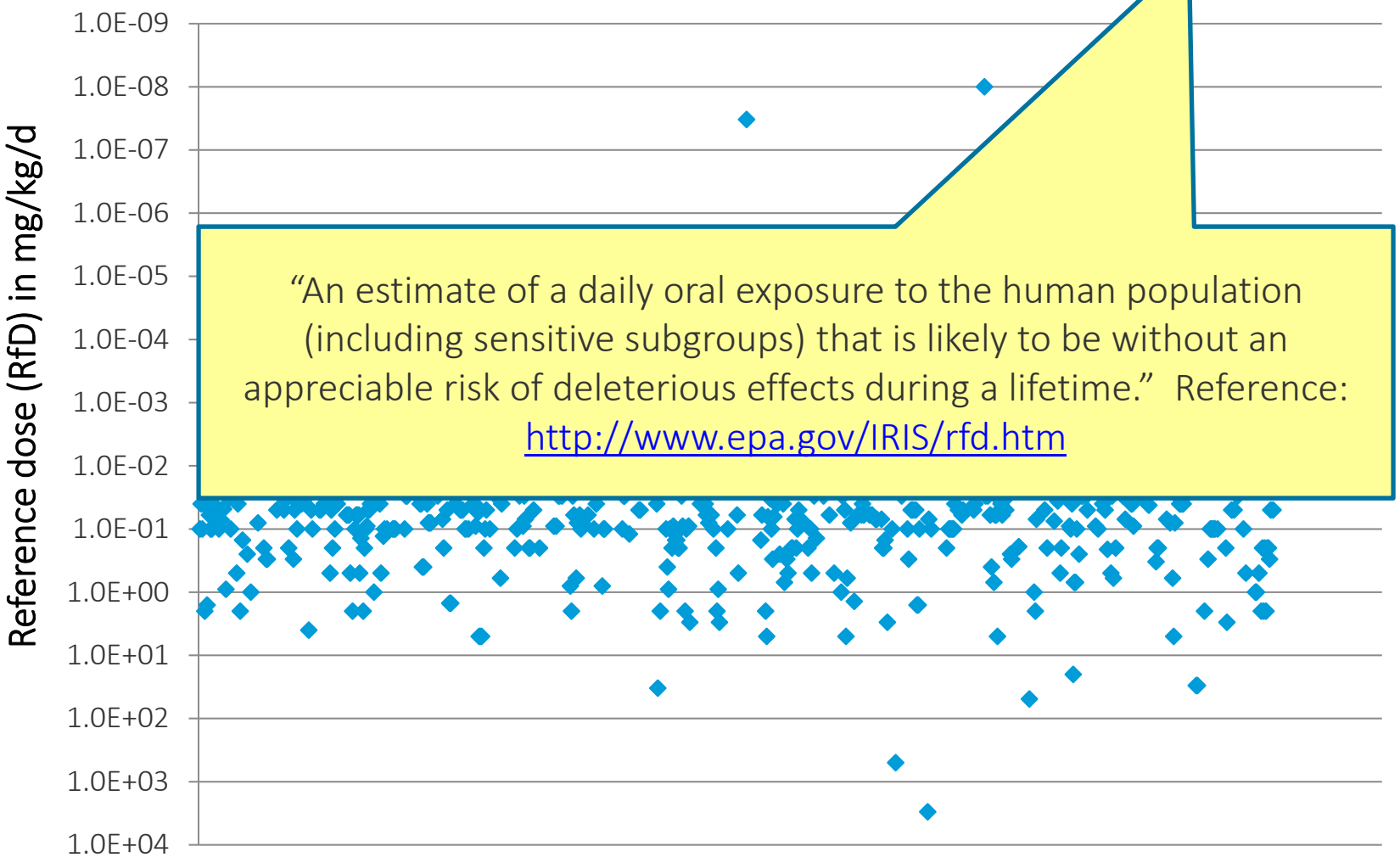
Development of Relative Ranking System
Universe of Chemicals with Regulatory Defined RfDs



Source USEPA RSL and TCEQ PCL tables

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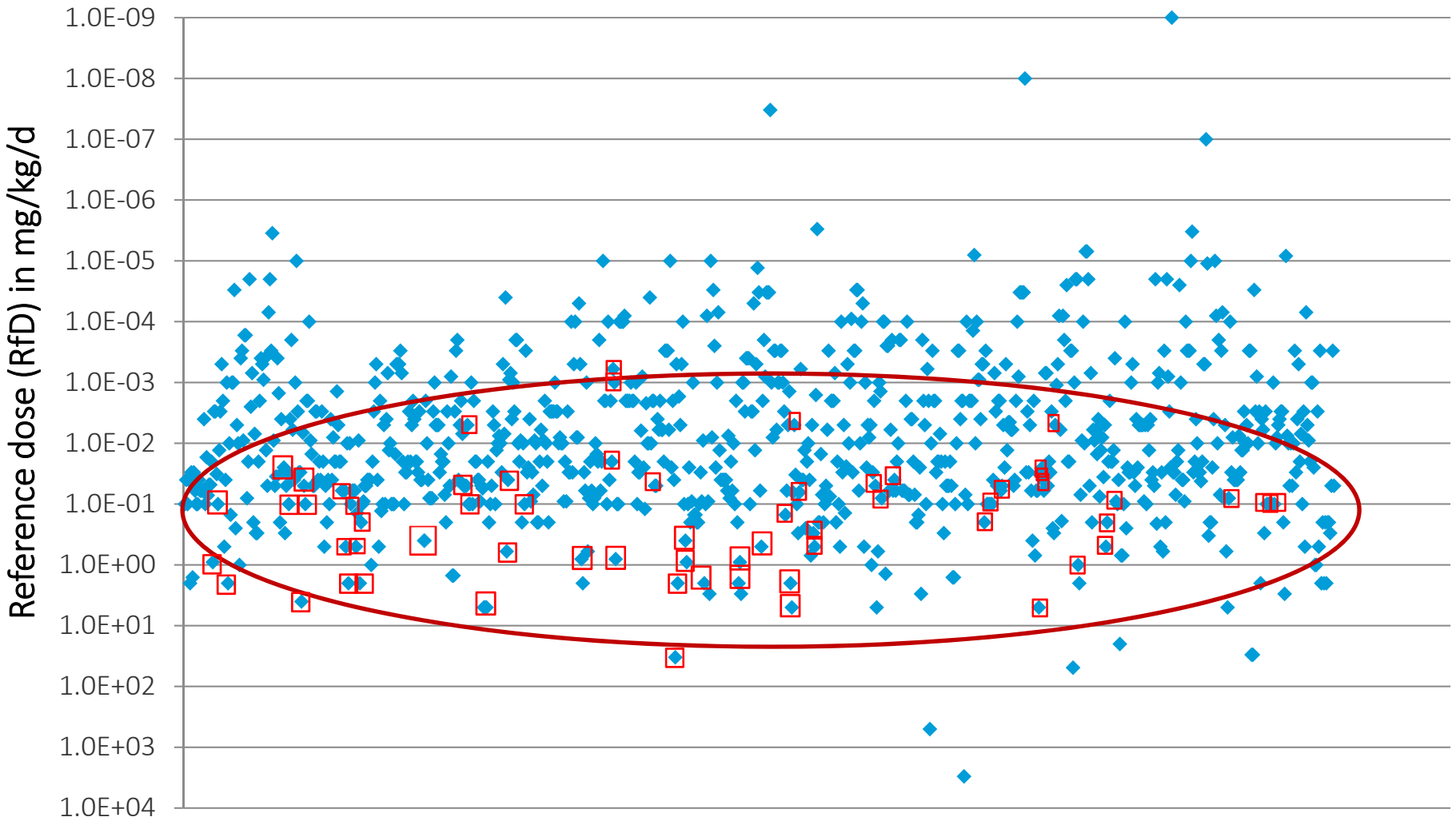
Development of Relative Ranking System
Potential Metabolites with Regulatory Defined RfDs



Source: US EPA RSL table and TCEQ TRRP table

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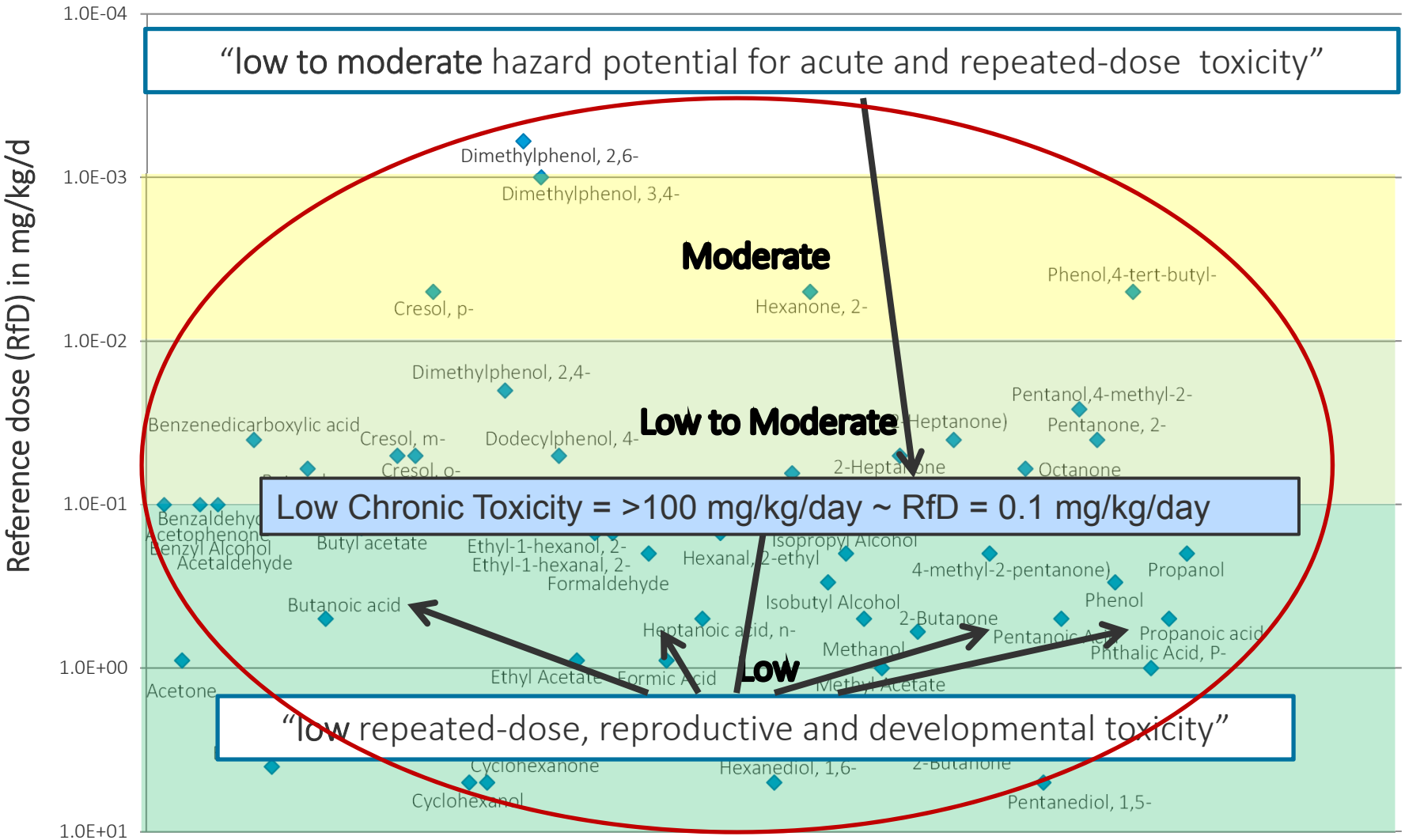
Potential Metabolites
USEPA OPPT ChAMP Relative Ranking System



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Expected Structural Families and Classes

Polar Family	Specific Structural Class	Expected Chronic Oral Toxicity to Humans
Alcohols (and diols)	(n- and alkyl) alcohols	?
	(alkyl) cyclic alcohols	?
	(alkyl) polycyclic alcohols	?
	(alkyl) aromatic alcohols	?
	(alkyl) polyaromatic alcohols	?
Acids (and esters)	(n- and alkyl) acids/esters	?
	(alkyl) cyclic acids/esters	?
	(alkyl) polycyclic acids/esters	?
	(alkyl) aromatic acids/esters	?
	(alkyl) polyaromatic acids/esters	?
Ketones	(n- and alkyl) ketones	?
	(alkyl) cyclic ketones	?
	(alkyl) polycyclic ketones	?
	(alkyl) aromatic ketones	?
	(alkyl) polyaromatic ketones	?
Aldehydes	(n- and alkyl) aldehydes	?
	(alkyl) cyclic aldehydes	?
	(alkyl) polycyclic aldehydes	?
	(alkyl) aromatic aldehydes	?
	(alkyl) polyaromatic aldehydes	?
Phenols	(alkyl) phenols	?
	Phenol	?

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Relative Toxicity Ranking Weight of Evidence Example: (n- and alkyl) acids/esters

Polar Family	Specific Structural Class	Expected Chronic Oral Toxicity to Humans
Acids (and esters)	(n- and alkyl) acids/esters	Low

RfD	(n- and alkyl) acids/esters	Reference
5.0E-01	Acrylic Acid	US EPA RSL Table
1.0E+00	Methyl Acetate	US EPA RSL Table
9.0E-01	Ethyl Acetate	US EPA RSL Table
5.0E-01	Butanoic acid	TCEQ TRRP PCL Table
1.4E-01	Butyl acetate	TCEQ TRRP PCL Table
9.0E-01	Formic Acid	US EPA RSL Table
5.0E-01	Heptanoic acid, n-	TCEQ TRRP PCL Table
2.0E+00	Hexanedioic Acid	US EPA RSL Table
6.4E-02	Hexanoic acid	TCEQ TRRP PCL Table
5.0E-01	Pentanoic Acid	TCEQ TRRP PCL Table
5.0E-01	Propanoic acid	TCEQ TRRP PCL Table
1.0E-01	C6-C22 n-alkyl carboxylic acids	LOW TOX (EPA)
1.0E-01	C4-C15 Linear alkyl diacids	LOW TOX (EPA)
1.0E-01	C8-C12 n-alkyl dioic acids	NOAEL>100 mg/kg/d
1.0E-01	C5-C28 Neoacids	NOAEL>100 mg/kg/d

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Relative Ranking for Expected Toxicity to Humans

Polar Family	Specific Structural Class	Expected Chronic Oral Toxicity Relative Ranking
Alcohols (and diols)	(n- and alkyl) alcohols	Low (RfD≥0.1; i.e., 0.1 to 1.0 or higher)
	(alkyl) cyclic alcohols	Low
	(alkyl) polycyclic alcohols	Low
	(alkyl) aromatic alcohols	Low
	(alkyl) polyaromatic alcohols	Low to moderate (0.1>RfD≥0.01)
Acids (and esters)	(n- and alkyl) acids/esters	Low
	(alkyl) cyclic acids/esters	Low
	(alkyl) polycyclic acids/esters	Low
	(alkyl) aromatic acids/esters	Low
	(alkyl) polyaromatic acids/esters	Low to moderate
Ketones	(n- and alkyl) ketones	Low to moderate
	(alkyl) cyclic ketones	Low
	(alkyl) polycyclic ketones	Low
	(alkyl) aromatic ketones	Low to moderate
	(alkyl) polyaromatic ketones	Low to moderate
Aldehydes	(n- and alkyl) aldehydes	Low to moderate
	(alkyl) cyclic aldehydes	Low to moderate
	(alkyl) polycyclic aldehydes	Low to moderate
	(alkyl) aromatic aldehydes	Low to moderate
	(alkyl) polyaromatic aldehydes	Low to moderate
Phenols	(alkyl) phenols*	Moderate (0.01>RfD≥0.001)
	Phenol	Low

*2,4- and 3,5- DTBP are assigned low toxicity based on USEPA toxicity data for the di-substituted alkylphenol (2009).

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Ranking System Application to Polar Metabolites Identified by GCxGC in One Field Sample

5H-Inden-5-one, octahydro-, trans-	Ketone	(alkyl) polycyclic ketones	Low
Acetic acid, (2,4-xylyl)-	Acid/Ester	(n- and alkyl) acids	Low
Benzaldehyde	Aldehyde	(alkyl) aromatic aldehydes	Low to moderate
Benzenepropanoic acid, á,á-dimethyl-	Acid/Ester	(alkyl) aromatic acids	Low
Bicyclo Octan-3-one	Ketone	(alkyl) polycyclic ketones	Low
Hexanal	Aldehyde	(n- and alkyl) aldehydes	Low to moderate
Cyclohexaneethanol, 2-methylene-	Alcohol	(alkyl) cyclic alcohols	Low
Cyclohexanone, 3,5-dimethyl-, cis-	Ketone	(alkyl) cyclic ketones	Low
Cyclopropanecarboxylic acid, cyclohexylmethyl ester	Acid/Ester	(alkyl) polycyclic acids	Low
Ethanol, 2-(2-butoxyethoxy)-	Alcohol	(n- and alkyl) alcohols	Low
2-Octanone	Ketone	(n- and alkyl) ketones	Low to moderate
Hexanoic acid	Acid/Ester	(n- and alkyl) acids	Low
Hexanoic acid, 2,2-dimethyl-	Acid/Ester	(n- and alkyl) acids	Low
Nonanoic acid, methyl ester	Acid/Ester	(n- and alkyl) acids	Low
Octanoic Acid	Acid/Ester	(n- and alkyl) acids	Low
Pentanal, 2,2-dimethyl-	Aldehyde	(n- and alkyl) aldehydes	Low to moderate
Pentanoic acid, 2-methyl-	Acid/Ester	(n- and alkyl) acids	Low
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	Acid/Ester	(n- and alkyl) acids	Low
Tridecanoic acid, methyl ester	Acid/Ester	(n- and alkyl) acids	Low

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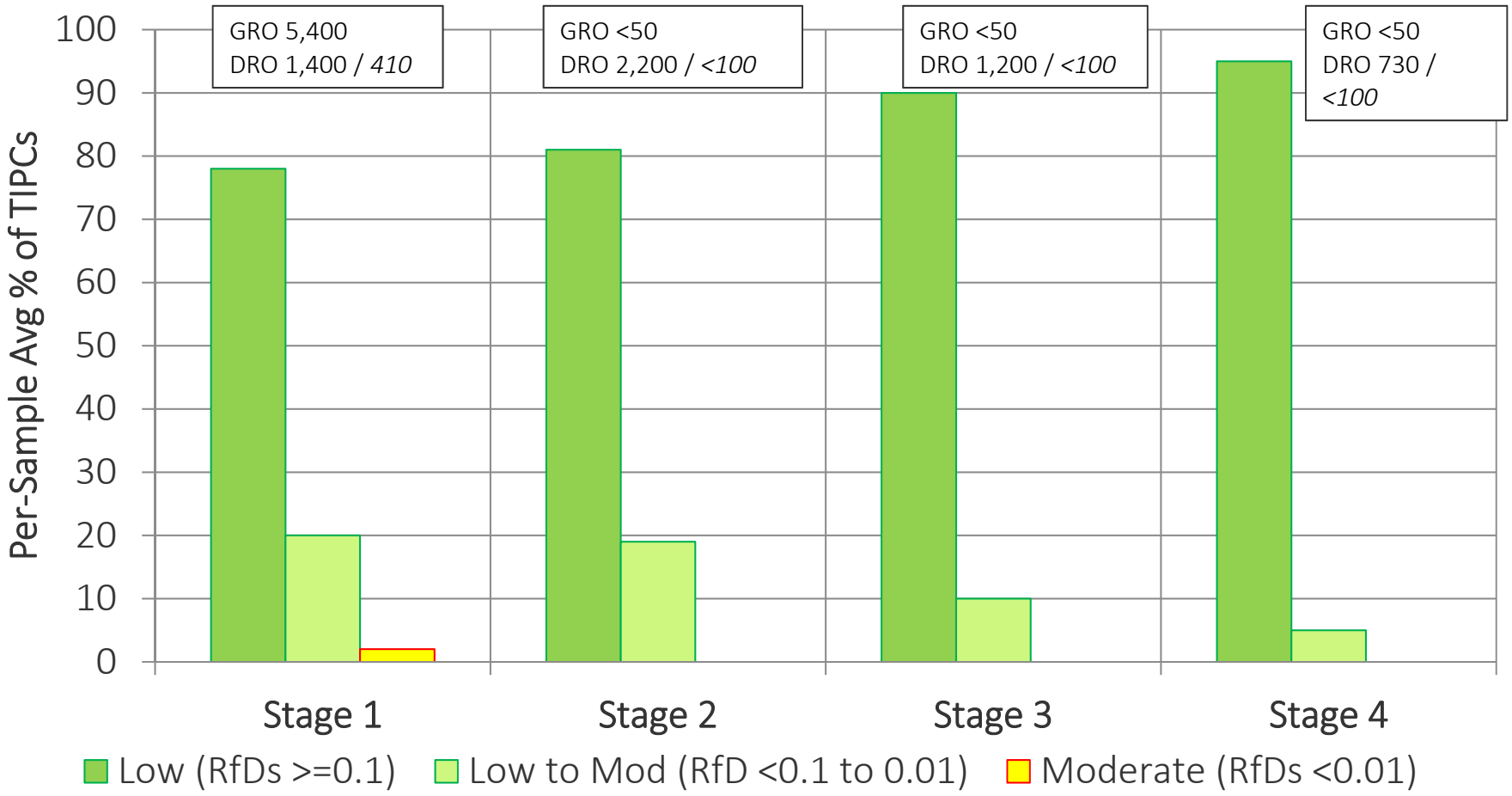
Average Distribution of Human Health Toxicity Profile:

Proportion of "Low" toxicity increases as biodegradation continues



DRO concentration (ug/L) without/*with* SGC is the average for the population representing he stage. Results are for samples without entrained product collected 2011 – 1Q2015.

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Potential Toxicity of Polar Metabolites Study Conclusions

- ✓ **Whole mixture testing** of polar metabolites found in groundwater at fuel release sites indicates:
  - No evidence that *polar metabolites* cause chronic toxicity to freshwater aquatic species
    - When toxicity was observed in the tests conducted, background water quality appears to be the major factor
  - *In vitro* assays demonstrate that these mixtures do not appear to have a significant potential to cause estrogenic effects or DNA damage
- ✓ Evaluation of the potential hazard to human health from the **components** of these complex mixtures indicates:
  - The vast majority of identified metabolites are expected to be of "Low Toxicity"
  - The trend with continued biodegradation is towards even lower toxicity.

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