Nature and Estimated Toxicity of Polar Metabolite Mixtures in Groundwater Quantified as Extractable “TPH” at Biodegrading Fuel Release Sites

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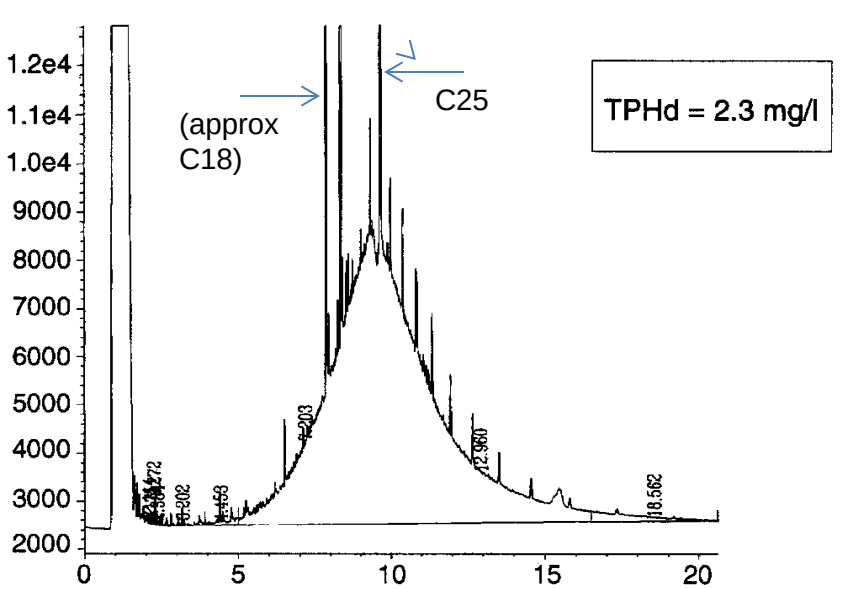
Background and Purpose of Work

- Groundwater at petroleum release sites is frequently analyzed using **Extractable TPH (e.g., BC Method EPH [LEPH, HEPH]; USEPA Method 8015B [TPHd/DRO], etc)**
  - GW sample is extracted using an organic solvent. Extract is analyzed using GC-FID; measures **all organics** extracted from the sample within specified **Boiling Point range or "Carbon Number range" (LEPH C10-19; HEPH C19-32; TPHd/DRO C10-28)**
- Polar metabolites from biodegradation (oxygen-containing non-HCs [*acids, alcohols, ketones, aldehydes, phenols*]) will be measured as "EPH" unless a SGC is used on the extract
  - SGC separates the hydrocarbons (which are "non-polar") from the non-hydrocarbons (which are "polar")

Background and Purpose of Work

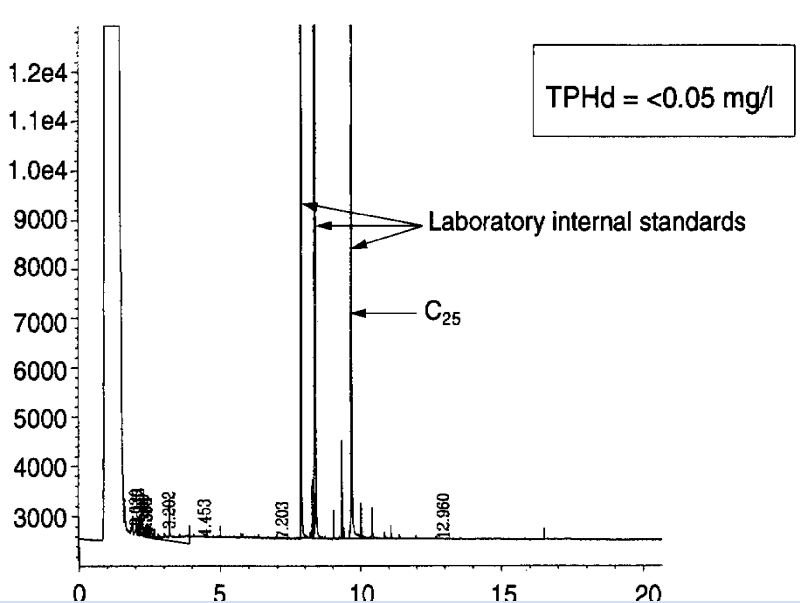
- **Up to 100%** of the organics in groundwater at sites with biodegrading petroleum can be **polars** and not dissolved hydrocarbons
- **Regulatory water quality objectives** (WQOs) for EPH are typically based on the properties of the **hydrocarbons** assumed to be present (e.g., aromatics), and **not on the properties of polar compounds**
- SGC recommended years ago to resolve "apples to oranges" technical conflict: comparing EPH results that **included polar compounds** to **hydrocarbon-based** WQOs

Why SGC is necessary to separate hydrocarbons and polars at sites with biodegrading sources



a. Groundwater sample without SGC
("TPHd" 2.3 mg/l includes hydrocarbons and polars; note mass >C14 and hump)

(Zemo and Foote 2003)



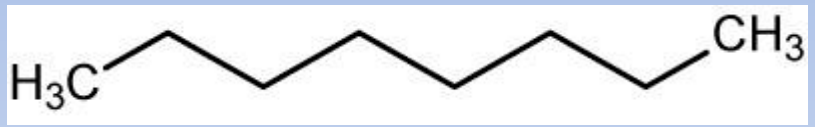
b. Duplicate sample after SGC
(100% "TPHd" was polars; hydrocarbons not detectable in this sample)

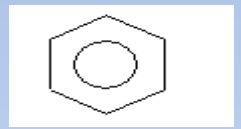
Background and Purpose of Work (cont.)

- SGC has been **inconsistently** accepted for groundwater samples
- Many have claimed that there is **uncertainty regarding the nature and toxicity of the polar compounds** that are removed by the SGC
  - Analytical methods were not available in the 1990s/early 2000s to identify compounds in complex mixture of polars
- This study was conducted to:
  - **provide a more detailed understanding of the polar compounds actually present in groundwater**, and
  - provide the technical basis and data needed for consistent acceptance of SGC prior to EPH analysis

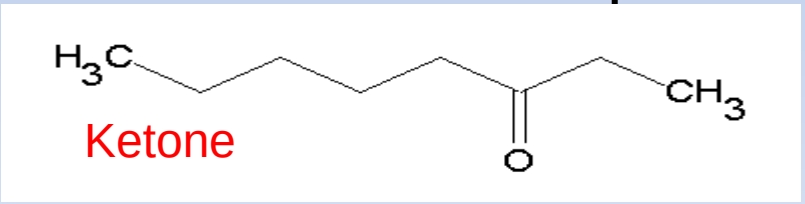
Hydrocarbons vs. Polars

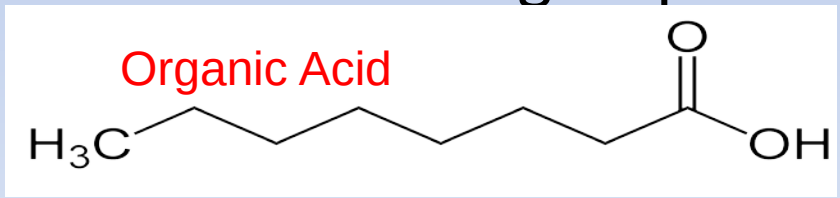
Hydrocarbons contain only carbon and hydrogen

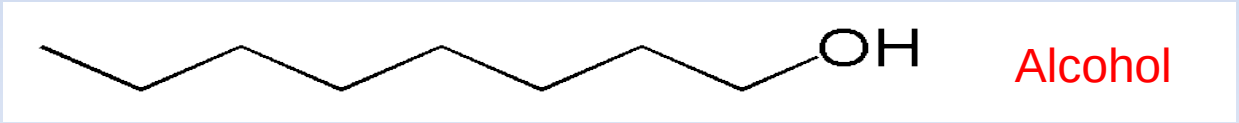




Polars contain a polar atom or functional group







Polars and the Natural Attenuation Paradigm

- Polar biodegradation byproducts (esp. organic acids) in groundwater have been studied by USGS and others for many years to document natural attenuation
- Presence of polar metabolites is **direct evidence** that intrinsic biodegradation of residual hydrocarbons is occurring
- Intrinsic and enhanced biodegradation is a widely-accepted remedial method for petroleum releases
  - Wiedemeier et al. 1995 ; ASTM 1998; USEPA 1999; many others

Biochemistry: **5 families** and **22 classes** of polars expected from biodegradation

Hydrocarbons are oxidized to organic acids and then converted to CO_2 and water. Intermediate oxidation steps can result in acids/esters, alcohols, phenols, ketones and aldehydes

Polar Family	Specific Chemical Class
Alcohols	(alkyl) alcohols
	(alkyl) cyclic alcohols
	(alkyl) polycyclic alcohols
	(alkyl) aromatic alcohols
	(alkyl) polyaromatic alcohols
Acids (and esters)	(alkyl) acids/esters
	(alkyl) cyclic acids/esters
	(alkyl) polycyclic acids/esters
	(alkyl) aromatic acids/esters
	(alkyl) polyaromatic acids/esters

Polar Family	Specific Chemical Class
Ketones	(alkyl) ketones
	(alkyl) cyclic ketones
	(alkyl) polycyclic ketones
	(alkyl) aromatic ketones
	(alkyl) polyaromatic ketones
Aldehydes	(alkyl) aldehydes
	(alkyl) cyclic aldehydes
	(alkyl) polycyclic aldehydes
	(alkyl) aromatic aldehydes
	(alkyl) polyaromatic aldehydes
Phenols	phenol
	alkylphenols

Human Health Toxicology

- Searched USEPA, United Nations, and other regulatory agencies for data on **individual compounds** and **classes** of polars
- Developed **RfD-Based toxicity ranking system** for 22 classes of metabolites consistent with existing regulatory systems
- Identified **individual ("target") polar compounds** for quantitative analysis based primarily on potential toxicity, chemical structure and availability of chemical standards:
  - 57 individual target compounds in 2011
  - 76 individual target compounds in 2012 and thereafter

Estimated Toxicity for Polar Compound Classes

Polar Family	Specific Chemical Class	Expected Chronic Oral Toxicity to Humans
Alcohols (and diols)	(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)	(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	(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	(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

Analytical Chemistry: Sample Analyses Protocol

All samples extracted with DCM using routine method for TPHd

(USEPA Method 3510; separatory funnel, same as BC EPH method)

Analyzed extracts with and without SGC using all methods below

Rinsed the SG column with methanol;

Analyzed methanol rinse using all methods except TPHd

Quantitative Analyses

Commercial Lab
TPHd /DRO (C10-28)
Method 8015 GC-FID
With and without SGC

Commercial Lab
Target Analytes
Modified 8270 GC-MS
Authentic standards

Qualitative Analyses

Commercial Lab
Library Search
GC-MS
Top 40 TICs

Research Lab
Two Dimensional GC
GCxGC-MS
Match 75%+; S/N> 5
All 4 extracts/rinse for each sample.

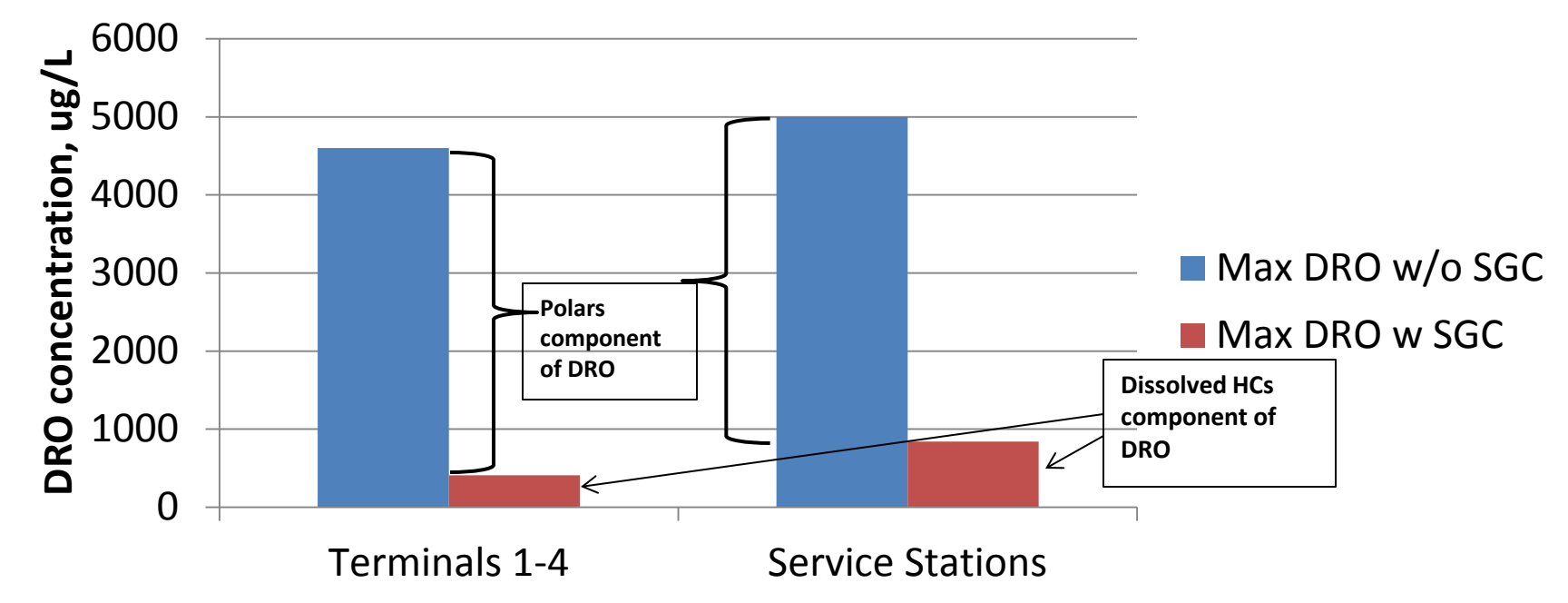
Field Study

- 2011 : Collected 22 gw samples at 5 fuel terminals with low/no BTEX plumes (highly biodegraded)
  - analyzed each sample for all parameters and NA parameters
  - published these results in 2013
- 2012: repeated sampling and analyses at 5 terminals
- 2013: expanded study to UST sites with elevated BTEX plumes (less biodegraded condition); collected 18 gw samples at 5 UST sites
- *2013 and 2014: conducted aquatic toxicity testing on gw samples containing polars from upland terminals*

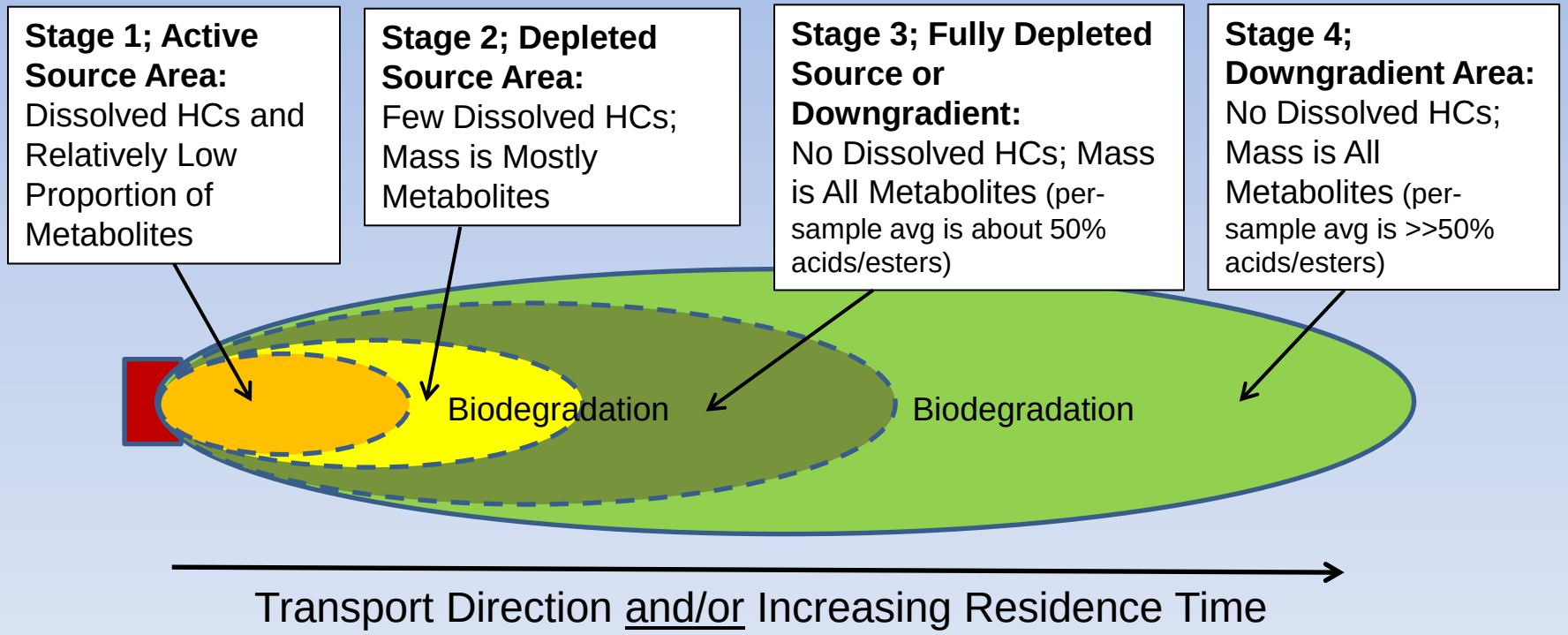
Study Summary: Life-Cycle of a Dissolved "EPH" Plume

- NOTES:
  - Individual target metabolites were very infrequently detected using GC-MS (RLs typically 10 ug/L); commercial lab GC-MS library search identified relatively few compounds in each sample.
  - Data shown here are from GCxGC-MS results for tentatively identified compound family and structural class; not quantitative, but likely identified peaks at single-digit ug/L.
  - More than 1,300 unique individual polar metabolites were identified using GCxGC-MS.
  - Results shown here are for samples that did not contain a separate-phase product component

Study Summary: “EPH” in both types of plumes was primarily Polars; when present, concentrations of dissolved diesel-range hydrocarbons were low



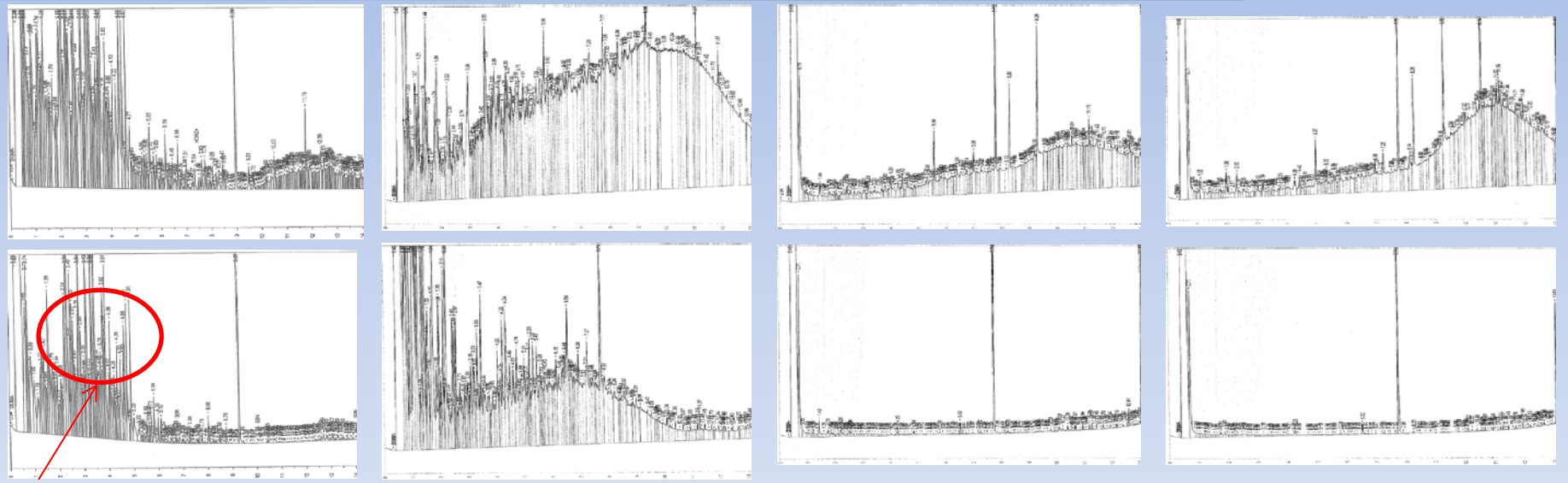
Study Summary: "Stages" for the Components of a Dissolved EPH (extractable organics) Plume



Example DRO Chromatograms w/o and w/SGC

Stage 1: Active Source
GRO 7,400; DRO 850 / 640

Stage 3: Fully Depleted/Dngrdt
GRO <50; DRO 3,300/<50



Dissolved C10 - C14 HCs present

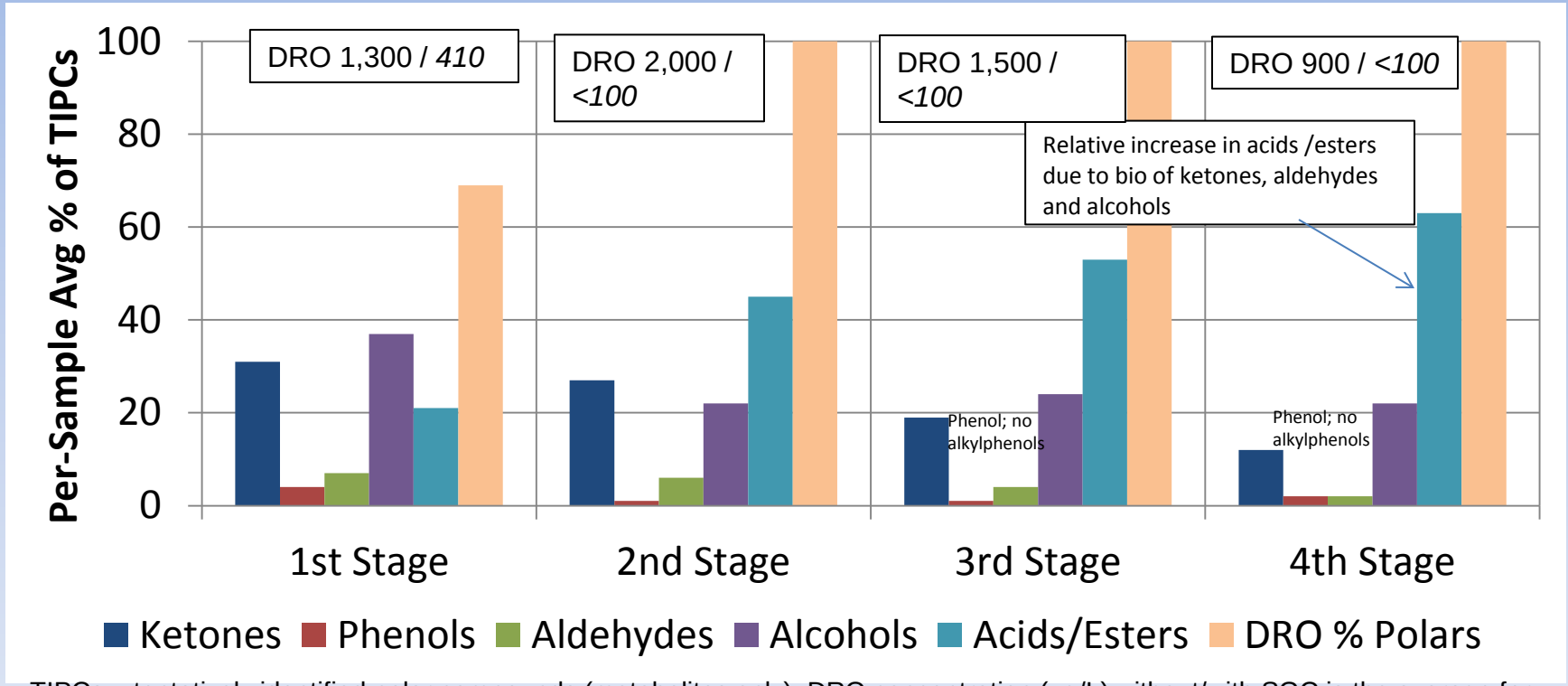
Stage 2: Depleted Source
GRO 1,000; DRO 6,000 / 800

Stage 4: Downgradient
GRO <50; DRO 470/<50

GRO/DRO concentrations (ug/L) are for example well for which chromatogram is shown.

Average Distribution of Metabolite Families:

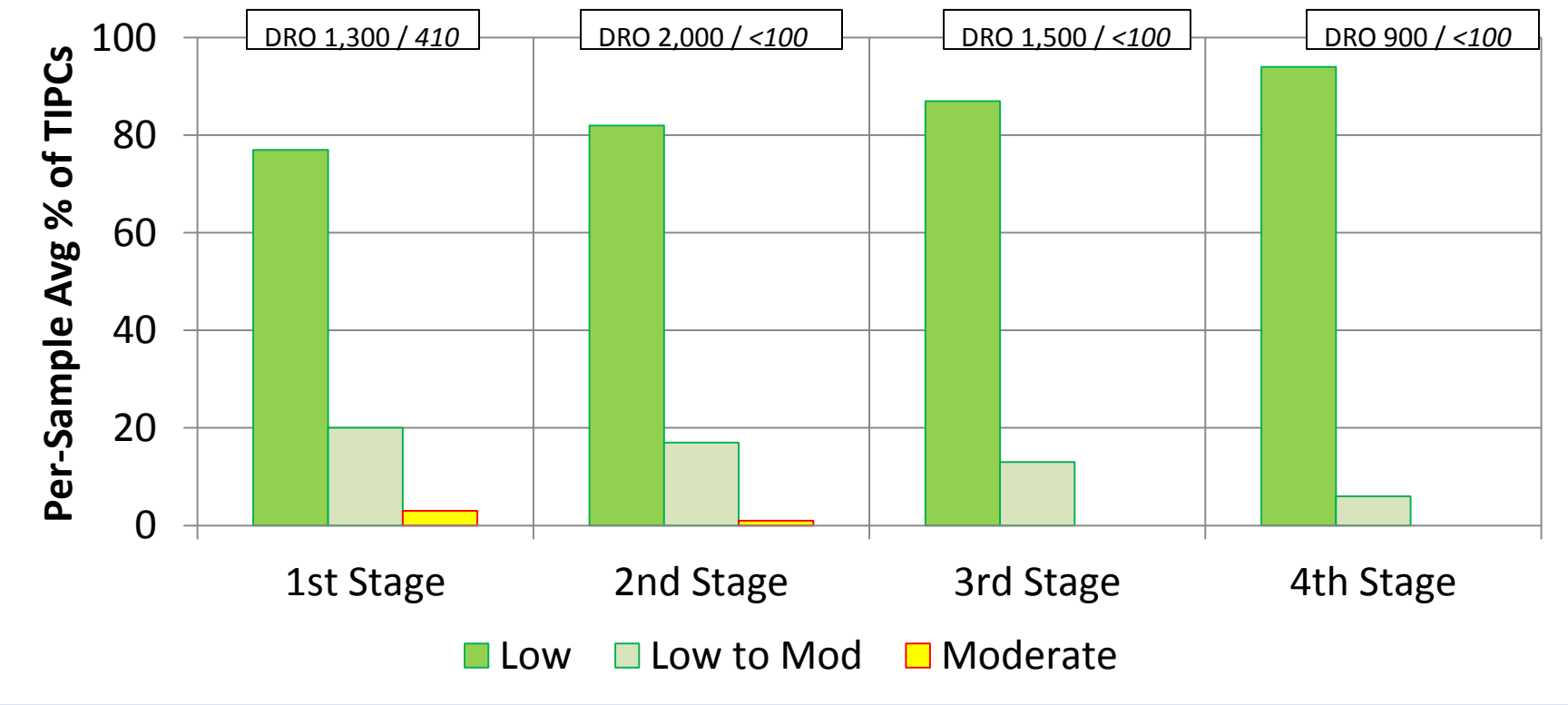
Proportion of acids/esters increases as biodegradation continues



TIPCs = tentatively identified polar compounds (metabolites only). DRO concentration (ug/L) without/with SGC is the average for the population representing the stage.

Average Distribution of Toxicity Profile:

Proportion of "Low" toxicity increases as biodegradation continues

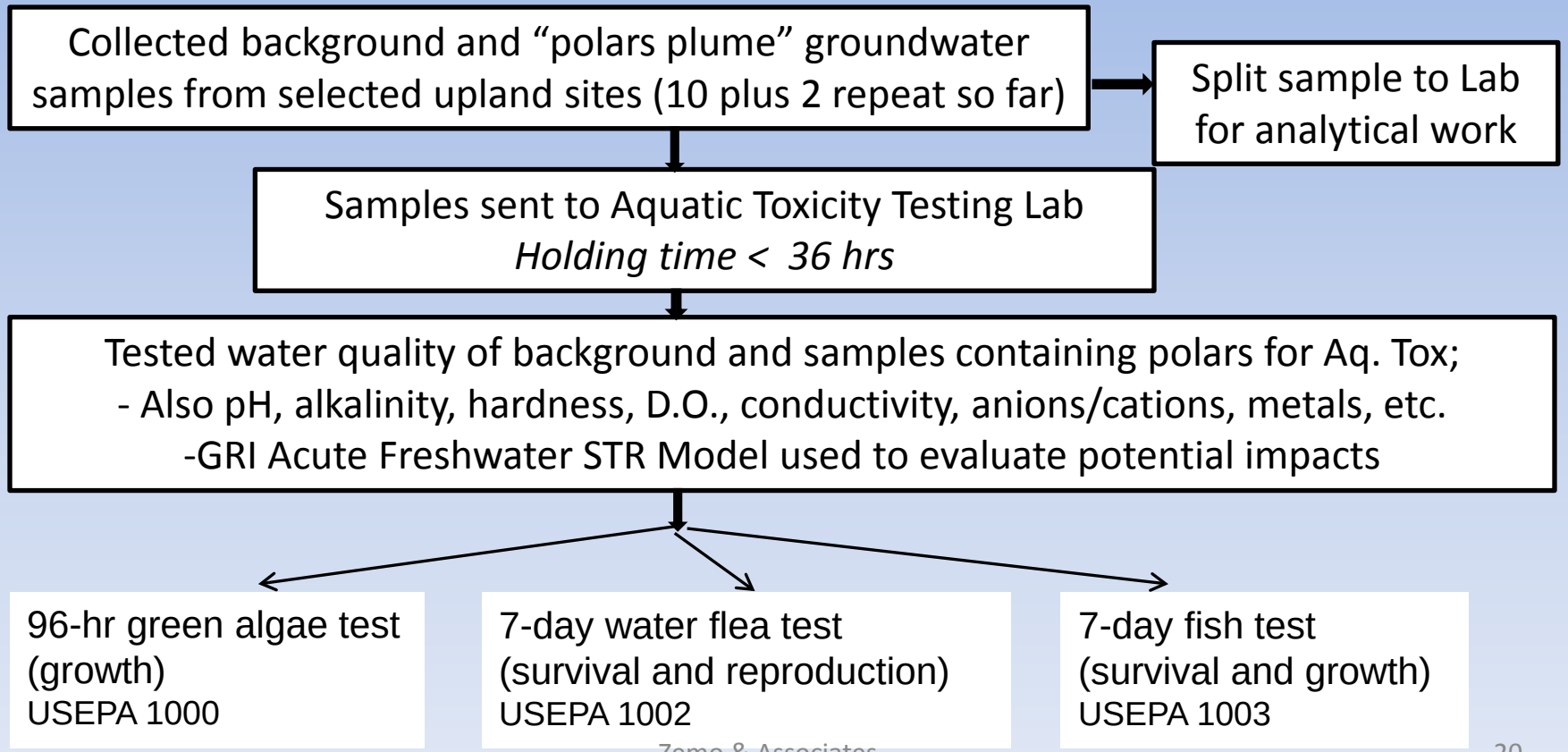


TIPCs = tentatively identified polar compounds (metabolites only). DRO concentration (ug/L) without/with SGC is the average for the population representing the stage.

Conclusions

- Overall trend with continued biodegradation of the metabolites is toward increasing proportion of acids/esters, and an ever-lower human-health toxicity profile
- Vast majority of identified metabolites are in “Low Toxicity” classes
  - RfDs of **0.1 to 1.0 (or higher)** vs aromatic hydrocarbons with RfDs of 0.03 to 0.04
- Metabolite mixtures in gw are unlikely to pose a significant risk to humans
- Study findings validate natural attenuation paradigm
- *SGC should be used routinely to separate the polars from hydrocarbons when it is necessary to compare EPH results to hydrocarbon-based WQOs*

Aquatic Toxicity Testing

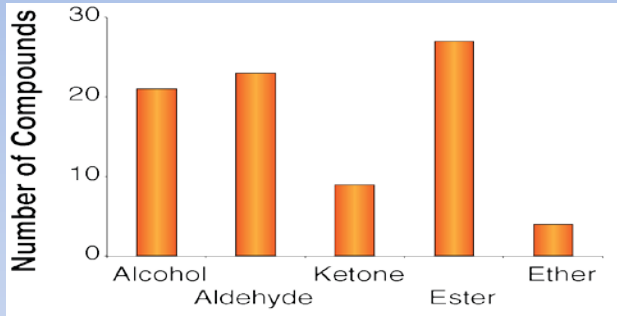


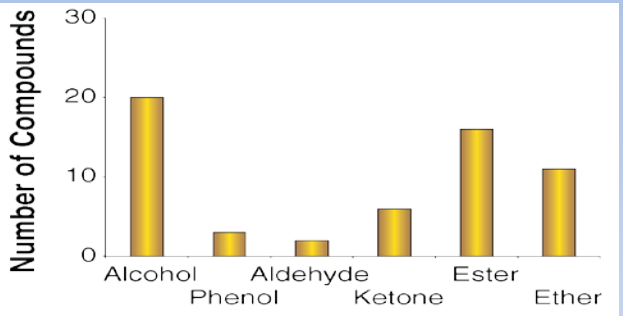
Aquatic Toxicity Tests: Results

- Tested “worst-case” conditions: impacted groundwater
- **Polar metabolites are not increasing the toxicity of the groundwater**
  - No difference in aquatic toxicity profile between upgradient and downgradient samples at most sites
- Toxicity primarily seen due to **background water quality**
- Toxicity **is not correlated** with the concentration of polars in sample (no dose-response)
- The complex mixtures of polar metabolites, at concentrations typically found at petroleum release sites, are unlikely to pose a significant risk to aquatic receptors

Risk Management Thoughts

Petroleum Metabolites are Not Unique; these types of compounds are part of our everyday lives

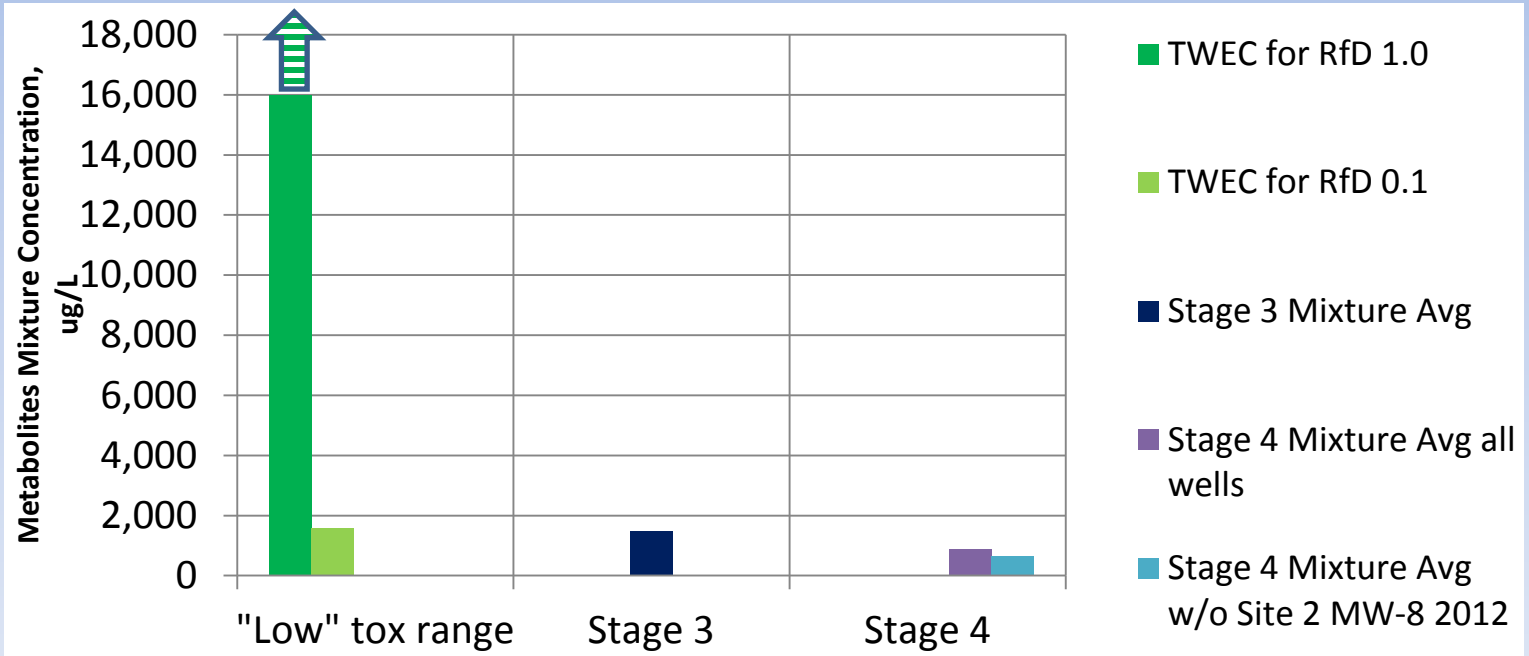




GCxGC of foods and beverages have identified the same classes of compounds and structures consistent with those found in this study

Risk Management Thoughts

Average GW concentrations are below the range of potential "Tap Water Equivalent Concentrations (TWECs) for "Low" toxicity classes. Toxicity profile decreases over time.



TWEC calculated using USEPA equation for gw ingestion and respective RfD

Status

- Peer-Reviewed Papers Published: **3** (2 GWM&R; 1 ES&T)
- Technical Presentations (with Abstracts): **SETAC** 2011; **AEHS** West Coast 2012; **SOT** Annual Mtg 2013 (poster, *blue ribbon*); **Battelle Bio** 2013; **INEF** 2013; **SETAC** 2013; **AEHS** 3/2014 (session); **SETAC** poster at 11/2014 mtg (aquatic tox testing)
- Presentations to Regulatory Agencies ongoing: CA RWQCBs; CA DTSC; CA State Water Board; CUPA (2012 and 2014); WA Ecology NWRO and SWRO; WV DEQ; AK DEC; HI DOH; UT DEQ
- Preparing several more manuscripts:
  - Human and aquatic toxicity evaluation;
  - Post-2011 results and broader risk management evaluation;
  - Various short technical notes, etc.

Questions?

- This project was funded under Chevron's Remediation Technology Development Initiative (RTDI)
- Results from initial (2011) sampling at the fuel terminals were peer-reviewed and published in:
  - Zemo et al, 2013. "**Nature and Estimated Human Toxicity of Polar Metabolite Mixtures in Groundwater Quantified as TPHd/DRO at Biodegrading Fuel Release Sites**". *Ground Water Monitoring & Remediation, v33 n4: 44-56.* DOI: 10.1111/gwmr.12030
  - Mohler et al, 2013. "**Non-Targeted Analysis of Petroleum Metabolites in Groundwater using GCxGC-TOFMS**". *Environmental Science & Technology, v47 n18*: 10471-10476
  - Zemo et al, 2013. "**Comparison of Shake and Column Silica Gel Cleanup Methods for Groundwater Extracts to be Analyzed for TPHd/DRO**". *GWM&R, v33 n4: 108-112* DOI: 10.1111/gwmr.12032

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