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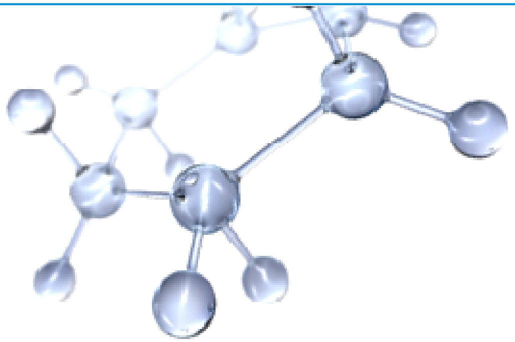
Assessing oil toxicity: methods & models



University of Florida Institute of Oceanography

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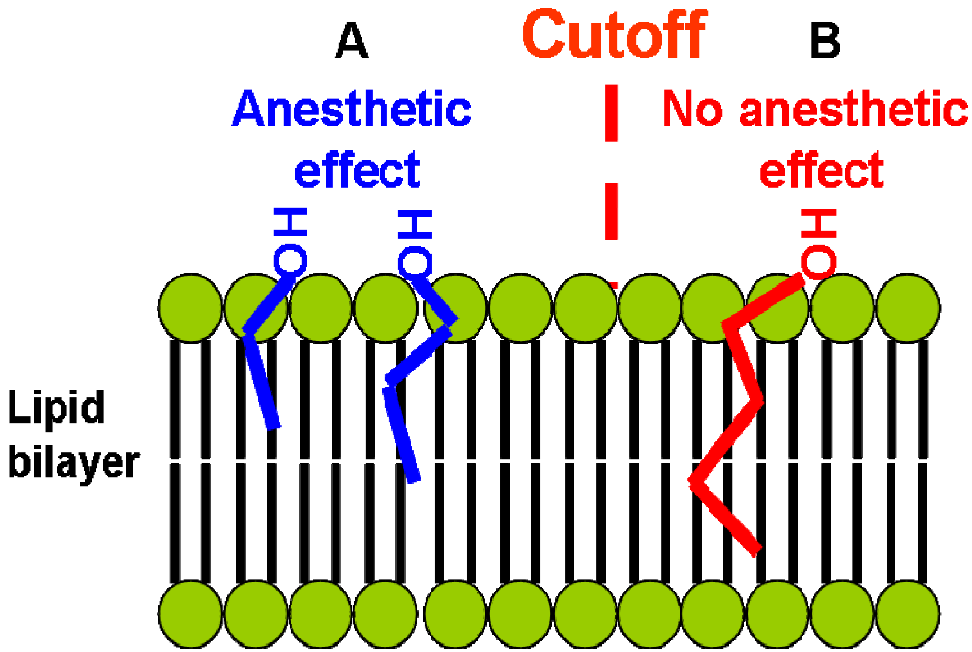
September 27, 2011

Outline

- Toxicity assessment of single hydrocarbons to aquatic/marine life
  - Target lipid model
- Methods for testing complex hydrocarbons, e.g. crude oil
  - Water Accomodated Fraction (WAF) test procedure
- Tools for predicting toxicity
  - Additive toxic unit model
  - Biomimetic extraction analysis
- Influence of chemical dispersants on oil toxicity
- Additional issues
  - Photo-enhanced toxicity
  - Bioaccumulation of PAHs in foodchain
- Summary & research needs

Narcosis

- Non-specific, perturbation of membrane function that results in decreased activity (e.g. ventillation, oxygen consumption, heart rate), immobilization and ultimately death to organisms
- Applicable to many classes of chemicals including hydrocarbons
  - Minimum level or "baseline" toxicity independent of exposure route
  - Shown to correlate with substance hydrophobicity until toxicity "cut-off"



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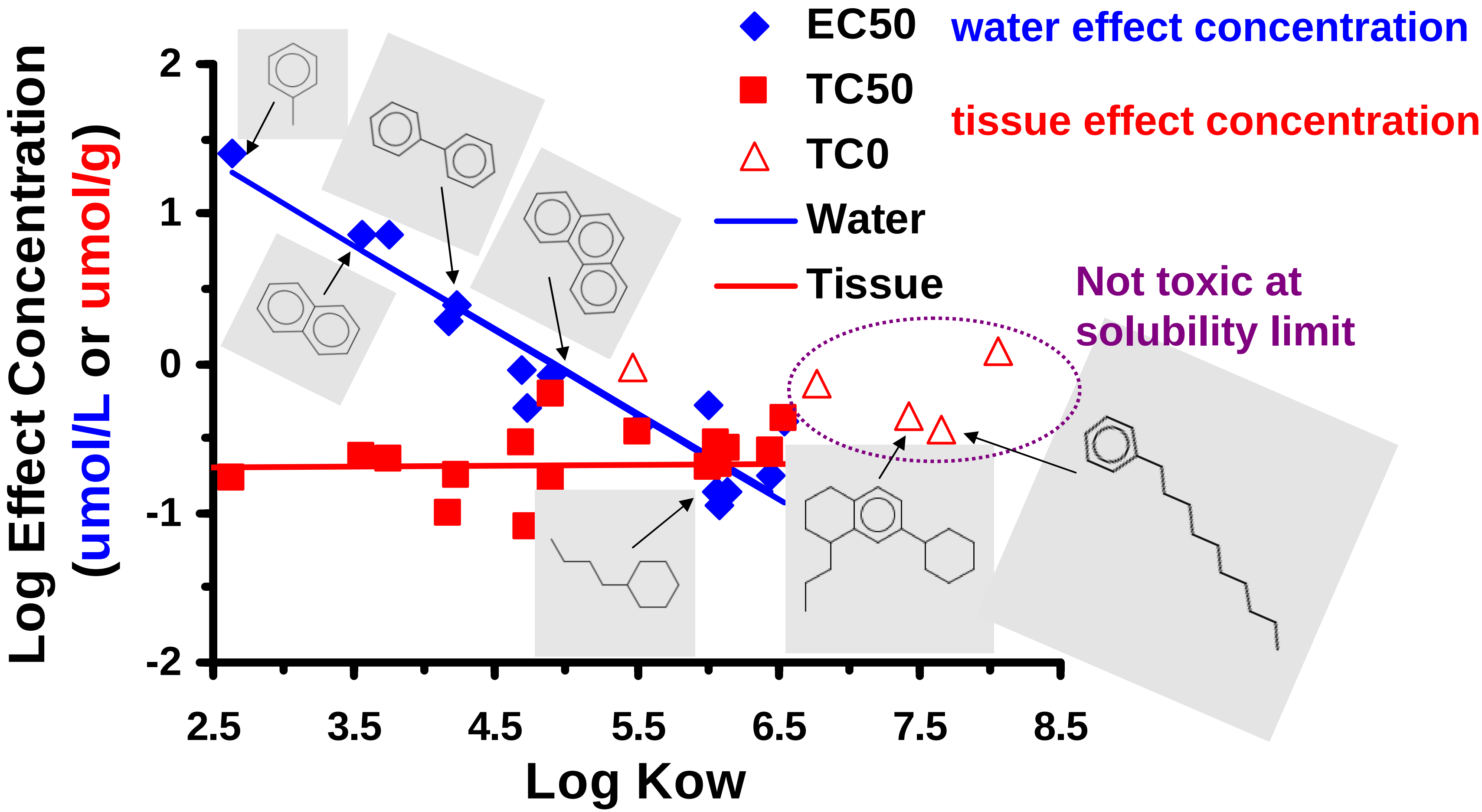
Inhibition of Mussel Filtration Rate



Source:
Donkin et al. (1991) Sci. Tot. Env. 109:461; Smith et al. (2001) ET&C 20:2482

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Predicting Narcosis using the Target Lipid Model

CTLBB for a chemical is determined as:

$$CTLBB = LC_{50} \times KT_{L\text{-}W} \qquad (1)$$

Rearranging and taking logs:

$$\log(EC_{50}) = \log(CTLBB) - \log(KT_{L\text{-}W}) \qquad (2)$$

Based on linear-free energy relationships:

$$\log(KT_{L\text{-}W}) = a_o + a_1 \log(K_{ow}) \qquad (3)$$

Substituting (3) into (2) yields:

$$\log(EC_{50}) = \log(CTLBB) - a_o - a_1 \log(K_{ow}) \qquad (4)$$

Response **Organism** **Hydrocarbon**

CTLBB = critical target lipid body burden (mmol/kg octanol)
EC_{50} = aqueous concentration that causes a 50% response (mmol/L)
$KT_{L\text{-}W}$ = target lipid water partition coefficient (L/kg lipid)
K_{ow} = octanol water partition coefficient (L/kg octanol)
a_o ,a_1 = emprical constants that relate partitioning at target site to octanol

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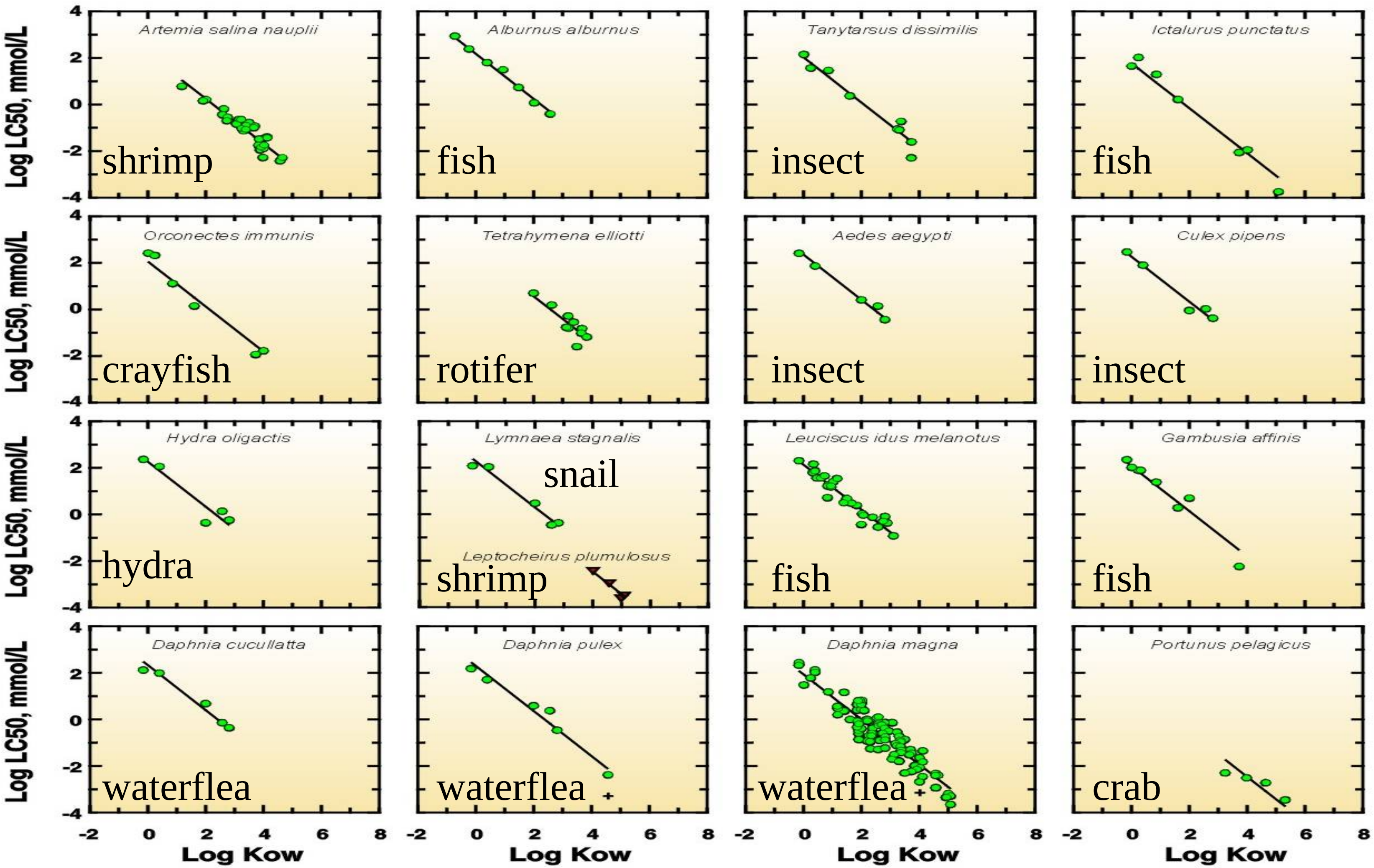
Calibration of TLM using Acute Toxicity Data Sets



Source: DiToro, McGrath, & Hansen (2000) ET&C 19:1951–1970.

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Results of TLM calibration

- Quantitative relationships developed for 56 species
  - amphibians, fish, invertebrates, algae, microbes / aquatic & marine
  - ca. 1000 reliable acute toxicity tests for 250+ chemicals
    + aliphatic hydrocarbons, alcohols, ethers, ketones, mono-, and poly-aromatic hydrocarbons including halogenated structures than span a log(K_{OW}) range from 0 to 6
- a_o chemical class dependent
  - a_o = 0 for most HCs (baseline); = 0.35 for PAHs (2X potency)
  - attributed to polar interactions that increase affinity for target site
- a_1 constant across narcotic chemicals!
  - a_1 = 0.936
- Intercept [log (CTLBB)] is species-dependent
  - used to define species-sensitivity distribution (next slide)

Source: McGrath & DiToro (2009) ET&C28:1130

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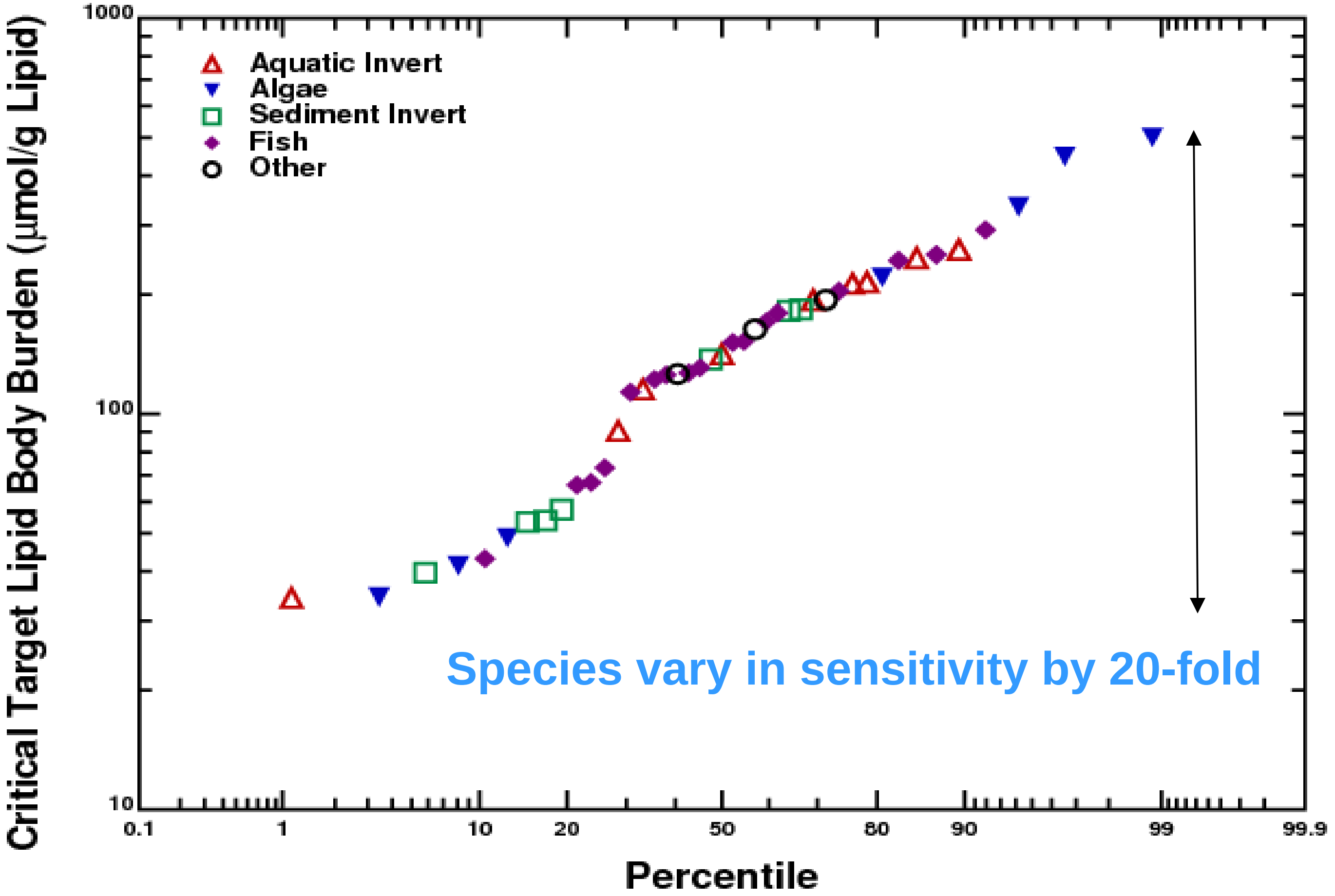
CTLBB Species - Sensitivity Distribution



Source: McGrath & DiToro (2009) ET&C 28:1130

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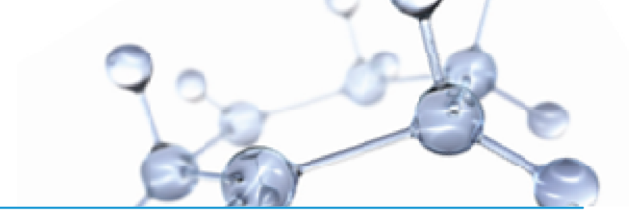
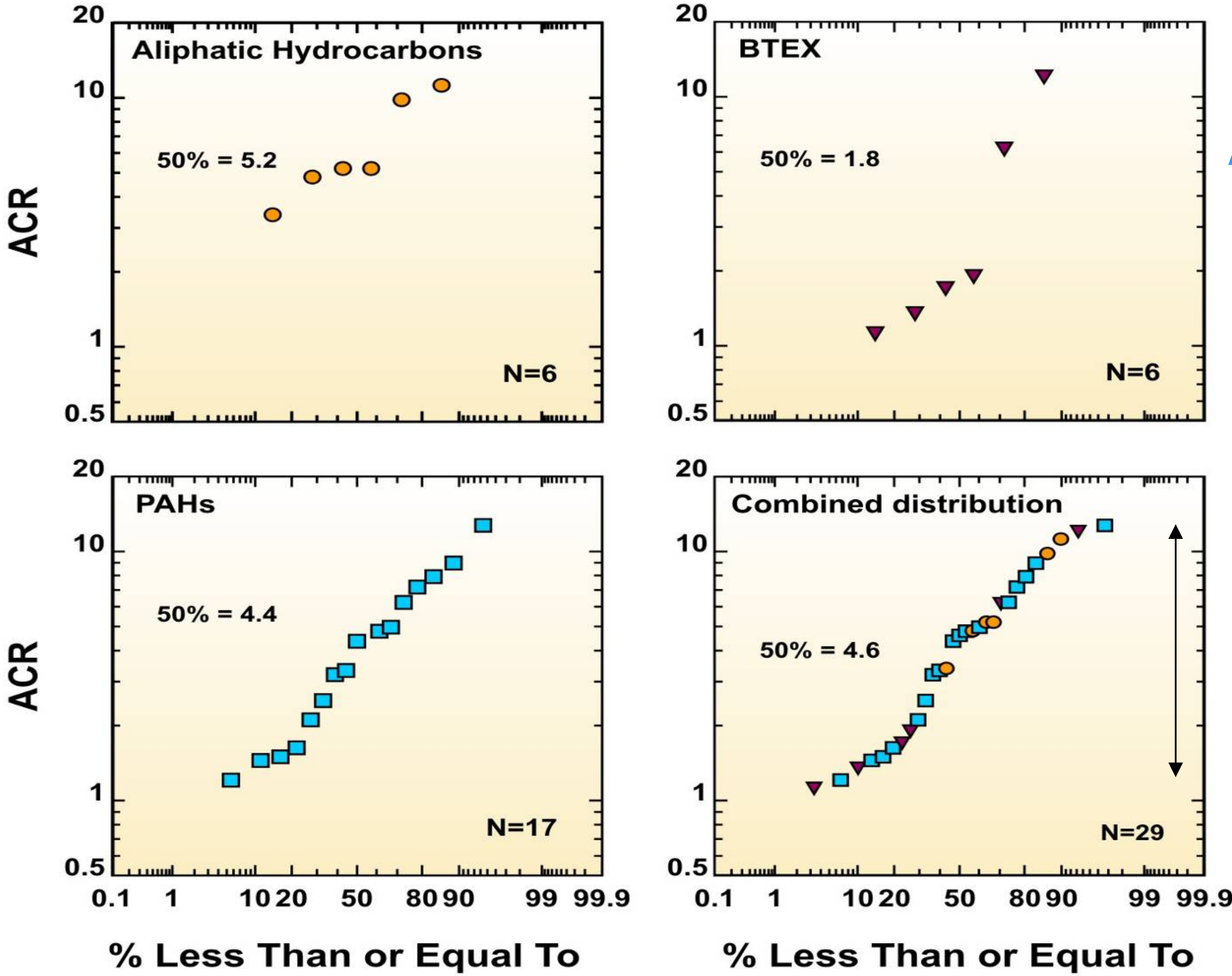
Extrapolation to Chronic Effects





Acute to Chronic Ratio
ACR =
acute L/EC_{50}
÷
chronic NOEC/EC_{10}

For hydrocarbons ACRs vary 10-fold

<u>Source</u>:
McGrath & DiToro (2009) ET&C 28:1130

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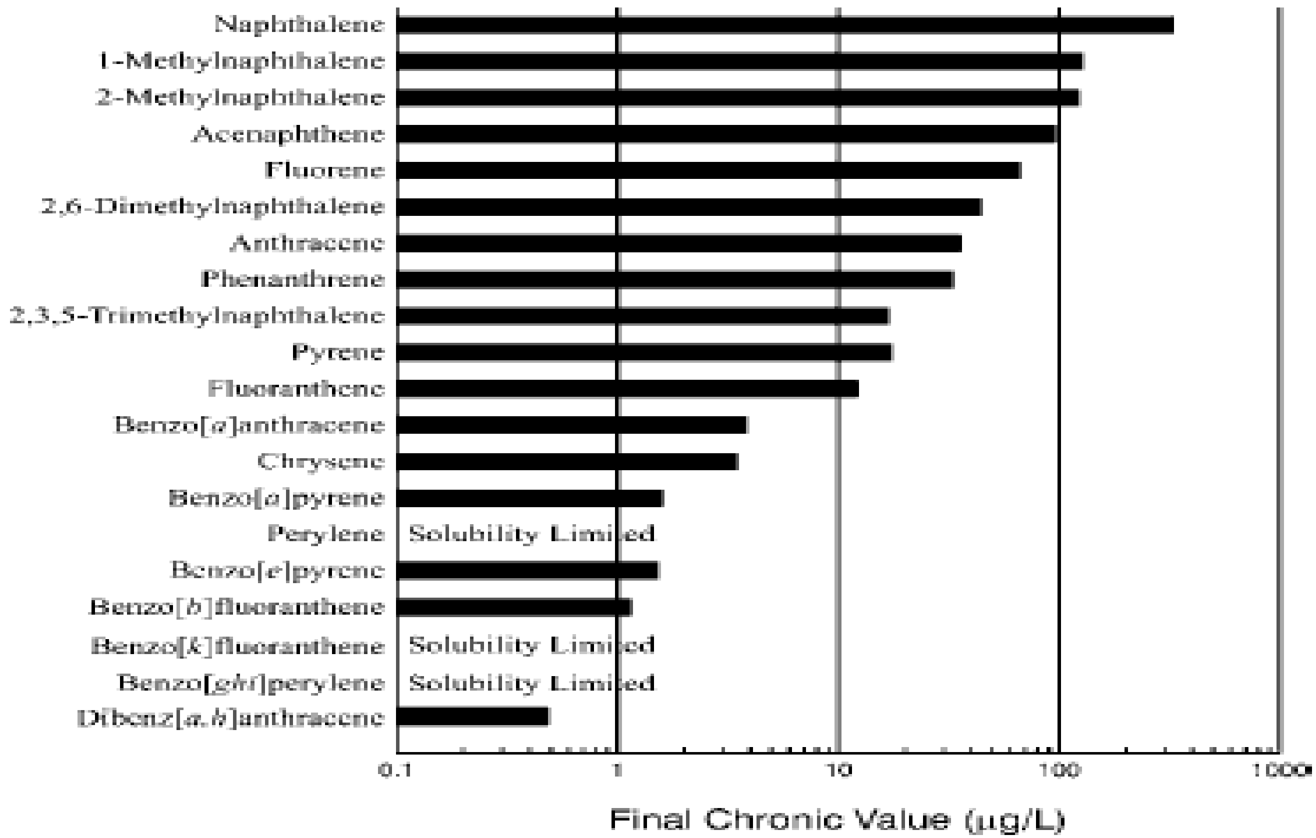
Derivation of Water Quality Criteria

- Final Chronic Value (mmol/L) is given by:

$$\log(FCV) = \log(CTLBB_{5th}) - 0.936 \log(K_{ow}) - a_0 - \log(GMACR)$$

CTLBB5th = 5th percentile of CTLBB species-sensitivity distribution
GMACR = Geometric Mean Acute to Chronic Ratio



Source:
DiToro et al. (2007)
ET&C 26:24

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Testing Complex Substances

- Water Accomodated Fraction (WAF): An aqueous medium containing the fraction of the petroleum product that remains in the aqueous phase once mixing is terminated and phase separation has occurred
  - WAF = soluble phase (dissolved fraction) + droplets (colloidal fraction)
  - WAFs are prepared at multiple oil-water ratios (i.e. Loadings)
  - Test method described by OECD guidance document
    - + http://www.epa.gov/endo/pubs/ref-2_oecd_gd23_difficult_substances.pdf

- Practical Considerations:
  - How to add the test substance to dilution water?
  - How to mix?
  - How long to equilibrate?
  - How long for phase separation after mixing?
  - How to sample WAFs for testing?
  - How to expose test organisms and express test results?

Outline of WAF Test Procedure

- Add a measured volume (liquids) or weight (solids) of substance to known volume of water in a sealed test vessel
  - Contains 5-10% headspace to allow mixing & includes Teflon coated stir-bar
  - Equipped with port at bottom for sampling WAFs with low density (floating) or glass siphon tube in middle for sampling high density (sinking) products

- Stir oil-water solution on magnetic stir plate at a rate that provides good mixing but prevents emulsion formation
  - Use mixing rate that creates < 10% vortex of static depth of oil-water solution
  - Typically stir at room temperature (22 ± 2 °C)

- Continue mixing until equilibrium is obtained
  - Take periodic samples for chemical analysis
    - + TOC, Solvent extraction coupled with UV Spectroscopy/GC-FID or MS
    - + Solid phase microextraction (SPME) coupled with GC-FID or MS
  - 48-96 hrs generally sufficient for most complex petroleum substances

WAF Preparation of Liquids

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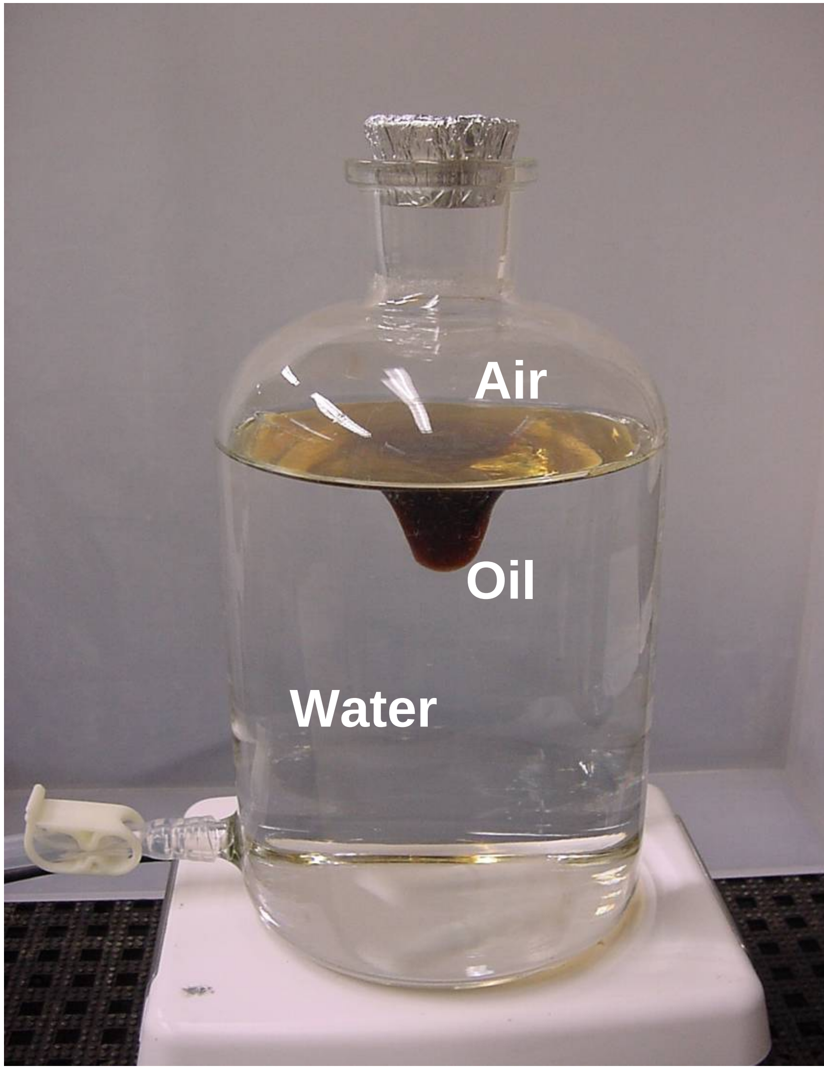
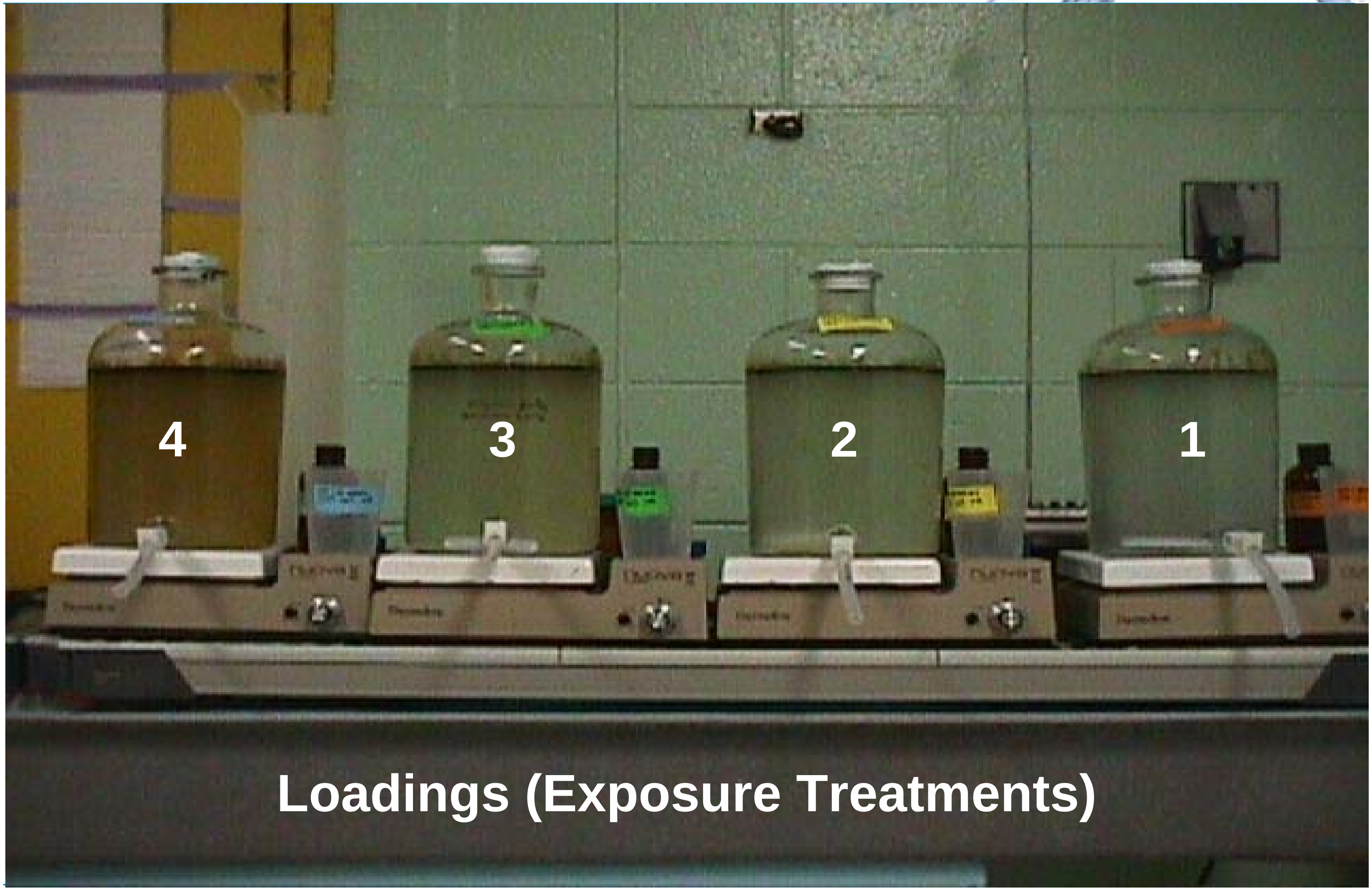
WAF Vessel / Mixing



Preparation of WAFs for Toxicity Testing



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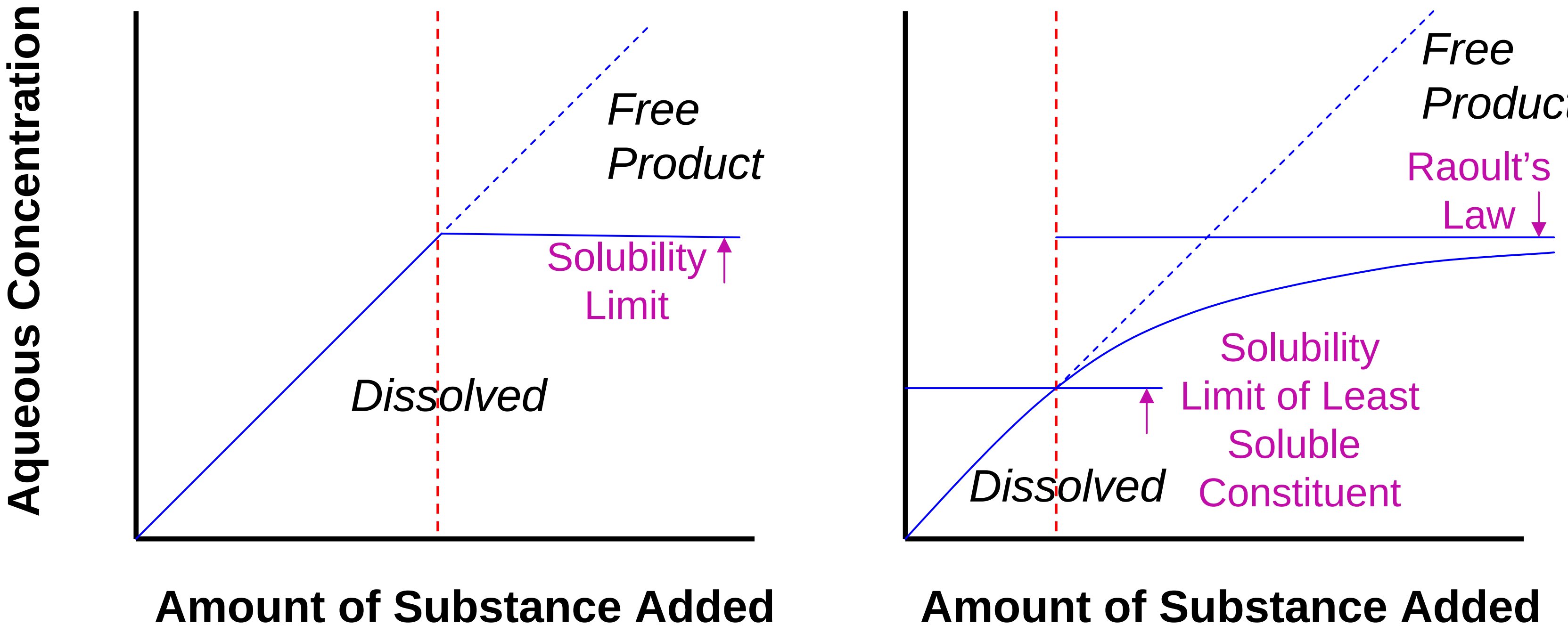
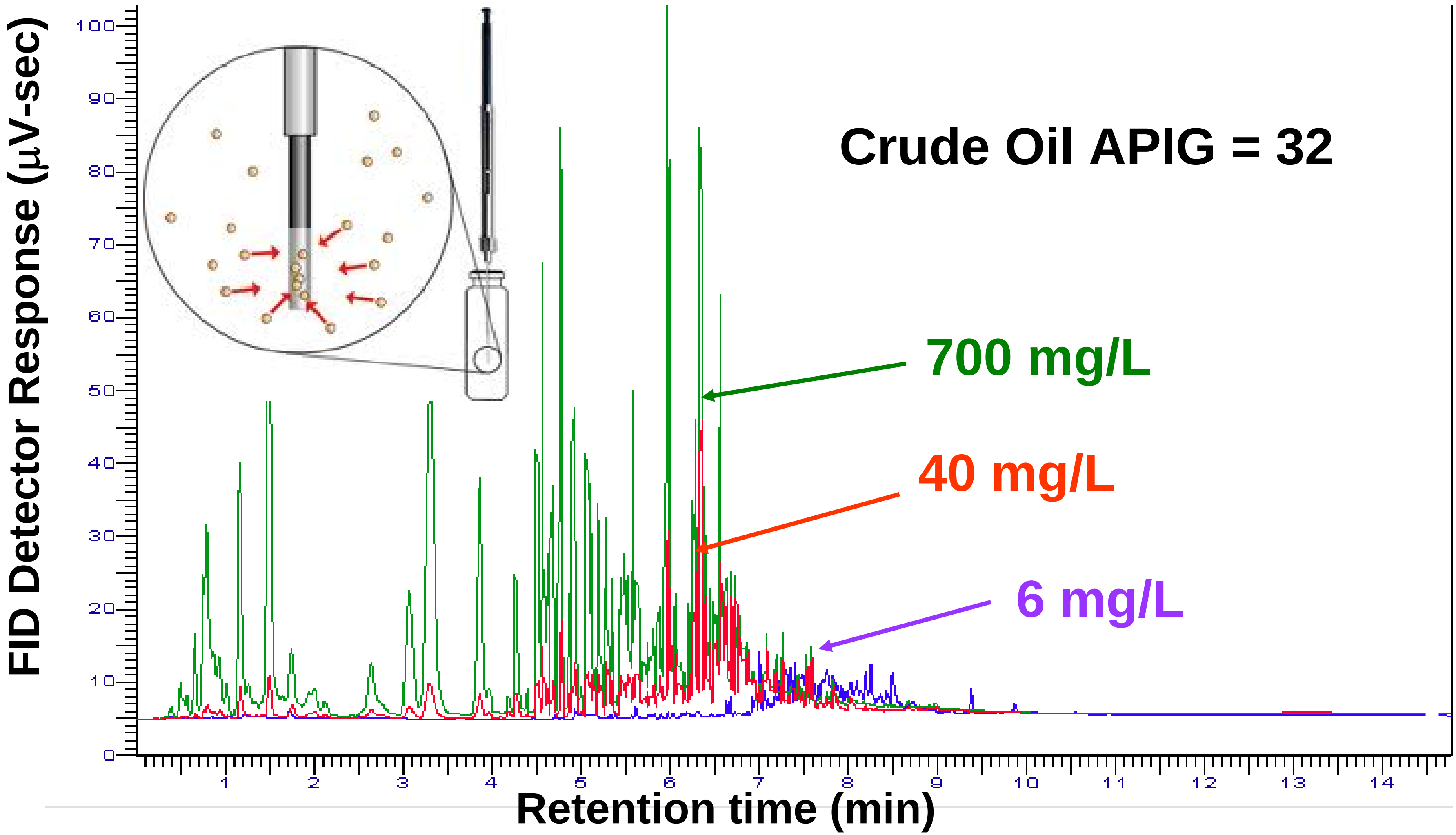
Aqueous Solubility Behavior

Single Hydrocarbon | **Multi-Component Oil**



SPME Fiber Chromatograms for Crude Oil WAFs

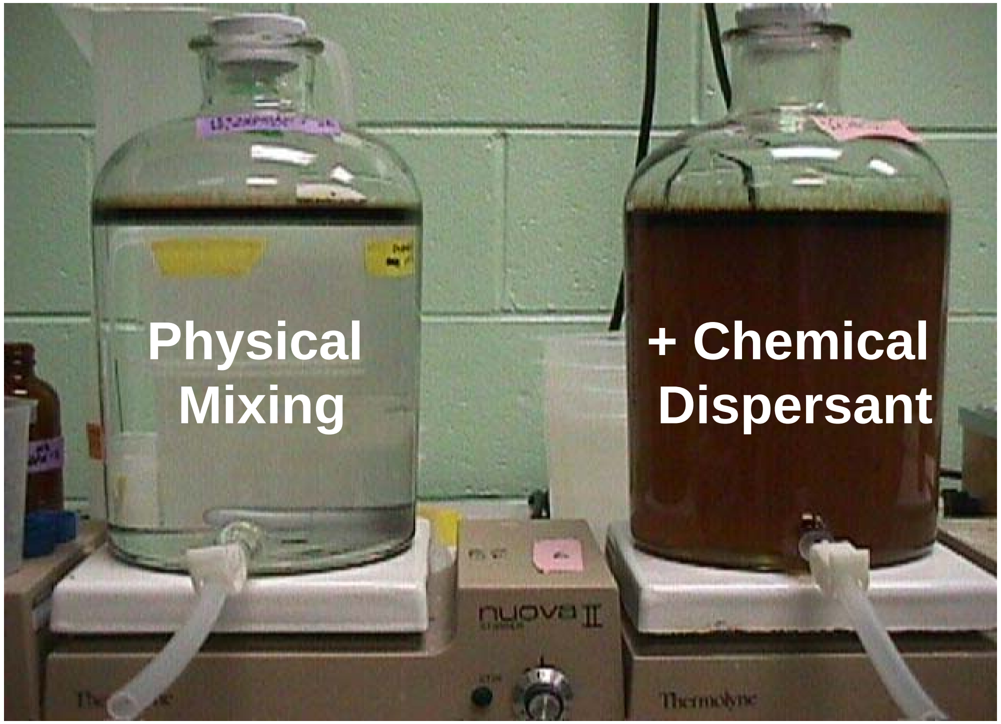


Outline of WAF Test (Cont'd)

- Stop mixing / allow phase separation
  - Typically allow 1 hour unless adjustment to different temperature required (e.g. trout studies) which may require longer periods
- Withdraw solution from WAF test system
  - Discard first 100 mls
  - Collect sample for toxicity testing by directly transferring WAF via gravity flow to air tight exposure vessels to which test organisms are introduced
  - Need to consider oxygen depletion concerns especially for fish
    - + Use static renewal exposure design
    - + Add pure oxygen
  - Need to consider pH changes for algae
    - + Increase buffering capacity of test media
- Observe test organism response to WAFs

Chemical Disperants

- Designed to exhibit low aquatic toxicity
  – Less toxic than the oil to be dispersed
- Increases amount of oil in aqueous test media
  – Augments “effective” loading potentially increasing dissolved or ‘bioavailable” hydrocarbon concentrations
  – Increases undissolved hydrocarbon, i.e. droplets



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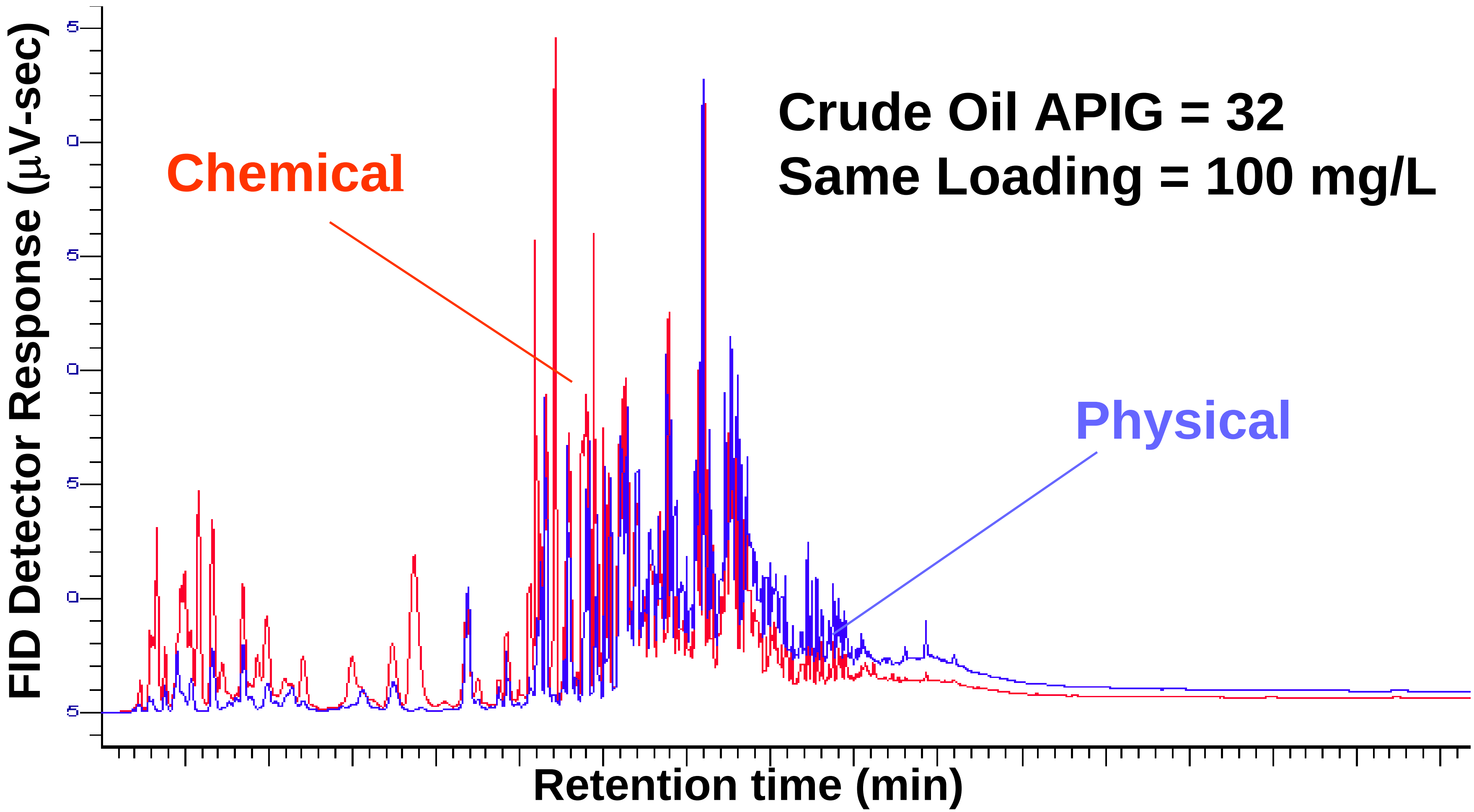
SPME Chromatogram Comparison for Physical & Chemical Dispersion



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Other Approaches

- Use of Water Soluble Fractions (WSF)
  - Filter WAF to remove undissolved oil
    + Potential for removal of dissolved constituent
    + Adds significant effort to test
    + Can be used to investigate role of physical effects associated with highly dispersed WAFs

- Use of WAF / WSF dilutions
  - Prepare WAF / WSF at a given loading (e.g. 10 g oil /L water)
  - Make serial dilutions of the WAF / WSF
  - Exposure test organisms to WAF / WSF dilutions
  - Express toxicity in terms of % dilution
  - Traditionally used in oil spill studies

Cautionary Note: A 1:100 dilution of a 10g/L WAF ≠ 100 mg/L WAF since amount and composition of hydrocarbons will differ

Tools for Predicting Toxicity

- Additive Toxic Unit Model
  - Given detailed composition of oil simulate composition of aqueous hydrocarbons in WAF test system
  - Use TLM to calculate species-specific toxicity to all predicted hydrocarbons in WAF
  - Calculate additive contribution of each hydrocarbon to toxicity

$$\mathrm{TU}_{\mathrm{i}} = \frac{C_{w,i}}{C^{*}_{w,i}} \quad \text{and} \quad \text{Total TU} = \sum_{i=1}^{n} \mathrm{TU}_{\mathrm{i}}$$

where:

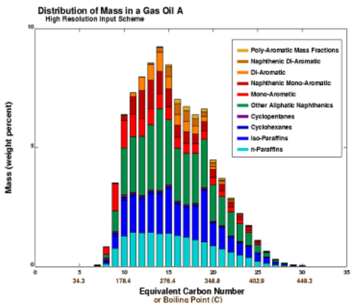
$C_{w,i}$ = aqueous concentration of hydrocarbon *i* predicted in WAF

$C_{w,i}^{*}$ = aqueous effect concentration (e.g., LC_{50}) of hydrocarbon *i*

TU < 0.3	Toxicity Unlikely
0.3< TU <2.0	Toxicity Uncertain
TU > 2.0	Toxicity Likely

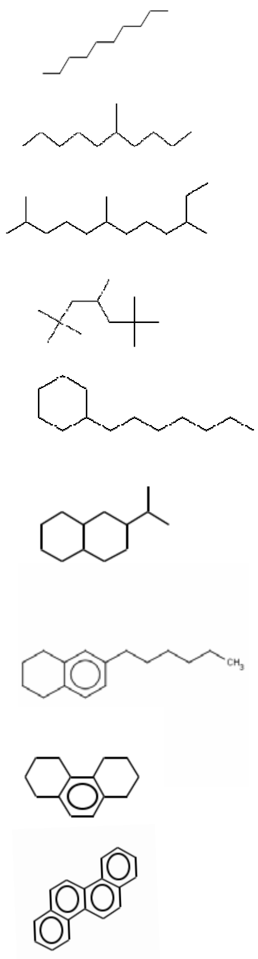
Overview of PETROTOX Model

Petroleum Product Composition



Initial Petroleum Product Loading

Model Structure Library



WAF Model

Air

Henry's Law

Water

Raoult's Law

Oil (NAPL)

WAF Hydrocarbon Concentrations

$C_{1,\,water}$

$C_{2,water}$

$C_{3,water}$

.....

.....

$C_{n,water}$

Toxic Units $= \sum_{i=1}^{n} (C_i \,/\, LC_{50i}) = 1?$

No

Effect Model

Target Lipid Model

Hydrocarbon Toxicities

LC_{50} 1, water

LC_{50} 2, water

LC_{50} 3, water

.....

LC_{50} n, water

Select new loading & repeat until convergence

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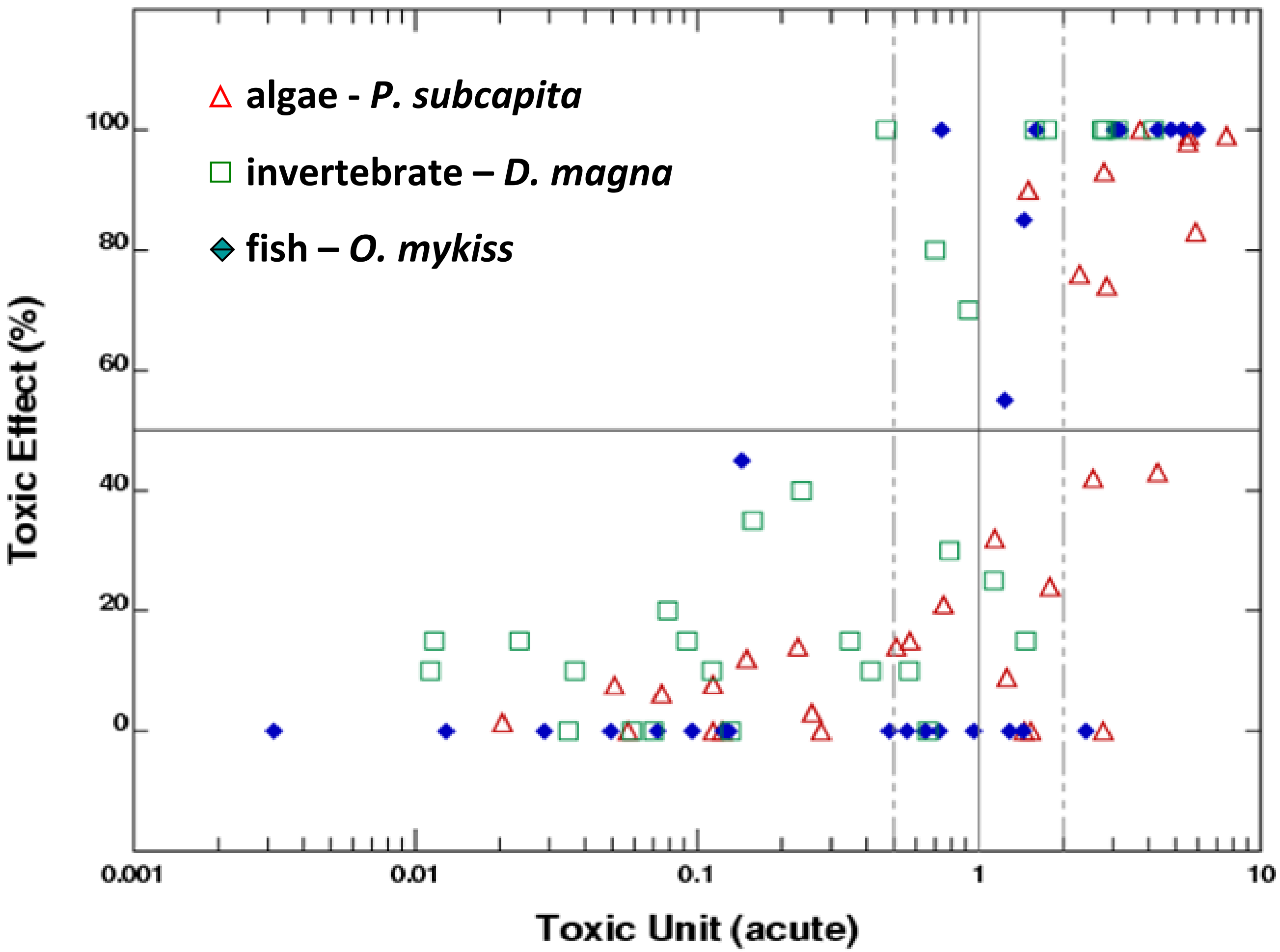
See: http://www.concawe.be/content/default.asp?PageID=778

Use of TLM to Predict Acute Toxicity of Gasolines



Source:
McGrath et al. (2005)
ET&C 24:2382–2394

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Tools for Predicting Toxicity (Cont'd)

- Biomimetic Extraction Analysis:
  - Ecotoxicity occurs when {molar} in organism lipid exceeds a critical threshold, i.e., CTLBB
  - For given organism / endpoint, CTLBB is ~ constant for different hydrocarbons which act by a common mode of action
  - Ecotoxicity of hydrocarbon mixtures is additive i.e., CTLBB concept applies to complex petroleum products
  - SPME fibers serve as a surrogate for organism target lipid
  - Total amount of hydrocarbons that sorb from a petroleum contaminated sample (e.g. WAF) to SPME fiber used for quantitative toxicity prediction

Mysid Toxicity Case Study

- Prepare physically and chemically dispersed WAFs
  - Five crude oils, no. 2 fuel oil
  - Two dispersants
  - Multiple oil loadings
- Measure SPME fiber concentrations associated with each WAF
  - Equilibrate fiber in WAF for 24 hrs
  - Inject fiber into GC/FID
  - Quantitate using molar response of C_2-naphthalene
  - Express results as umol/ml PDMS = mM PDMS
- Determine 48-hr acute toxicity using *Mysidopsis bahia*

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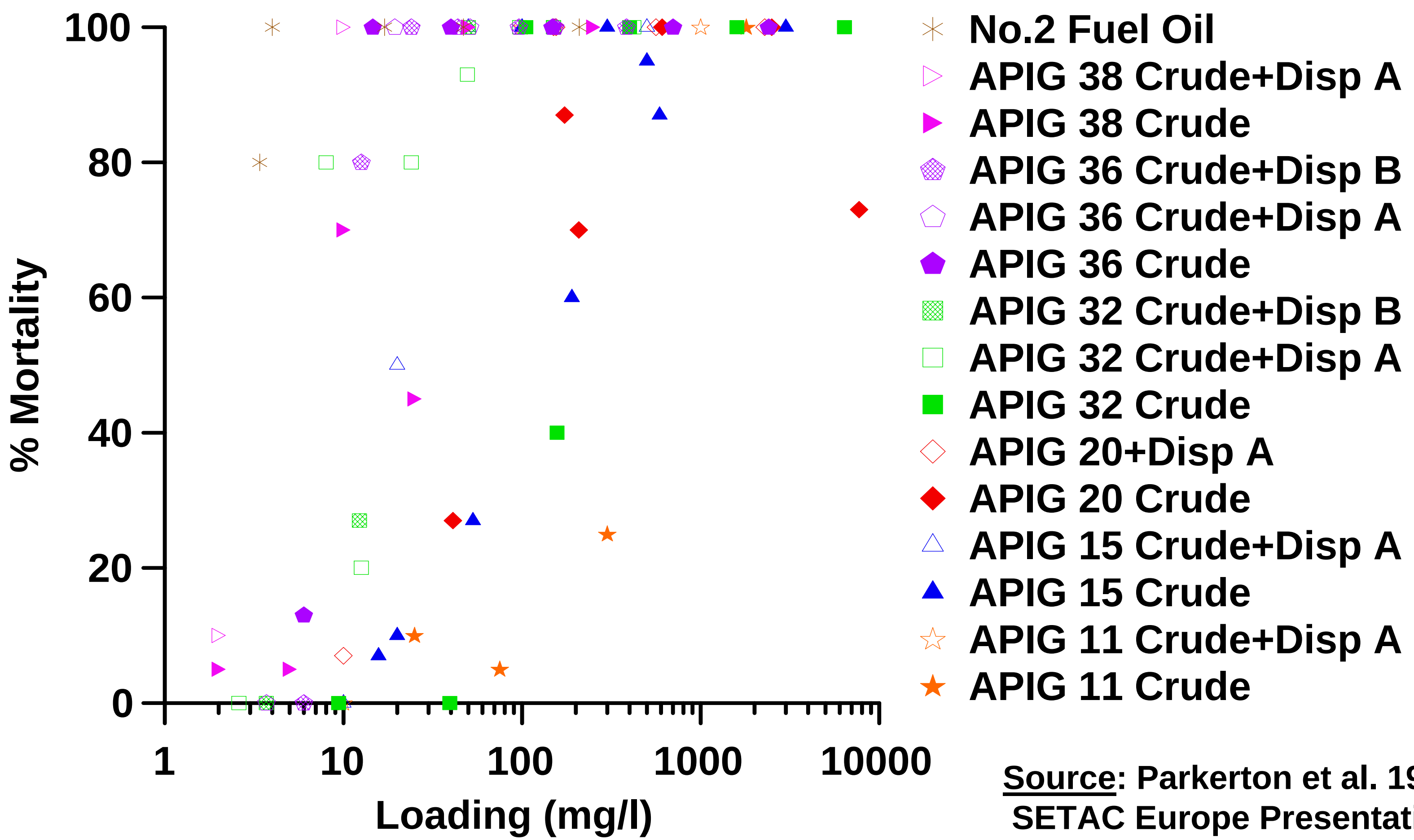
Mysid Toxicity vs Oil Loading

- Toxicity highly variable across treatments



Source: Parkerton et al. 1999
SETAC Europe Presentation

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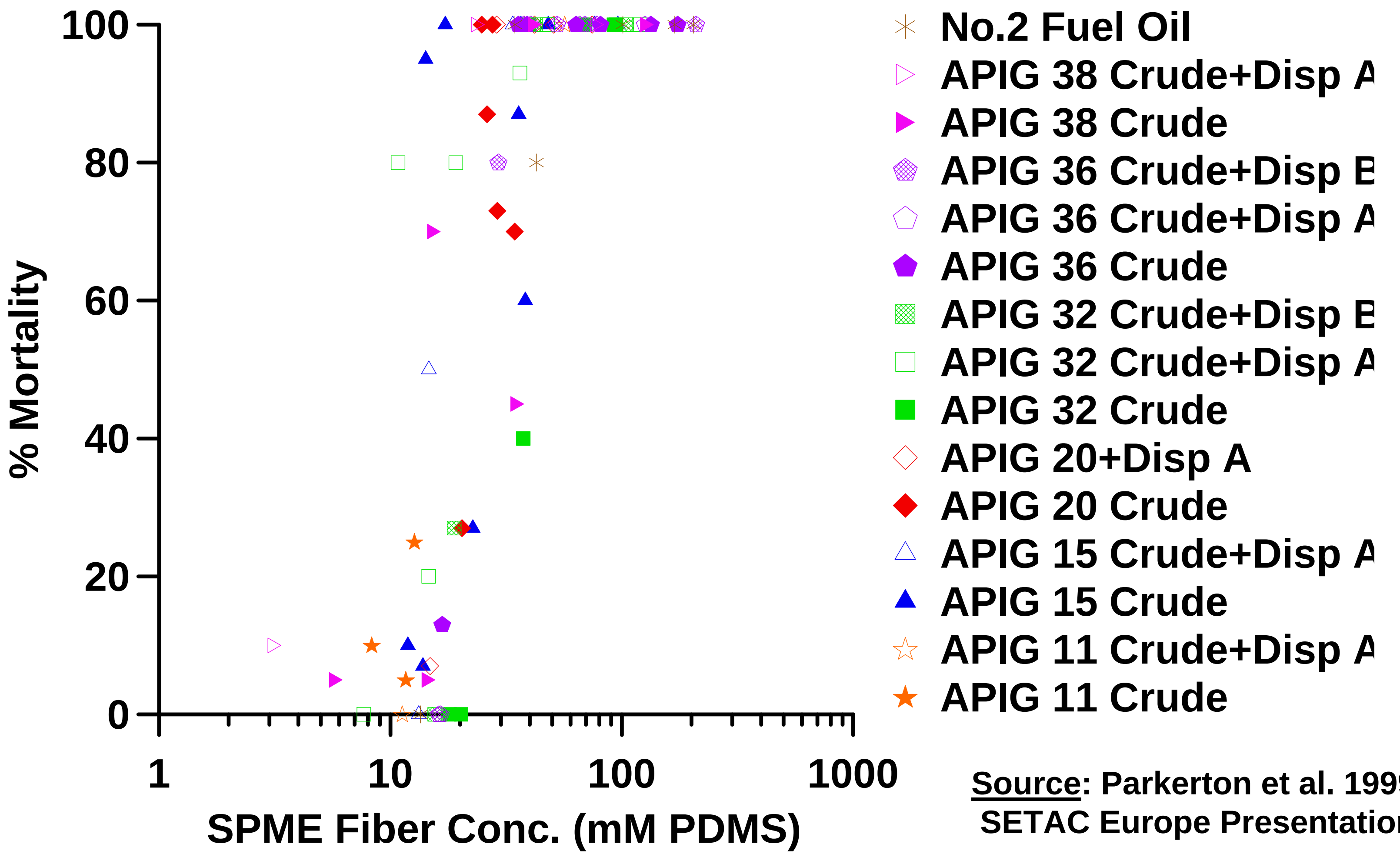
Mysid Toxicity vs C_{Fiber}

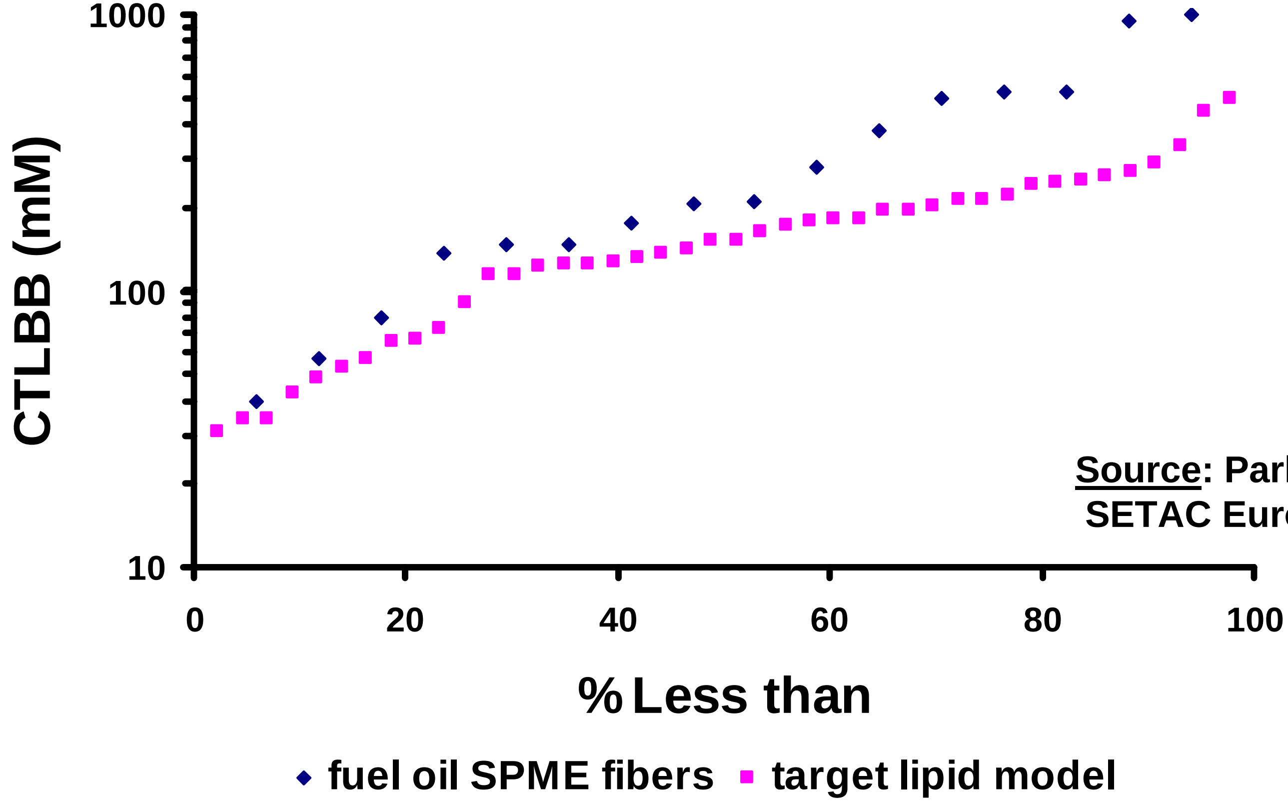
- Clear dose-response across treatments; dispersed oil not different



Source: Parkerton et al. 1999 SETAC Europe Presentation

Further Validation Efforts

- Prepare WAFs using no. 2 fuel oil at different loadings
- Determine C_{Fiber} and toxicity for different test species
- Use C_{Fiber} – toxicity responses to estimate critical fiber burdens (CFBs)
- Translate CFBs into CTLBBs given $K_{TL\text{-}W}$ / $K_{PDMS\text{-}W}$ ~ 8



Source: Parkerton et al. 2009 SETAC Europe Presentation

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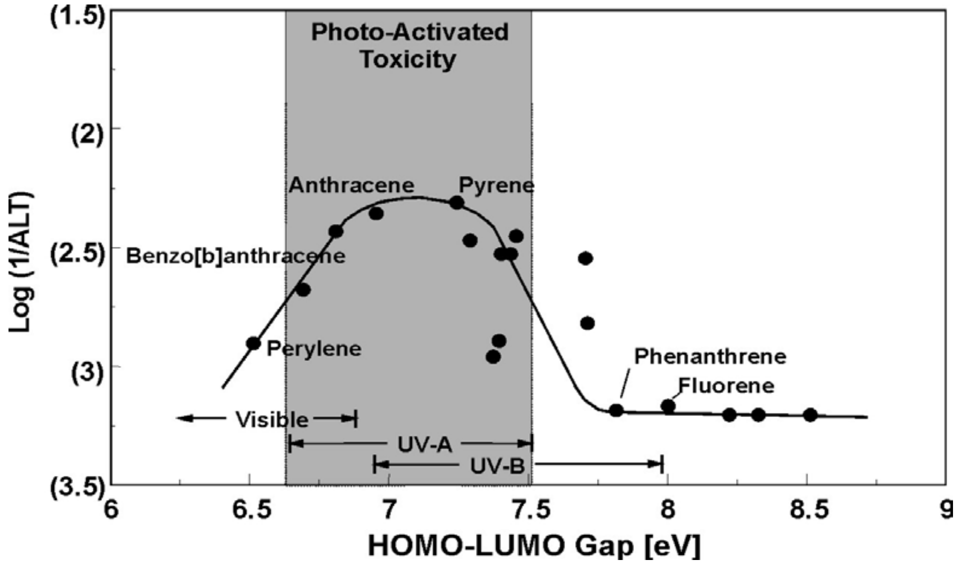
Photo-Enhanced Toxicity

- Selected PAHs shown to be more toxic in lab in presence of UV light



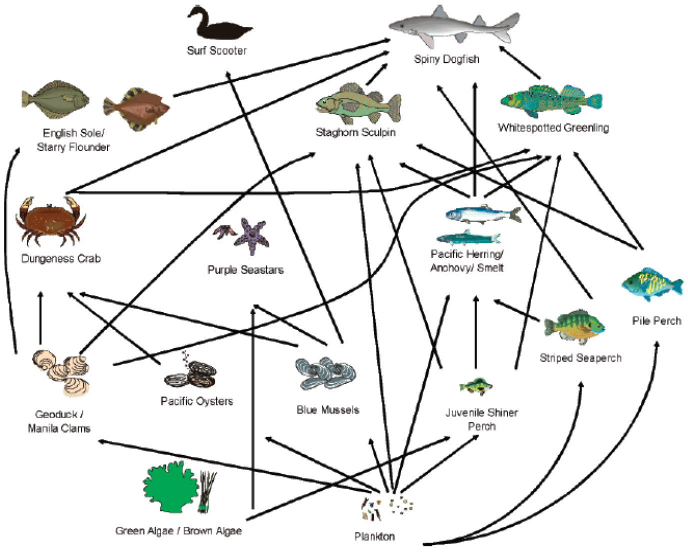
Mount et al., (2001) Linking exposure and dosimetry to risk from photo-activated toxicity of PAHs. Presented at the 2001 Annual SETAC Meeting. Baltimore, MD.

- Toxicity predicted by product of UV intensity and PAH tissue residue
  - UV intensity depends on location, season, time of day, water clarity; decreases exponentially with water depth
  - PAH tissue residue depends on PAH exposure concs and organism

- Influence of UV light on PAH toxicity offset by photodegradation
  - Estimated aqueous photolysis half-life for anthracene ca. minutes to days

Bioaccumation of PAHs in Foodchain

- Selected PAHs known to be carcinogenic/mutagenic, e.g. benzo(a)pyrene, dibenz(a,h,)anthracene, chrysene
- Bioconcentration at base of foodweb limited by dissolved PAH concs.
- Subsequent transfer to higher organisms mitigated by biotransformation processes
  - PAHs shown to biodilute, not biomagnify in foodweb
    - + Lab Biomagnification Factors (BMFs)
    - + Field Trophic Magnification Factors (TMFs)



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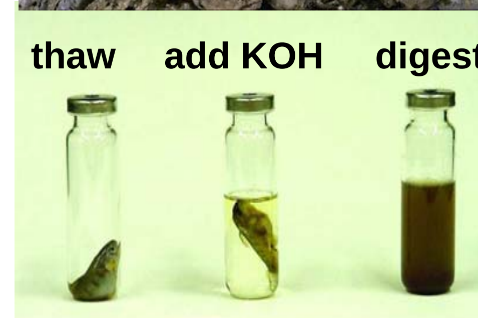
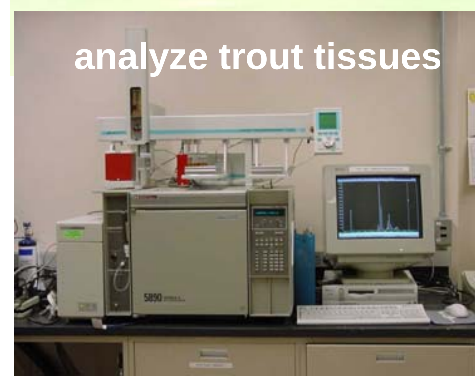
Lab Dietary Bioaccumulation Test

- Spike hydrocarbons to commercial fish diet
  - Lipid content of diet 15%
  - Spike liquids directly, solids in corn oil
- Confirm dietary concentrations analytically
- Feed 3% ration of spiked diet to trout or carp (1-5 grams; 2-4% lipid) for 7 to 10 days (uptake)
- Transfer exposed fish to clean food (depuration)
- Analyze fish at different depuration times e.g. 0, 1, 3, 7, 14, 21 days
- Use hexachlorobenzene as positive control





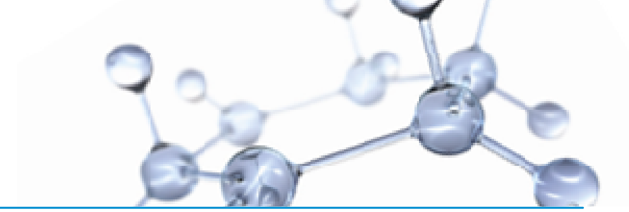
Bioaccumulation Data Analysis

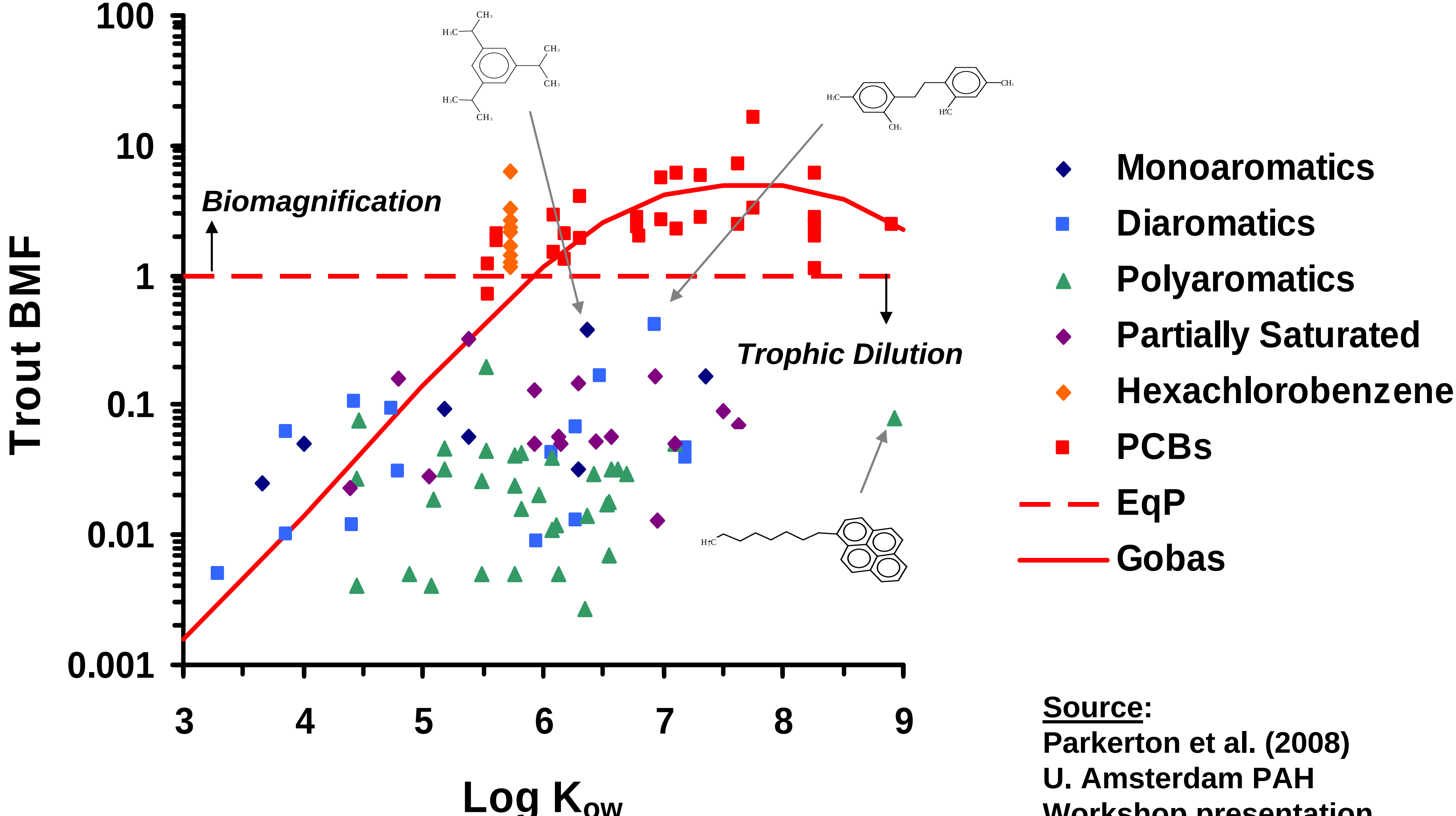
- Use experimental depuration data to deduce:
  - Growth-corrected half-life ($t_{1/2}$)
    - + Derived from slope of depuration plot & fish growth rate
  - Assimilation efficiency from diet (α)
    - + Derived from intercept of depuration plot & first-order model
  - Biomagnification factor (BMF)

$$BMF = \frac{C_{fish,\,lipid}}{C_{diet,\,lipid}} = \frac{\alpha\, I_{diet}\, t_{1/2}}{0.693} \frac{L_{diet}}{L_{fish}}$$

BMF < 1	Trophic Dilution
BMF = 1	Equilibrium Partitioning
BMF > 1	Biomagnification

Trout BMFs
for Aromatic Hydrocarbons





Source:
Parkerton et al. (2008)
U. Amsterdam PAH Workshop presentation

PCB data from Fisk et al. 1988 ET&C 17:951

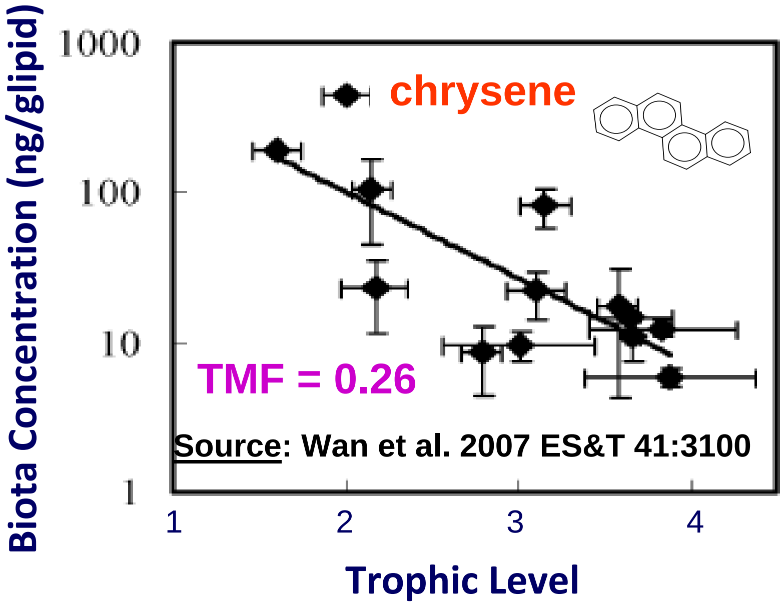
Field Bioaccumulation Assessment

- Collect field organisms from foodweb: analyse tissues for chemical and nitrogen isotopes
  - nitrogen isotopes used to determine trophic level (TL)
- Regress chemical concentration against TL to determine trophic magnification factor (TMF)
  - mean increase (biomagnification) or decrease (biodilution) of chemical / TL

$$\text{Log } C_{lipid} = a + b\,(\text{Trophic Level})$$

$$TMF = 10^{b}$$

TMF < 1	Trophic Dilution
TMF = 1	Equilibrium Partitioning
TMF > 1	Biomagnification



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Literature TMFs for PAHs

PAH	TMF Ref =1	TMF Ref =2	TMF Ref =3
benz[a]anthracene	0.20	0.75	0.83
benzo[a]pyrene	0.24	0.75	0.80
benzo[e]pyrene	0.25	0.86	0.57
benzofluoranthene	0.27	0.84	0.69
benzo[ghi]perylene	0.66	0.75	0.72
chrysene	0.26	0.66	0.65
fluoranthene	0.11	0.72	0.60
indeno-123-cd]pyrene	0.81	0.75	0.80
dibenz[ah]anthracene	0.85		
perylene	0.24	0.67	0.77
phenanthrene	0.43	0.82	0.75
pyrene	0.17	0.74	0.62

Ref =1 Wan Y, Jin X, Hu J, Jin F. (2007). Trophic dilution of polycyclic aromatic hydrocarbons (PAHs) in a marine food web from Bohai Bay, North China. *Environ Sci Technol* 41:3109-3114.

Ref =2 Nfon, E., Cousins, I.T., et al. (2008). Biomagnification of organic pollutants in benthic and pelagic marine food chains from the Baltic Sea. *Sci Total Environ* 397:190-204.

Ref =3 Takeuchi, I., Miyoshi, N., et al. (2009). Biomagnification profiles of polycyclic aromatic hydrocarbons, alkylphenols and polychlorinated biphenyls in Tokyo Bay elucidated by d13C and d 15N isotope ratios as guides to trophic web structure. *Mar Poll Bull* 58:663-671.

Summary

- The target lipid model provides a quantitative framework for predicting the acute and chronic toxicity of single and complex hydrocarbons

- The WAF test procedure is the preferred test method for assessing the aquatic toxicity of complex petroleum substances
  - method endorsed by OECD
  - accounts for multi-component dissolution behavior

- Passive sampling methods (e.g. SPME fibers) that quantify dissolved hydrocarbons in WAFs provide simple analytical tool to support testing and toxicity prediction

- Chemical dispersants exhibit low toxicity but can increase the bioavailability of hydrocarbons in the oil being dispersed
  - can result in increased WAF toxicity in lab studies
  - offset by role bioavailability plays in reducing field exposures, e.g. dilution, biodegradation

- Photo-enhanced toxicity and bioaccumulation in foodweb depends on dissolved PAH concentrations in the field; significance further limited by:
  - UV attenuation in water column and photodegradation
  - biodilution in the foodchain

Research Needs ?

- Develop reliable CTLBBs and ACRs for additional GOM species, e.g sponges, corals for which limited data are available

- Develop data and improved models for characterizing toxicity of aromatic hydrocarbons on survival, growth and reproduction of key GOM species under time-variable exposure and field conditions, e.g. temperature, UV light, oxygen

- Link toxicity and population models to predict population-level responses

- Further investigate analytical and short-term toxicity screening tests for use in future spill response

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Selected Publications

Parkerton, T.F. A. D. Redman, J. A. McGrath, D. K. Letinski, E. J. Febbo, R. G. Manning, M. H. Comber, D. M. Di Toro (2011) Extension and validation of the target lipid model for deriving predicted no effect concentrations for hydrocarbons, Submitted to *Environ. Toxicol. Chem.*

Burkhard, L.P., J.A. Arnot, M. R. Embry, K. J. Farley|, R. A. Hoke, M. Kitano, H. A. Leslie, G. R. Lotufo, T. F. Parkerton, K. G. Sappington, G. T. Tomy (2011), Comparing laboratory and field measured bioaccumulation endpoints, *Integrated Environmental Assessment & Management*. DOI: 10.1002/ieam.244

McElroy, A.E., M. G. Barron, N. Beckvar, S. B. Kane Driscoll, J. P. Meador, T. F. Parkerton, T. G. Preuss, J. A. Steevens (2011). A review of the tissue residue approach for organic and organometallic compounds in aquatic organisms, *Integrated Environmental Assessment & Management* 7:50-74.

Hook, S.E., M. A. Lampi, E. J. Febbo, J. A. Ward, T. F. Parkerton (2010). Hepatic gene expression in rainbow trout (*Oncorhynchus mykiss*) exposed to different hydrocarbon mixtures, *Environ. Toxicol. Chem.* 29:2034-2043.

Hook, S.E., M. A. Lampi, E. J. Febbo, J. A. Ward, T. F. Parkerton (2010). Temporal patterns in the transcriptomic resonse of rainbow trout, *Oncorhynchus mykiss*, to crude oil, *Aquat. Toxicol*. 99:320-329.

Arnot. J., D. Mackay, T. F. Parkerton, R. Zaleski, C. S. Warren (2010) Multimedia modeling of human exposure to chemical substances: the role of food web biomagnification and biotransformation, *Environ. Toxicol. Chem.* 29:44-55.

Weisbrod,A.V., K. B. Woodburn, A. A Koelmans,T. F Parkerton. A. E McElroy, K. Borga (2009). Evaluation of bioaccumulation using *in-vivo* laboratory and field studies, *Integrated Environmental Assessment and Management*, 5(4):598–623

van de Meent,D., A. Hollander, M. Comber, T. Parkerton (2009) Environmental fate factors and human intake fractions for risk assessment of petroleum products, *Integrated Environmental Assessment & Management*, 6:135-144.

Arnot, J., D. Mackay, T.F. Parkerton, M. Bonnell (2008). A database of fish biotransformation rates, *Environ. Toxicol. Chem.* 27:263–2270.

. Parkerton, T., J. Arnot, K. Woodburn, A. Weisbrod, C. Russom, R. Hoke, T. Traas, M. Bonnell, L. Burkhard, M. Lampi (2008). Guidance for evaluating *in-vivo* fish bioaccumulation bata; *Integrated Environmental Assessment & Management*, 4(2):139–155.

Redman, A. J .McGrath, T. Parkerton, E. Febbo, D. Letinski, D. Winkelmann, D. DiToro (2007). Application of the Target lipid model for deriving predicted no effect concentrations for wastewater organisms, *Environ. Toxicol. Chem.* 26:102-112.

Foster. K.l., D. Mackay, T.F. Parkerton, E. Webster, L. Milford (2005). A five stage risk assessment strategy for mixtures: gasoline as a case study, *Environ. Sci. Technol.* 39:2711-2718.

McGrath JA, Hellweger FL, Parkerton TF, Di Toro DM. (2005). Application of the narcosis target lipid model to complex mixtures using gasolines as a case study, *Environ. Toxicol. Chem.* 24:2382-2394

McGrath, J.A., T.F. Parkerton, and D.M. Di Toro (2004). Application of the narcosis target lipid model to algal toxicity and deriving predicted no effect concentrations. *Environ. Toxicol. Chem*. 23:2503–2517.