Assessing oil toxicity: methods & models

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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. ventilation, 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”
Inhibition of Mussel Filtration Rate

![Graph showing inhibition of mussel filtration rate](image)

- **Log Kow**
- **Log Effect Concentration (umol/L or umol/g)**
- **EC50** water effect concentration
- **TC50** tissue effect concentration
- **TC0**
- **Water**
- **Tissue**

Source:
Predicting Narcosis using the Target Lipid Model

CTLBB for a chemical is determined as:

\[ \text{CTLBB} = \text{LC}_{50} \times \text{KT}_{L-W} \]  

(1)

Rearranging and taking logs:

\[ \log(\text{EC}_{50}) = \log(\text{CTLBB}) - \log(\text{KT}_{L-W}) \]  

(2)

Based on linear-free energy relationships:

\[ \log(\text{KT}_{L-W}) = a_0 + a_1 \log(K_{ow}) \]  

(3)

Substituting (3) into (2) yields:

\[ \log(\text{EC}_{50}) = \log(\text{CTLBB}) - a_0 - a_1 \log(K_{ow}) \]  

(4)

CTLBB = critical target lipid body burden (mmol/kg octanol)

EC\(_{50}\) = aqueous concentration that causes a 50% response (mmol/L)

KT\(_{L-W}\) = target lipid water partition coefficient (L/kg lipid)

K\(_{ow}\) = octanol water partition coefficient (L/kg octanol)

a\(_o\), a\(_1\) = empirical constants that relate partitioning at target site to octanol
Calibration of TLM using Acute Toxicity Data Sets

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

• $a_0$ chemical class dependent
  – $a_0 = 0$ 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)

CTLBB Species - Sensitivity Distribution

Species vary in sensitivity by 20-fold

Extrapolation to Chronic Effects

Acute to Chronic Ratio

\[ \text{ACR} = \frac{\text{acute } L/EC_{50}}{\text{chronic } \text{NOEC}/EC_{10}} \]

For hydrocarbons ACRs vary 10-fold

**Derivation of Water Quality Criteria**

- Final Chronic Value (mmol/L) is given by:
  \[
  \log (FCV) = \log(CTLBB_{5th}) - 0.936 \log(K_{ow}) - a_0 - \log (GMACR)
  \]

  \(CTLBB_{5th}\) = 5\(^{th}\) percentile of CTLBB species-sensitivity distribution

  \(GMACR\) = Geometric Mean Acute to Chronic Ratio

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

- **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

![Image of a glass tube on a digital scale]
WAF Vessel / Mixing
Preparation of WAFs for Toxicity Testing

Loadings (Exposure Treatments)
Aqueous Solubility Behavior

Single Hydrocarbon

- Aqueous Concentration
- Amount of Substance Added
- Dissolved
- Free Product
- Solubility Limit

Multi-Component Oil

- Aqueous Concentration
- Amount of Substance Added
- Dissolved
- Free Product
- Raoult’s Law
- Solubility Limit of Least Soluble Constituent
SPME Fiber Chromatograms for Crude Oil WAFs

Crude Oil APIG = 32

Retention time (min)

FID Detector Response (µV-sec)

700 mg/L
40 mg/L
6 mg/L
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
SPME Chromatogram Comparison for Physical & Chemical Dispersion

Chemical

Physical

Crude Oil API G = 32
Same Loading = 100 mg/L

FID Detector Response (µV-sec)

Retention time (min)
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

\[
TU_i = \frac{C_{w,i}}{C_{w,i}^*} \quad \text{and} \quad \text{Total} \ TU = \sum_{i=1}^{n} TU_i
\]

where:
- \( C_{w,i} \) = aqueous concentration of hydrocarbon \( i \) predicted in WAF
- \( C_{w,i}^* \) = aqueous effect concentration (e.g., LC\(_{50}\)) of hydrocarbon \( i \)

- \( TU < 0.3 \) \hspace{1cm} \text{Toxicity Unlikely}
- \( 0.3 < TU < 2.0 \) \hspace{1cm} \text{Toxicity Uncertain}
- \( TU > 2.0 \) \hspace{1cm} \text{Toxicity Likely}
Overview of PETROTOX Model

Model Structure Library

Petroleum Product Composition

Initial Petroleum Product Loading

Air
Henry’s Law

Water
Raoult’s Law

Oil
(NAPL)

WAF Model

WAF Hydrocarbon Concentrations

C_1, water
C_2, water
C_3, water
.....
C_n, water

Toxic Units = \sum_{i=1}^{n} \left( \frac{C_i}{LC_{50i}} \right) = 1?

Effect Model

Hydrocarbon Toxicities

LC_{50} 1, water
LC_{50} 2, water
LC_{50} 3, water
.....
LC_{50} n, water

Target Lipid Model

Select new loading & repeat until convergence

See: http://www.concawe.be/content/default.asp?PageID=778
Use of TLM to Predict Acute Toxicity of Gasolines

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 C₂-naphthalene
  – Express results as umol/ml PDMS = mM PDMS

• Determine 48-hr acute toxicity using *Mysidopsis bahia*
Mysid Toxicity vs Oil Loading

- Toxicity highly variable across treatments

% Mortality vs Loading (mg/l)

Source: Parkerton et al. 1999
SETAC Europe Presentation
Mysid Toxicity vs C\textsubscript{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 $C_{\text{Fiber}}$ and toxicity for different test species
- Use $C_{\text{Fiber}}$ – toxicity responses to estimate critical fiber burdens (CFBs)
- Translate CFBs into CTLBBs given $K_{\text{TL-W}} / K_{\text{PDMS-W}} \sim 8$

Source: Parkerton et al. 2009
SETAC Europe Presentation
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
Bioaccumulation 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)
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 ($\alpha$)
    + Derived from intercept of depuration plot & first-order model
  – Biomagnification factor (BMF)

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

<table>
<thead>
<tr>
<th>BMF</th>
<th>Description</th>
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<tr>
<td>&lt; 1</td>
<td>Trophic Dilution</td>
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<tr>
<td>= 1</td>
<td>Equilibrium Partitioning</td>
</tr>
<tr>
<td>&gt; 1</td>
<td>Biomagnification</td>
</tr>
</tbody>
</table>
Trout BMFs for Aromatic Hydrocarbons

Biomagnification

Trophic Dilution

Monoaromatics
Diaromatics
Polyaromatics
Partially Saturated
Hexachlorobenzene
PCBs
EqP
Gobas

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

\[
\log C_{\text{lipid}} = a + b (\text{Trophic Level})
\]

\[
TMF = 10^b
\]

- \( TMF < 1 \) Trophic Dilution
- \( TMF = 1 \) Equilibrium Partitioning
- \( TMF > 1 \) Biomagnification

Source: Wan et al. 2007 ES&T 41:3100
## Literature TMFs for PAHs

<table>
<thead>
<tr>
<th>PAH</th>
<th>TMF Ref =1</th>
<th>TMF Ref =2</th>
<th>TMF Ref =3</th>
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<tr>
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<td>phenanthrene</td>
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<tr>
<td>pyrene</td>
<td>0.17</td>
<td>0.74</td>
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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.
Selected Publications


