Tuzzy Talk # 7:

Effects of Oil, Dispersants and Dispersed Oil on Organisms

Part 1:

Introduction to Toxicology (Continued)

November 1, 2014

Nancy E. Kinner

University of New Hampshire

Center for Spills in the Environment

Mistake Correction!!!!!

From

Tuzzy Talk #6

October 4, 2014

PLANT CELL WALL

2

Powerpoint on Plant Cell Walls

University of Georgia

Debra Mohnen
Professor of Biochemistry and Molecular Biology

http://www.ccrc.uga.edu/~dmohnen/bcmb8020/PlantWall-06-2Page.pdf

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Tuzzy Talks

- **Tuzzy Talks Series 1: (March 2013)**
  - **What Is Crude Oil? and**
  - **What Happens When It Gets in Water?**
- **Tuzzy Talks Series 2: (Feb – April 2014)**
  - **Biodegradation of Crude Oil**
  - **Dispersants and Dispersed Oil (DDO)**
    - **Natural Dispersion**
    - **Chemically Enhanced Dispersion**
- **Tuzzy Talks Series 3 (Oct – Feb 2015)**
  - **Effects of Oil, Dispersants and Dispersed Oil on Organisms**

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Overview of Toxicology

What is toxicity?
What does toxicity mean?
How is toxicity measured?

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Toxicology

- Study of harmful effects
  - Caused by natural or synthetic pollutants
  - Ecotoxicology if effects are to members of ecosystem
    - Plants
    - Animals (including humans)
    - Microbes
- Individuals → Populations → Communities → Ecosystems → Ecoregions → Biosphere
- Within Individuals

  Biomolecules → Cells → Tissues → Organs → Organ Systems

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Ecotoxicology Field is Rapidly Changing!

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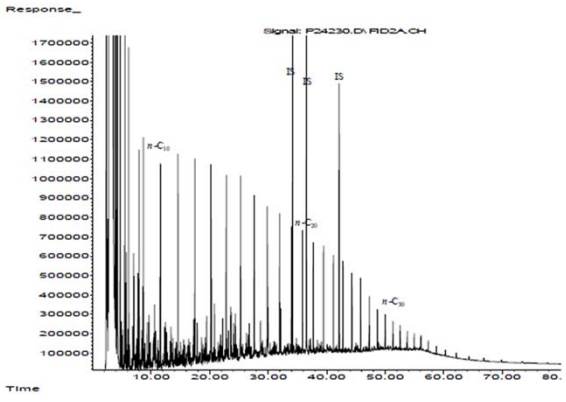
Crude Oil Composition

Figure 3. GC/FID chromatogram of North Slope crude oil.



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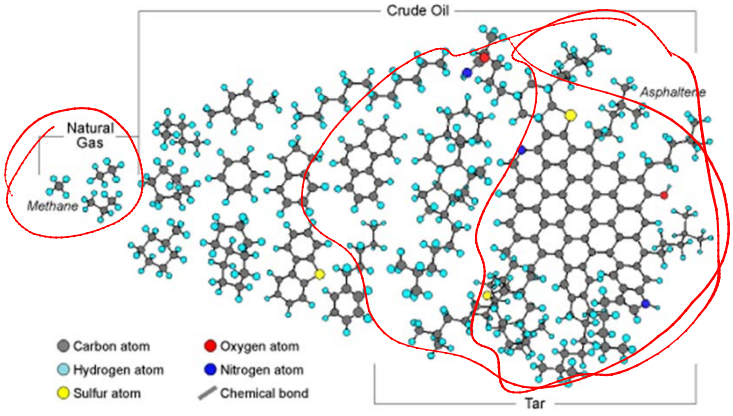
Crude Oil Composition

Examples of Some Organic Compounds in Petroleum



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Hydrocarbons from Oil

- **Most not very soluble in water**
- **Most very soluble in fats (lipids)**

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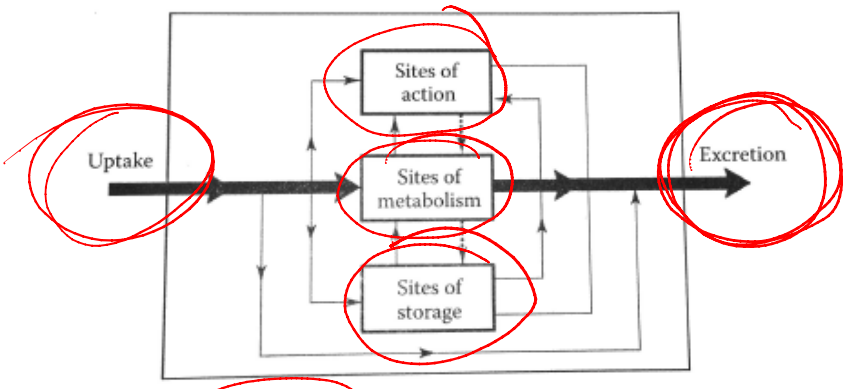
Model of Fate of Hydrocarbons in Organisms



*Lipophilic = "loves" lipids
Soluble in lipids (oil compounds)
Hydrocarbons are lipophilic

Principles of Ecotoxicology
C.H. Walker et al. (2012)

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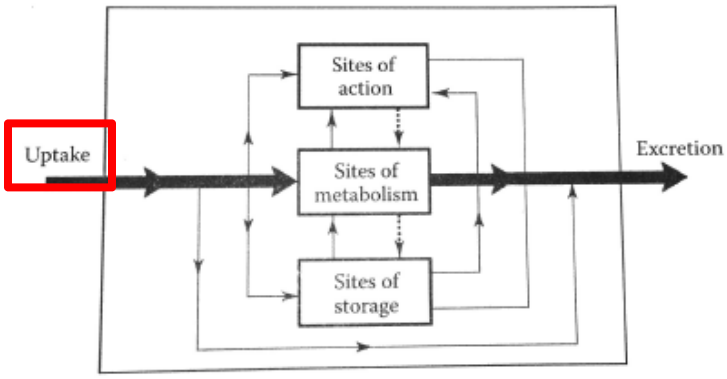
Model of Fate of Hydrocarbons in Organisms



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Uptake of Hydrocarbons

- Gross level of exposure uptake
  - How into organism's body
- Cellular level of uptake
  - How into cell
  - Across membranes

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Main Exposure/Uptake Routes of Hydrocarbons for Organisms (Into Body)

- Ingestion (digestive tract, gut)
- Skin → Absorption
- Inhalation → gills, lungs
- Contaminant interaction with surfaces of cells (skin, gut, gill, lung)

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Some Major Routes of Uptake into Body

- Fish
  - Gills - Dissolved or Suspended Contaminants in water
  - Digestive Tract - Food
- Aquatic Mammals and Birds
  - Digestive Tract - Food
- Aquatic Amphibians
  - Digestive Tract - Food
  - Skin – Dissolved or Suspended Contaminants in water

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Some Major Routes of Uptake into Body (Cont'd)

- Aquatic Invertebrates
  - Digestive Tract - Food
  - Respiratory Surface - Dissolved or Suspended Contaminants
- Plants
  - Leaves - Droplets or Particulates
    - Vapors
  - Roots - Dissolved Contaminants in Soil Water

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Distribution in Organism After Uptake

- Vertebrates via blood and lymph
- Invertebrates via hemolymph

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Major Routes of Uptake Cellular Level

- Move across membranes
- Through lipid bilayer
- Hydrocarbons soluble in lipid bilayer
- Cell contents mostly water
  - Hydrocarbons less soluble inside cell because of water

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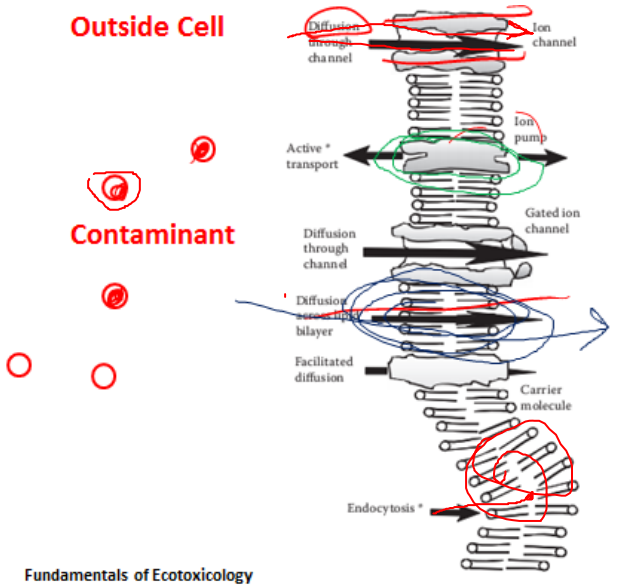
Contaminants Uptake Across Membranes



Fundamentals of Ecotoxicology
M.C. Newman (2010)

Movement of Hydrocarbons Across Membranes

- Large molecules can't move through
  - Must be degraded into small molecules first
- Temperature low means membrane less "fluid" so less movement across them

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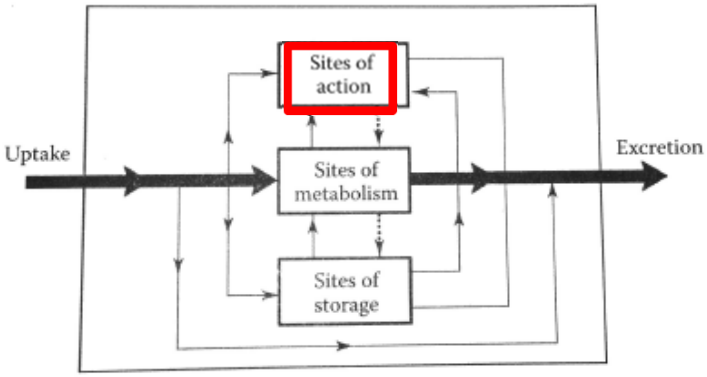
Model of Fate of Hydrocarbons in Organisms



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Soluble in lipids (oil compounds)

Hydrocarbons are lipophilic

Principles of Ecotoxicology
C.H. Walker et al. (2012)

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Contaminant Action

- Contaminant acts on organism
  - Contaminant interacts with cell's DNA or proteins
    - DNA is nuclear material (genes)
  - Contaminants interact with cell's structure
    - Membranes (lipid bilayer)

Enzyme

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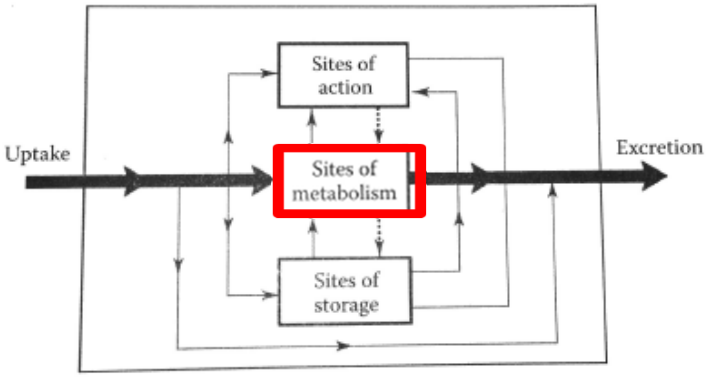
Model of Fate of Hydrocarbons in Organisms



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C.H. Walker et al. (2012)

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Contaminant Metabolism

- Organism acts on contaminant
  - Detoxifies contaminant
  - Can make it more toxic
  - Biotransformation of contaminant

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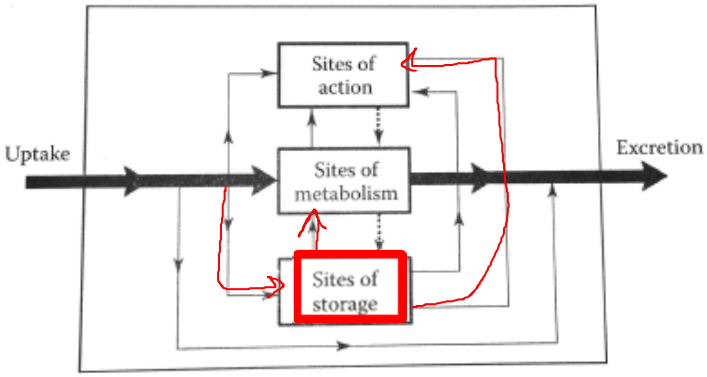
Model of Fate of Hydrocarbons in Organisms



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Soluble in lipids (oil compounds)

Hydrocarbons are lipophilic

Principles of Ecotoxicology
C.H. Walker et al. (2012)

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Contaminant Storage

- Contaminant is <u>not</u> acting on organism
- Organism is <u>not</u> acting on contaminant
- Contaminant is in "non-active" state in organism (stored)

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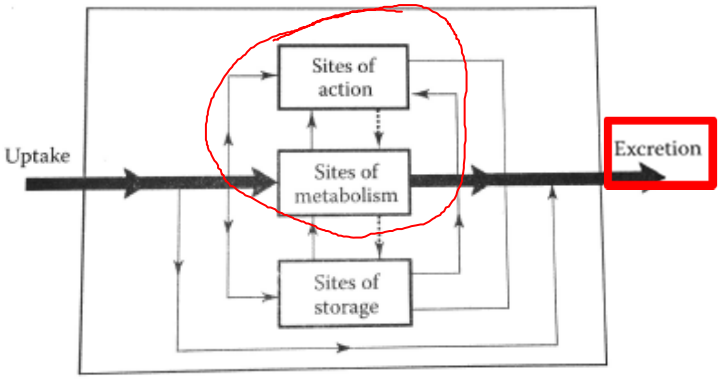
Model of Fate of Hydrocarbons in Organisms



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Soluble in lipids (oil compounds)

Hydrocarbons are lipophilic

Principles of Ecotoxicology
C.H. Walker et al. (2012)

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Contaminant Excretion

- **Contaminant may be excreted unchanged**
- **More likely contaminant excreted <u>after</u> biotransformation**

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Reality Check

- Usually not just one step
- May be multiple sites where things occur
- Complex!!!!

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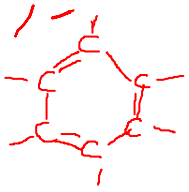
Toxicology of Hydrocarbons

- Most common hydrocarbon structures studied for toxicity are PAHs
  - Polycyclic aromatic hydrocarbons
  - Multiple aromatic rings
    - 2 – 7
  - Low molecular weight PAHs
    - More toxic
  - Higher weight PAHs
    - More carcinogenic (cancer – causing)
- Remain after weathering of crude oil



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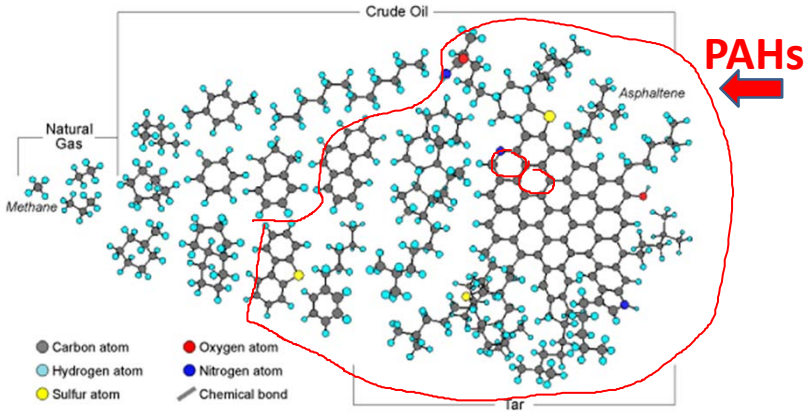
Crude Oil Composition

Examples of Some Organic Compounds in Petroleum



PAHs

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Interactive Effects of Contaminants

- Toxicity of individual compounds
- Toxicity of mixture of compounds
  - Antagonism (mixing decreases toxicity)
  - Additive (have similar effect)
  - Potentiation (mixture exceeds effect of each)

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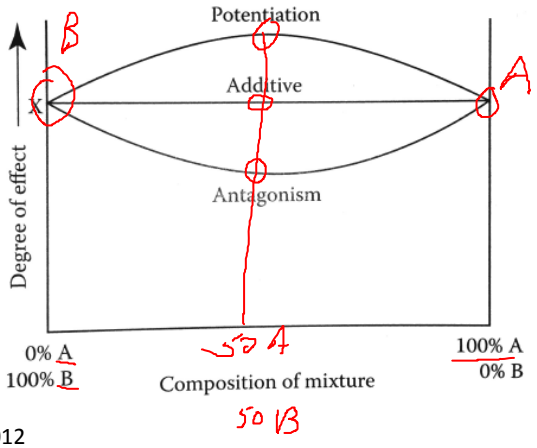
Toxic Effects of Mixtures



Walker et al., 2012

Toxicity at Multiple Levels in Organisms

- **Effects on DNA**
  - **Genotoxicity**
- **Effects on cell membrane**
- **Effects on organs**
- **Effects on whole organisms**

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Effects on DNA

- Genotoxicity - Damage the DNA
  - Mixed functions oxygenase enzymes (MFOs)
  - See Tuzzy Talk #6
- Genetic diseases in future generations

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Effects on Membranes

- Narcosis (caused by "narcotics" including hydrocarbons)
  - Theories of impacts to membranes
  - Contaminant alters physical and/or chemical properties of lipid bilayer
    - Less fluid, inhibits passage of substances in and out of cell
  - Contaminant interacts directly with protein associated with membrane
  - Contaminant alters interaction of lipid bilayer and protein inserted into it
  - Decrease function → hence narcosis
  - Disrupts energy generation across membranes

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Effects on Organs

- Cardiovascular or respiratory effects
  - Heart rate
  - Gas exchange rates

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Effects on Whole Organism

- Nervous systems impacts
- Behavioral impacts
  - Reaction to stimuli, swimming speed/stamina
  - Predation vulnerability
  - Impaired foraging
  - Reproductive effects
  - Reduced growth/efficiency
- Disease causing change in tissues
  - Skin ulcers, fin rot, etc.
  - Liver disease
  - Intestinal disease

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Effects on Whole Organism (Cont'd)

- Immunological Effects
  - Antibody production
  - Reduced phagocytosis of bacteria by white blood cells
  - Few studies on this
- Development Effects
  - Blue sac disease in larval fish
  - Pacific herring embryos + PAHs affect developing heart
    - Effect on heart rate and rhythm

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Effects on Whole Organism (Cont'd)

- Reproductive Effects
  - Low fertility
  - High embryo mortality
  - Hard to study because long term chromic effects difficult to monitor

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Toxicity Terms

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Lots of Toxicity Terms!!!

Key ones to follow are in next slides

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Toxicity = Capacity to Cause Adverse Effects on Living Organisms

Caused by exposure/dose of contaminant

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Lethal = Adverse Effect is Death

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Effective = Adverse Effect
But Not Death

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Exposure

- Amount of contaminant in organism's environment
  - Time (period)
  - Concentration of contaminant
- Units of exposure
  - Concentration:

$$\text{Water} = \frac{\text{mass contaminant}}{\text{volume of water}}$$

$$\text{Sediment} = \frac{\text{mass contaminant}}{\text{mass sediment}}$$

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Measured vs. Nominal Exposure Concentration

- Measured concentration in environment through exposure period
- Nominal: measured only at beginning of exposure period
- Some hydrocarbons volatile
  - Exposure concentration decreases over time
  - So nominal concentration is deceiving
- Must be Measured Concentration to be valid test

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Measure of Exposure

Lethal Concentration

$LC_{\%}$

Concentration of contaminant in environment which produces death in given % of exposed population of organisms in specified time

$$\text{Time } LC_{\%} = \frac{\text{mass contaminant}}{\text{volume of air or water}}$$

Example:

96hr LC_{50} Phenanthrene for *Chironomus sancticaroli* (flying insect) is 1.60 [95% Confidence Interval, 1.51 to 1.66] mg/L

(Morais et al., 2014)

Dose

- Amount of contaminant that enters organism
- Units of dose

$$\frac{\text{mass contaminant}}{\text{mass of organism's body}}$$

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Lethal Dose

LD_{50}

Dose of contaminant which produces death in given % of population of organisms which it is administered by any of a variety of methods

$$LD_{\%} = \frac{\text{mass contaminant}}{\text{volume of body weight}} \text{ method of administration}$$

Example:

LD_{50} Phenanthrene for Mouse (oral) = 700 mg/kg of body weight

Effective Concentration or Dose
EC or ED

- If adverse response is not death, then EC or ED
- $EC_{\%}$ at 96hr = Concentration where given % of organisms show effect
  - 96hr EC_{50}
- $ED_{\%}$ method of dose = Dose where given % of organisms show effect when administered with specified method
  - Oral ED_{50}

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Other Reported Values

- No Observed Effect Concentration (NOEC)
- No Observed Effect Dose (NOED)
- Lowest Observed Effect Concentration (LOEC)
- Lowest Observed Effect Dose (LOED)

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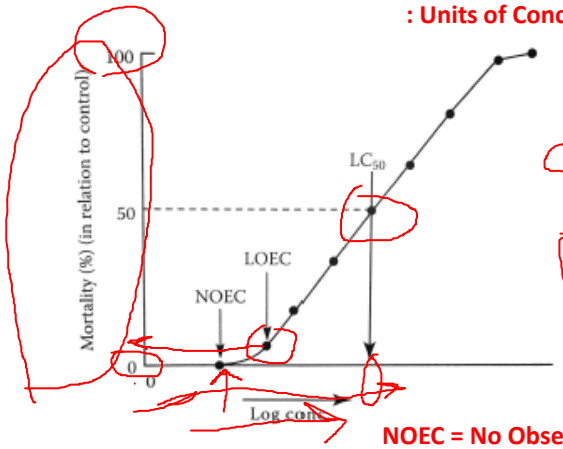
Must Specify: Exposure Period (Time) (check units)
: Contaminant
: Units of Concentration = mg contaminant / L of water



96 hr
Phen.
Lethal

NOEC = No Observed Effect Concentration
LOEC = Lowest Observed Effect Concentration
LC_{50} = Concentration where 50% of organisms show effect

Walker et al., 2012

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Confusion with These Measures of Toxicity

- Lower value means more toxic
- Less needed to cause a toxic effect – more toxic the substance is
- LD_{50} of 25 mg/kg is more toxic than is one of 7,000 mg/kg

$LD_{50} = \frac{25\ mg}{Kg\ body}$

Phen
Mouse
oral

Acute vs. Chronic

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Acute Toxicity

- Death or Effect occurs with relatively short time of exposure
  - Less than 2 to 4 days

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Chronic Toxicity

- **Death or Effect occurs over relatively long time of exposure**
  - **Typically greater than 10% of lifespan**
    - **If Lifespan = 10 yrs, then Chronic Toxicity occurs after at least 1 year exposure**
- **Acute = less than 2 to 4 days of exposure**

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Toxicity Testing

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Toxicity Testing

- Usually done in laboratories
- Requirements:
  - Lots of test water
  - Non-polluting or non-absorbing materials for conducting tests
  - Adequate space for culturing organisms, testing and measuring
  - Source of healthy test organisms

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Types of Toxicity Tests

- Duration of Test
  - Short
  - Intermediate (effects on various life stages)
  - Long (chronic)

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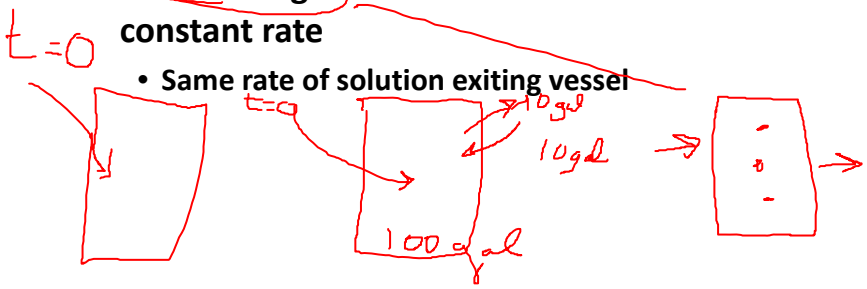
Types of Toxicity Tests

- Method of Adding Test Solution
  - Static: No change of solution
  - Renewal: Periodically remove some solution and add fresh solution in its place
  - Flow-through: solution flows into vessel at constant rate
    - Same rate of solution exiting vessel



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Types of Toxicity Tests

- Single Components
- Mixture of Components
- Environmental Conditions (e.g. Temperature, light, pH)
- Size of Vessel
  - Microcosm
  - Mesocosm
  - (Field studies)

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Quality Control for Toxicity Tests

- **Standard Operating Procedures (SOPs)**
- **Blind testing: analyst does not know experimental condition**
- **Random design: eliminate bias from individual test chambers**
- **Change of custody of materials and documents**

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What Can Go Wrong in Toxicity Testing

- **Lack of test array randomization**
- **Testing not blind**
- **Controls not tested**
- **Test chambers not identical**
- **Control mortality exceeds acceptable limits**
- **Test organisms not randomly assigned to test chambers**
- **Test organisms not from the same population**
- **Test organisms not all the same species**
- **Test organism holding time exceeded**
- **Water quality parameters out of range**

STD Methods (2005)

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Most Common Toxicity Tests

- **Static Tests**
  - **Organisms placed in chamber**
    - **No flow**
    - **Water not changed during test**
    - **Easy and inexpensive**
    - **<u>But</u>**
      - **Need initial and final concentration measured**
      - **Constant concentration?**
      - **Waste product build-up**

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Less Common Toxicity Tests

- **Flow – Through Tests**
  - **Continuous flow through of test water**
    - **Organism held in chamber**
    - **Eliminates issues of static tests**
    - **Cost, space, and waste high**
    - **Still must monitor that concentration in chamber is constant**

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Measures of Toxicity

- **Toxicity is measured as clinical "endpoints" which include**
  - **Mortality (death) =lethal**
  - **Teratogenicity (ability to cause birth defects)**
  - **Carcinogenicity (ability to cause cancer)**
  - **Mutagenicity (ability to cause inherited change in the DNA)**
- **Other Measurable Effects**
  - **Behavioral change**
  - **Disease**
  - **Physical or chemical change**

Examples of Short Term Toxicity Test for Fish

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Common Conditions to Maintain

- Light quality and intensity
- Period of light and dark
- pH of water
- Quality of water (e.g., metals, pesticides, chlorine)
- Contaminant concentration
  - Usually ≥ 5 concentrations
  - Control
- Dissolved oxygen adequate

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Variables for Short Term Acute Toxicity Tests for Fish

- Duration: 24, 48, 96 hr (typical)
- Flow conditions: static, renewal or flow through
- Temperature constant at __ ˚C
- Volume of Chamber
- Age of test organisms
- Organisms per test chamber
- # Replicate chambers per concentration or dose
- Feeding of fish
- Cleaning of chambers
- Endpoint
- Test acceptability ≤ 10% of controls show effect

Exposure

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Parameters Monitored in Test Chambers

- Water quality
- # of organisms alive (if lethality testing)
- Concentration of contaminant present
  - Should be constant
  - Not only normal (initial) measured concentrations
  - Constant/adequate conditions (e.g., temperature, light, food)

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Cumulative Data Generated (# Dead Fish/Total # Fish)

Time (Hours)	Concentration 1 Chamber 1			Concentration 2 Chamber 2			...	Concentration 5 Chamber 3			Control* Chamber 3		
0	0/10	0/10	0/10								0/10	0/10	0/10
24	1/10	0/10	0/10										
48	2/10	2/10	1/10										
72	2/10	3/10	2/10										
96	2/10	4/10	3/10								0/10	0/10	0/10
Total % Mortality	20%	40%	30%	48%	51%	54%							

* Less than 10% mortality in controls

Only use data if concentrations 1, 2, …5 maintained in desired range (Measured Concentrations)

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Exposure (Concentration) or Dose Response Curves

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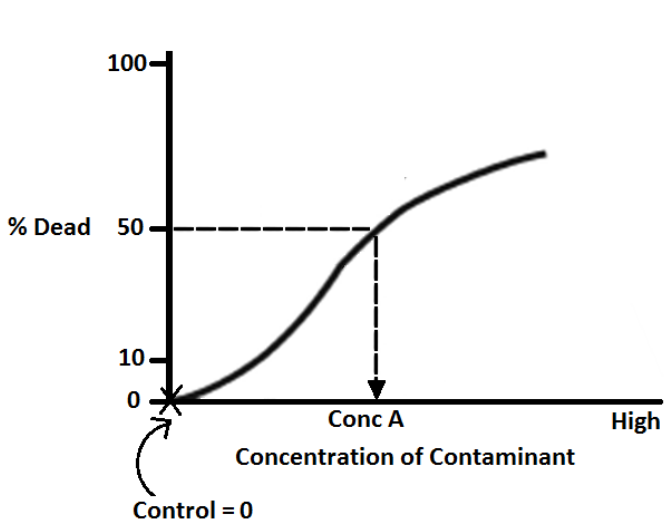

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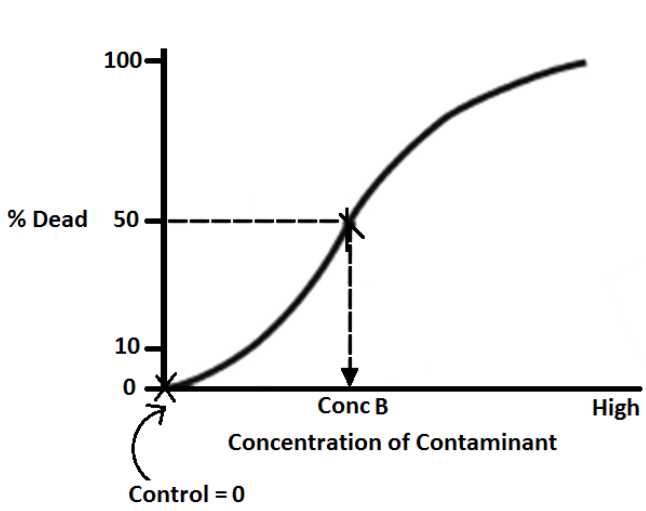

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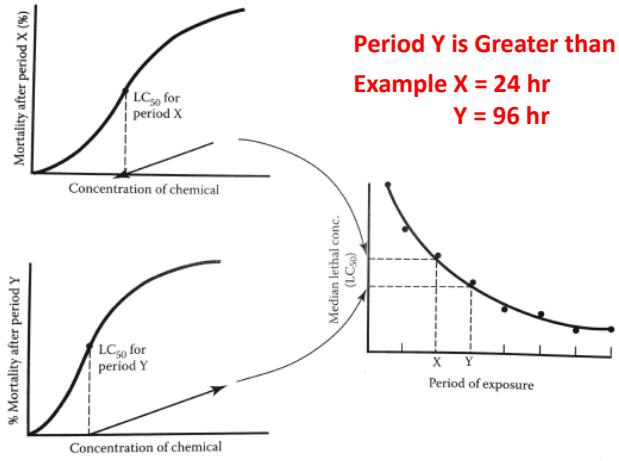
Exposure Response Curves

As Exposure Period Increases, LC_{50} Decreases



Period Y is Greater than Period X

Example X = 24 hr

Y = 96 hr

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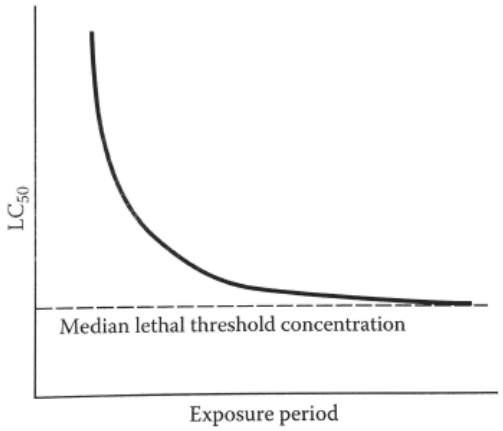
Walker et al., 2012

Mean Lethal Threshold LC_{50}



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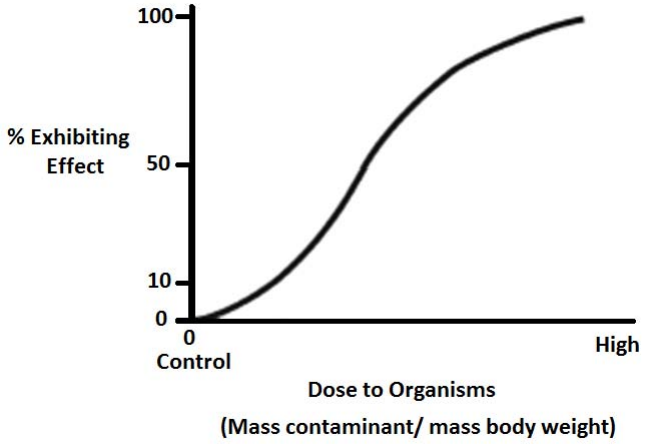
Walker et al., 2012

Dose Response Curves



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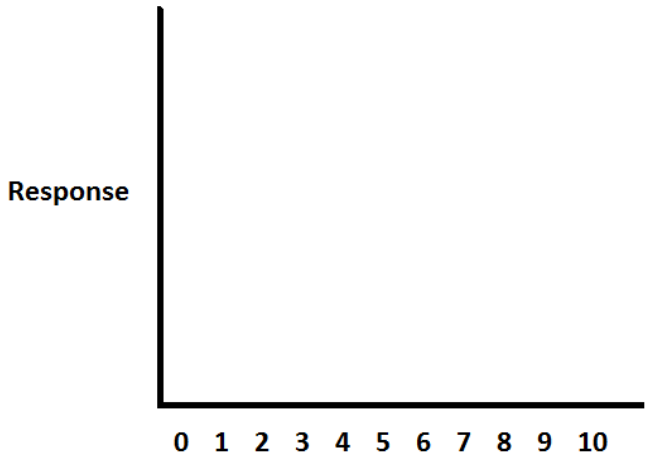
Things to Watch on Curves



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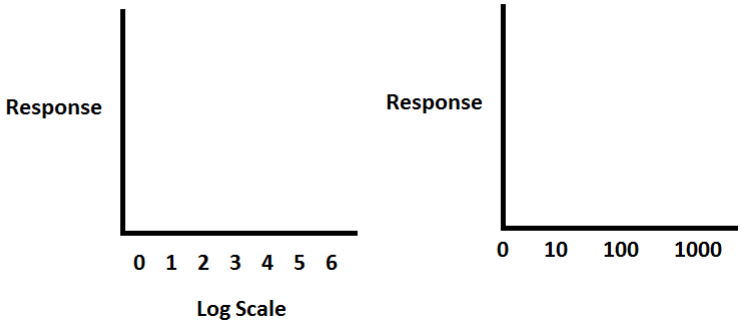
Logarithmic Scale

0 = 100
10 = 1
100 = 2
1000 = 3
10,000 = 4
1000,000 = 5
1,000,000 = 6



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Various Ways to Plot Response Curves

- **Curve fitting methods**
  - **Probit, Logit etc**
- **Methods to estimate LC_{50}**
  - **Litchfield Wilcoxon, Spearman-Karber**
- **Details <u>not</u> discussed in Tuzzy Talks**

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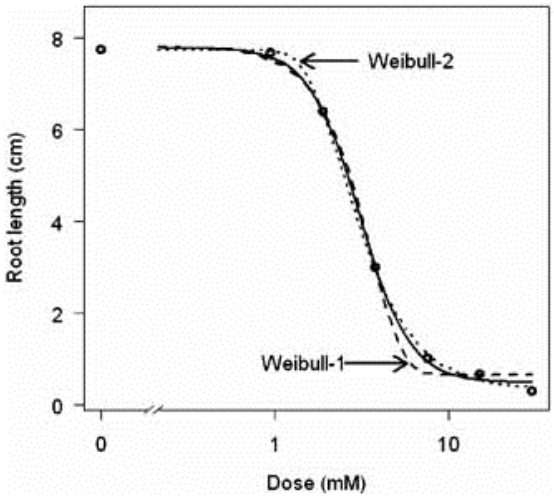
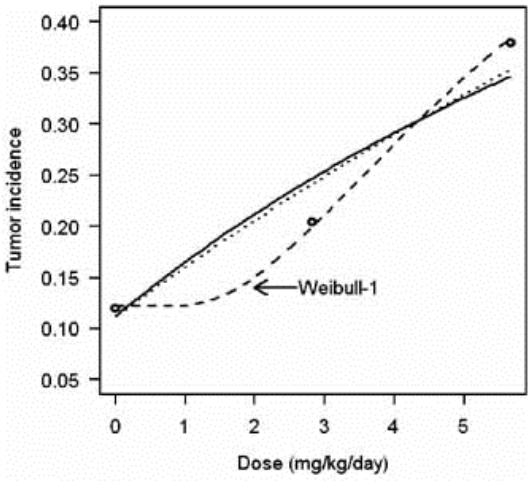
Toward a unified approach to dose–response modeling in ecotoxicology



Environmental Toxicology and Chemistry
Volume 29, Issue 1, pages 220-229, 18 SEP 2009 DOI: 10.1002/etc.7
http://onlinelibrary.wiley.com/doi/10.1002/etc.7/full#fig2

Toward a unified approach to dose–response modeling in ecotoxicology



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http://onlinelibrary.wiley.com/doi/10.1002/etc.7/full#fig3

Mathematics for Response Models

Model	Model function/Equation	Special cases
log-logistic	$c + \frac{d-c}{1+\exp(b(\log(x)-\log(e)))} = c + \frac{d-c}{1+\left(\frac{x}{e}\right)^b}$	Log-logit (logistic regression), Hill, Michaelis-Menten
log-normal	$c + (d-c)\Phi(b(\log(x) - \log(e))$ (Φ is the cumulative distribution function of the standard normal distribution)	Log-probit
Weibull-1	$c + (d-c)\exp(-\exp(b(\log(x) - \log(e))))$	Exponential, log-log link
Weibull-2	$c + (d-c)(1 - \exp(-\exp(b(\log(x) - \log(e)))))$	Complementary log-log link, first-order multistage, one-hit

The parameters c and d denote the horizontal asymptotes or limits, whereas the parameter b is the relative slope at the inflection point of the resulting dose–response curve. The parameter e corresponds to the dose where the inflection point is located. The original, untransformed dose is denoted x throughout.

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http://onlinelibrary.wiley.com/doi/10.1002/etc.7/full#fig3

Thank You So Much for Participating in These Talks

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Questions?

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