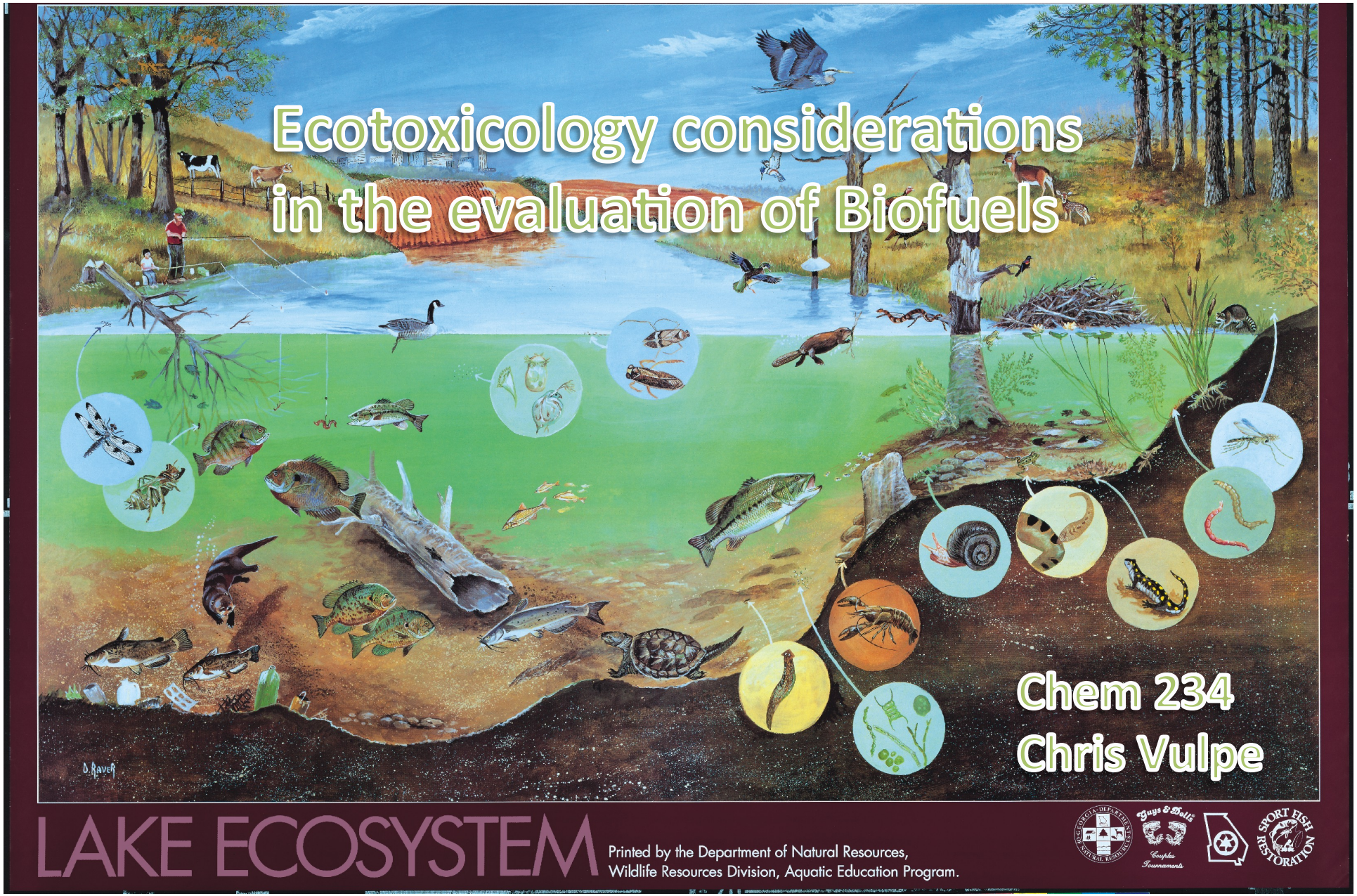
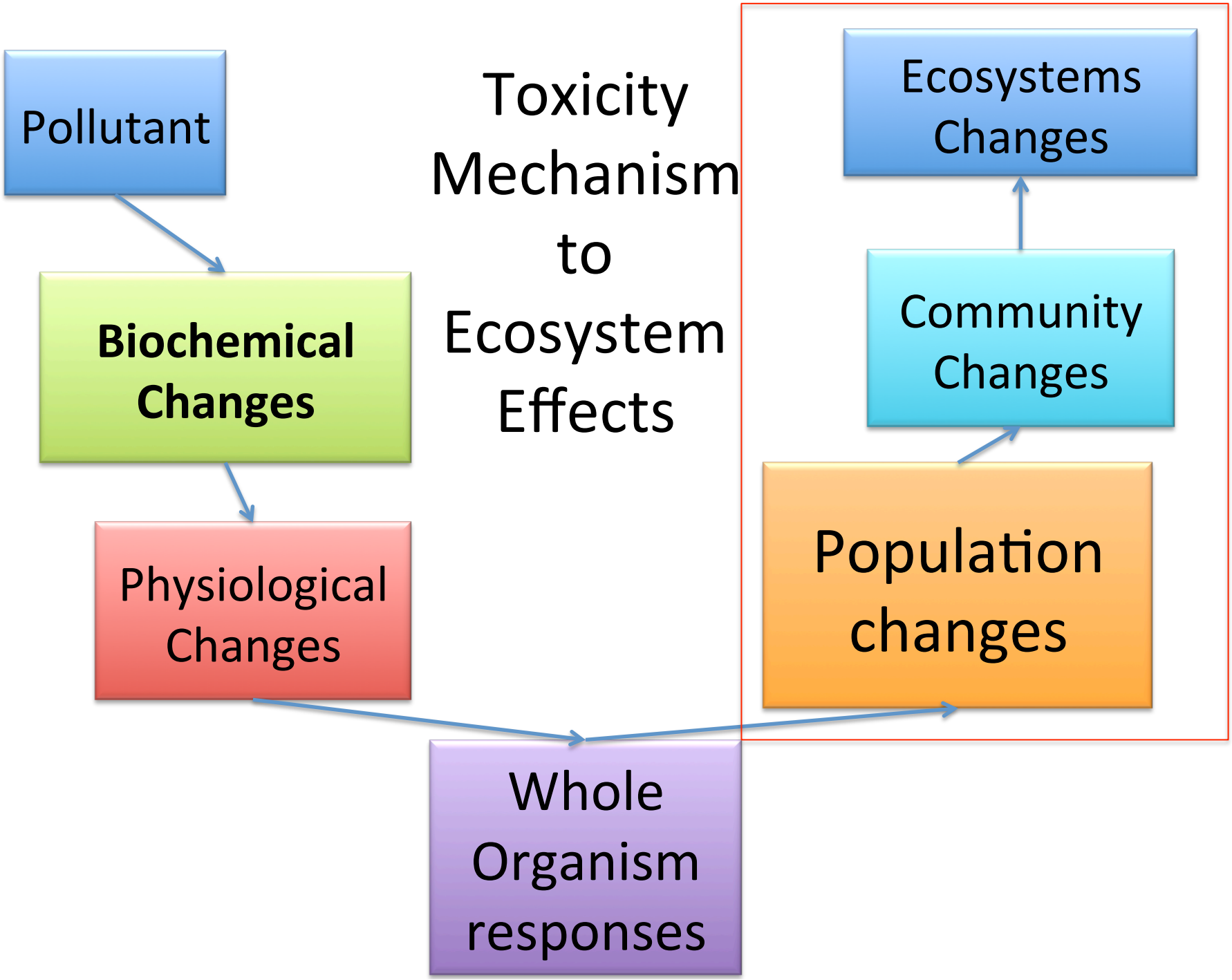


http://gastateparks.org/content/Georgia/parks/education/LakeEcosystem_Poster.jpg

ECOTOXICOLOGY

- Entry, distribution and fate of pollutants within the environment
- The entry and fate of pollutants in living organisms within an ecosystem
- The harmful effects of the chemical pollutants on the constituents (biotic & abiotic) of ecosystems (which include man)

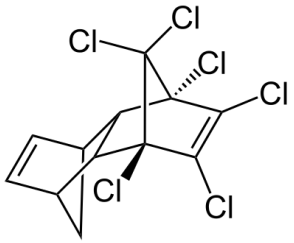
Toxicology	Ecotoxicology
Single target Species (Human)	Multiple Target Species
Individual Effects	Population level effects
Absorption	Environmental Release
Distribution	Environmental Fate Bioaccumulation/Bioconcentration Biomagnification
Metabolism	Organismal Metabolism
Elimination	Biodegradation Sequestration


Pollutant
Biochemical Changes
Physiological Changes
Toxicity Mechanism to Ecosystem Effects
Whole Organism responses
Population changes
Community Changes
Ecosystems Changes

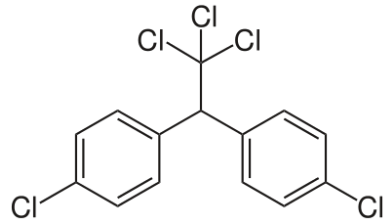
Chemical Assessment in Ecotoxicology

- The Big Three – PBT
  - Persistence (P) – does it stick around? Biodegraded? Is it very persistent?
  - Bioaccumulation (concentration & magnification) (B) – does it accumulate in organisms? Does the concentration increase from prey to predator?
  - Toxicity (T) – will it kill the organisms?

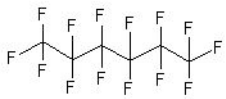
- Environmental Fate and Transport – where is it going? What is the relevant organism – air, water, sediment,
- Exposure scenarios – point source vs dispersed runoff - pulse, constant, only Tuesdays in March.

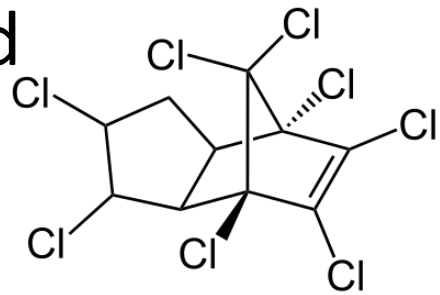


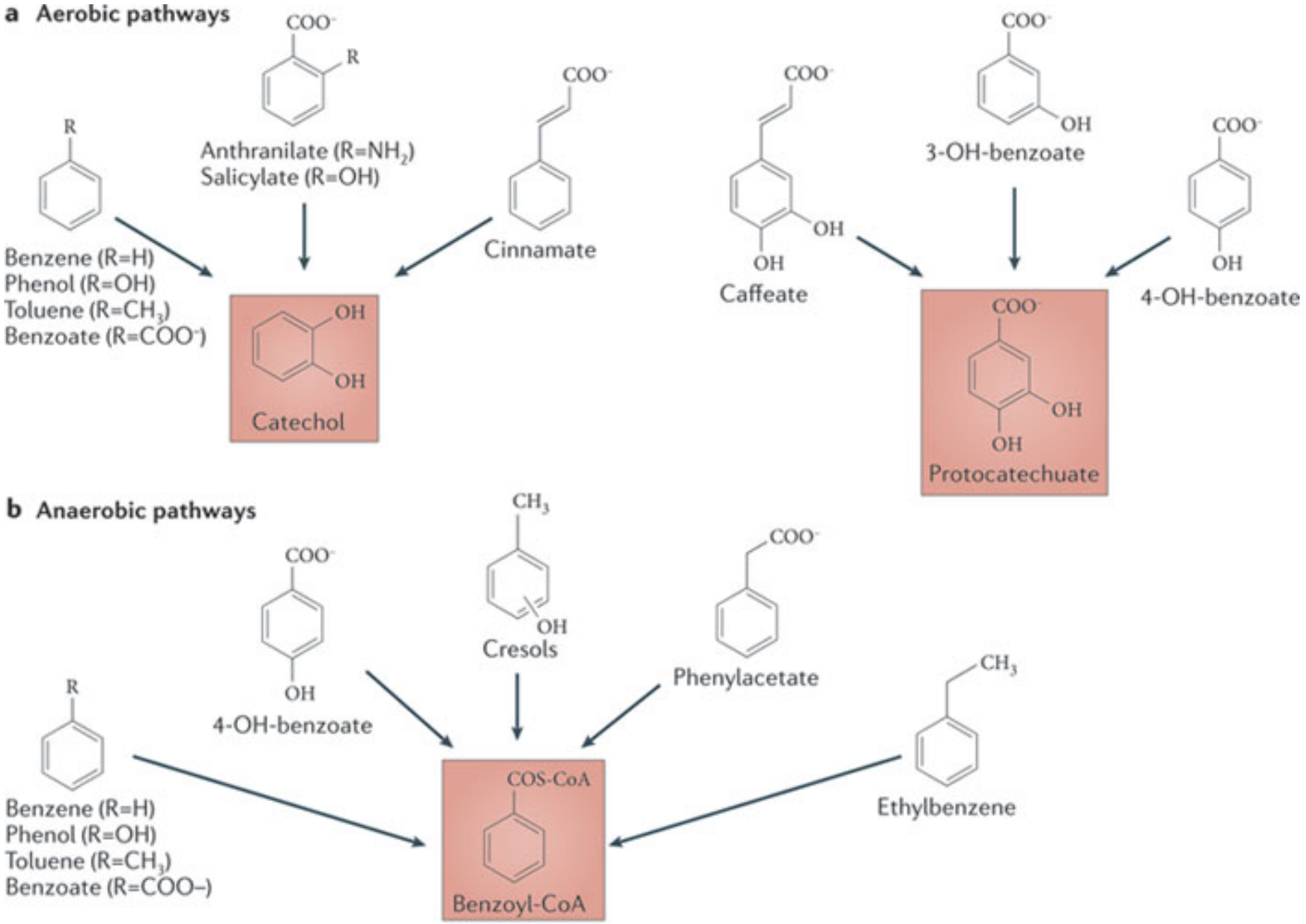
Persistence



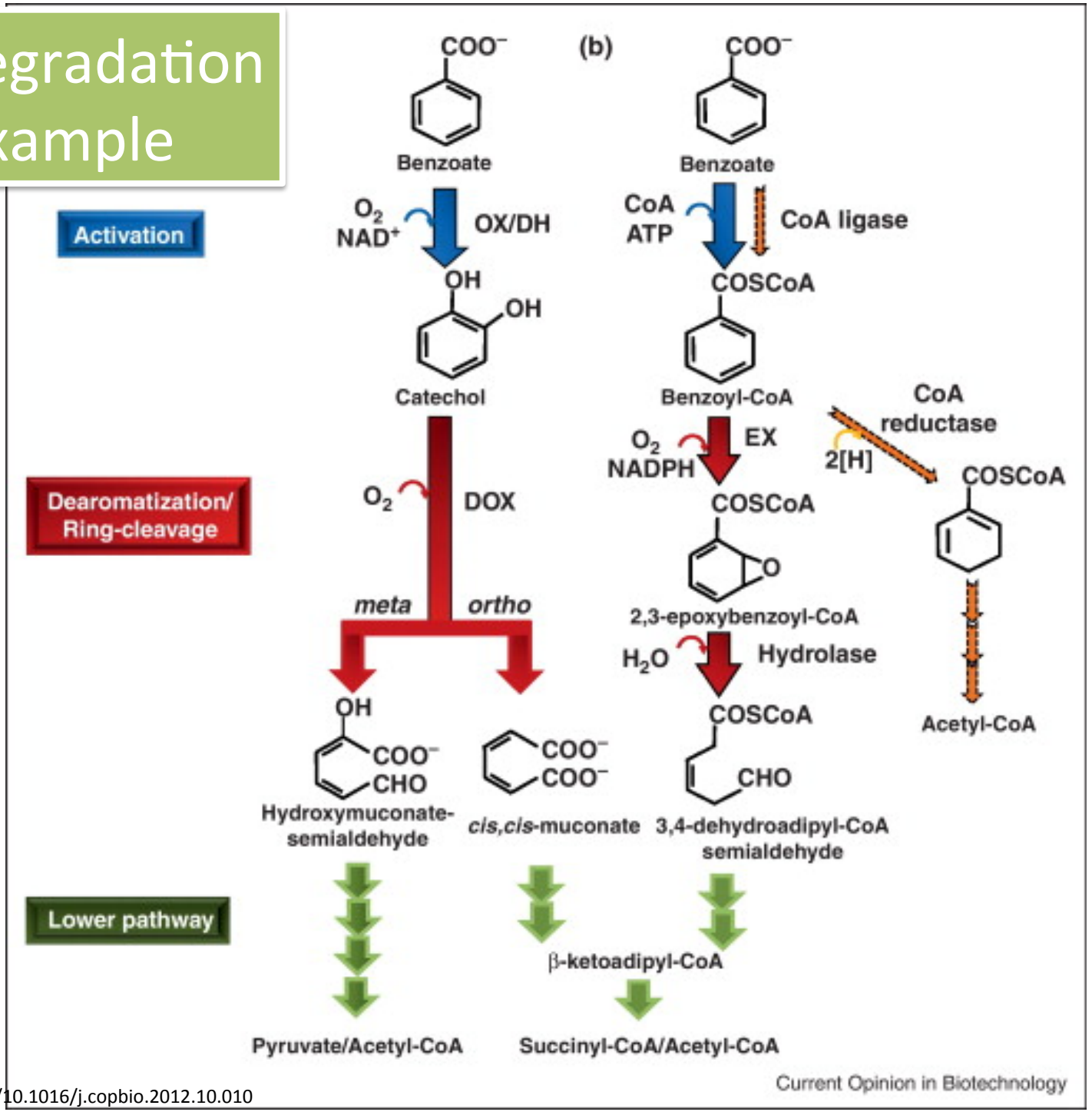
- Abiotic degradation – breakdown without organisms – e.g. photolysis
- Biodegradation – organism driven – usually microbes
- Community driven process – enzymes from multiple different species
- Co-metabolism – energy source
- Biometabolism – Aerobic vs Anaerobic Metabolism
- Recalcitrant compound –not metabolized





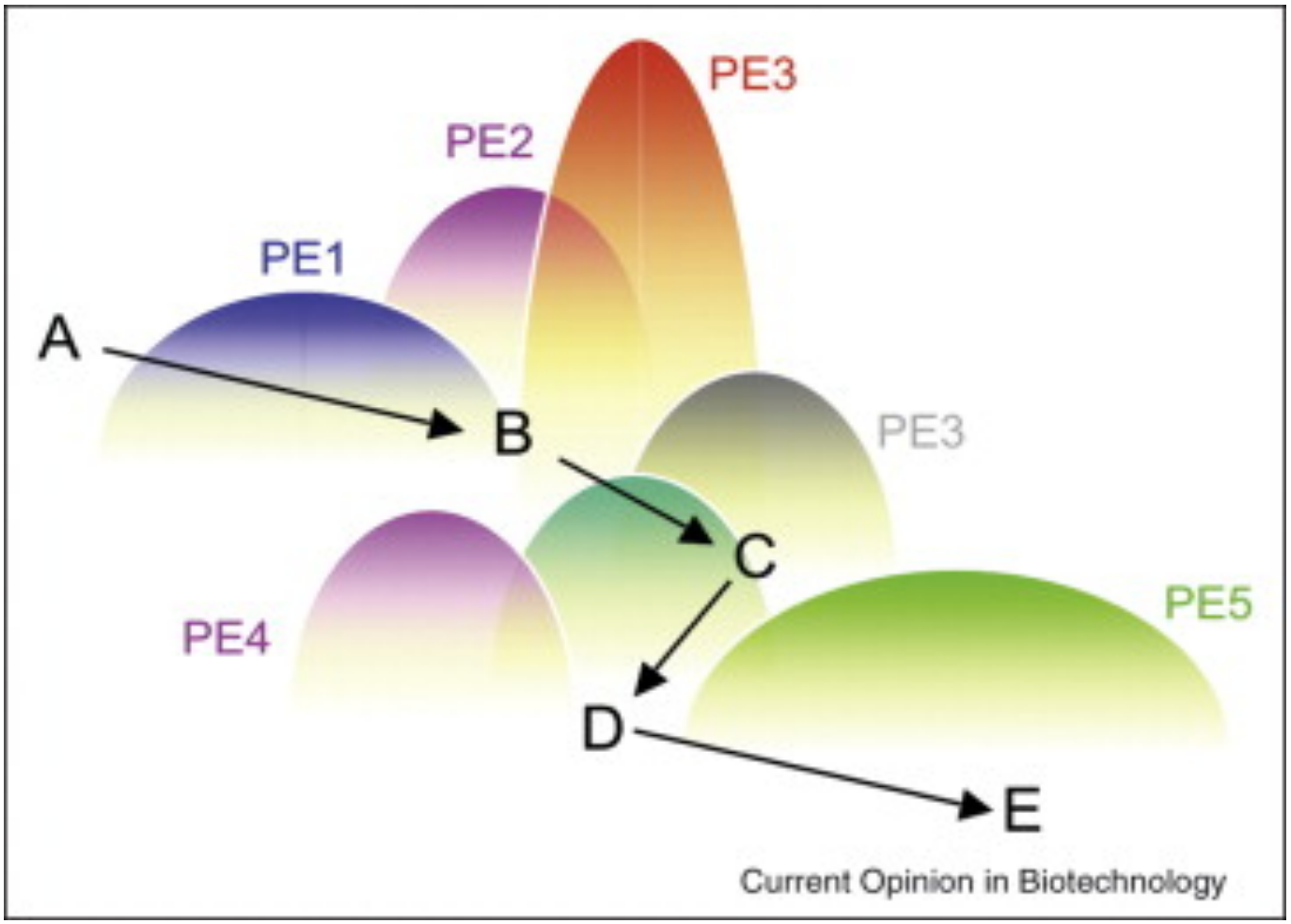

a Aerobic pathways
COO⁻
R
Anthranilate (R=NH2)
Salicylate (R=OH)
R
Benzene (R=H)
Phenol (R=OH)
Toluene (R=CH3)
Benzoate (R=COO⁻)
COO⁻
Cinnamate
OH
OH
Catechol
COO⁻
OH
OH
Caffeate
COO⁻
OH
3-OH-benzoate
COO⁻
OH
4-OH-benzoate
COO⁻
OH
OH
Protocatechuate
b Anaerobic pathways
COO⁻
OH
4-OH-benzoate
CH3
OH
Cresols
COO⁻
Phenylacetate
R
Benzene (R=H)
Phenol (R=OH)
Toluene (R=CH3)
Benzoate (R=COO–)
COS-CoA
Benzoyl-CoA
CH3
Ethylbenzene

Biodegradation Example

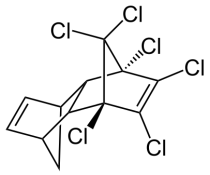


http://dx.doi.org/10.1016/j.copbio.2012.10.010

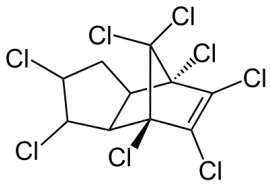
Biodegradation in a community of organisms



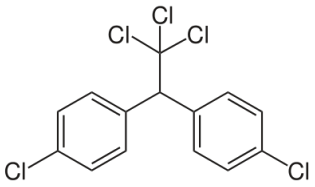
Current Opinion in Biotechnology Volume 19, Issue 6 2008 579 - 589



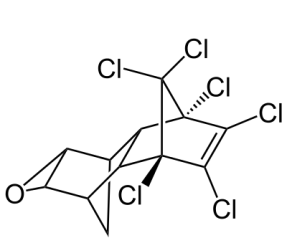
Aldrin



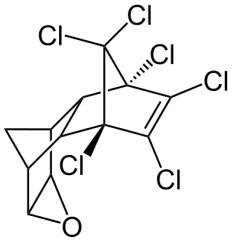
Chlordane



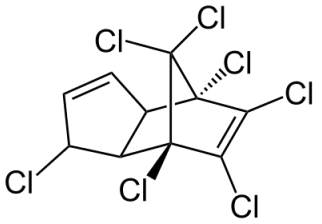
DDT



Dieldrin



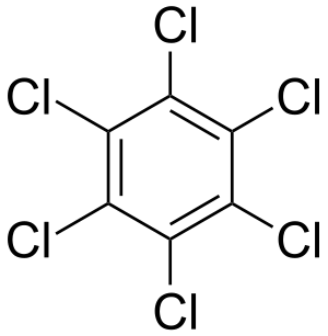
Endrin



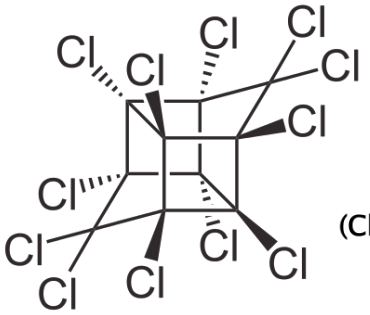
Heptachlor

Dirty Dozen

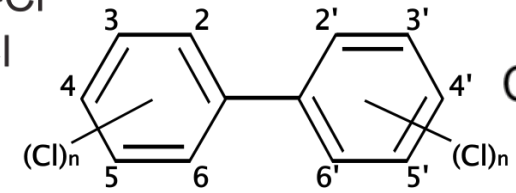
United Nations Environmental Program



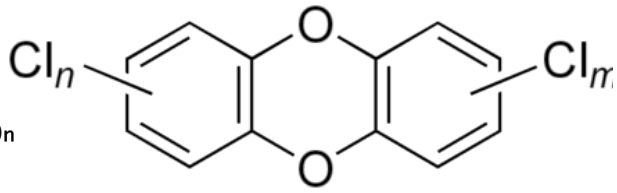
Hexachlorobenzene



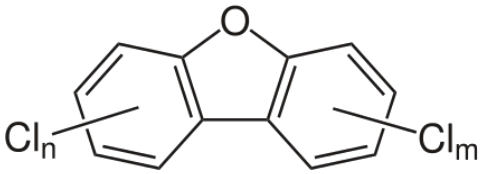
Mirex



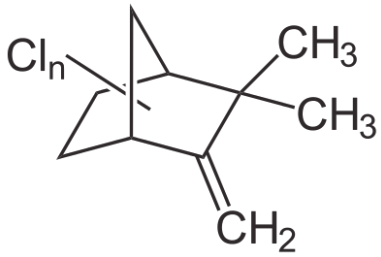
Polychlorinated Biphenyls (PCBs)



Polychlorinated dibenzo**dioxins**

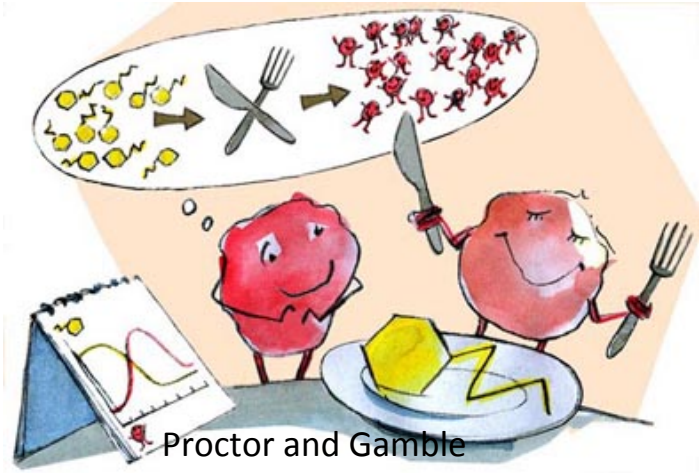


Polychlorinated dibenzofurans



Toxaphene

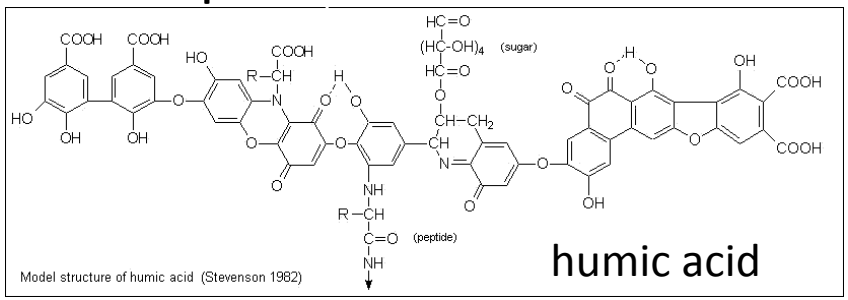
Barriers to Biodegradation



- Chemical structure not found in nature
  - do not or rarely exist as natural products or contain structural elements that cannot be synthesized biochemically
  - number and orientation of certain groups differ from naturally existing compounds. e.g. aliphatic or aromatic compounds that carry several methyl groups.
    - *ortho*-dimethyl or *vicinal*-trimethyl substituted aromatic compounds
    - branched aliphatic compounds like *tert*-butanol or *tert*-butylmethyl

Barriers to Biodegradation

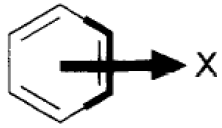
- low water solubility and high lipid solubility
  - Mass transfer limitations – absorption to organic matter – polycyclic aromatic hydrocarbons
  - Humic acids are thought to be complex aromatic macromolecules



- High molecular weight –
  - no transport into micro-organisms
- Lack of functional Groups –
  - Unsubstituted hydrocarbons
- Unfavorable thermodynamics
  - Monochlorinated compounds
- Steric hindrance-branching hydrocarbons
  - methyl-tert-butyl ether

Barriers to Biodegradation

- Electron donating functional groups
  - Oxygenases can't attack



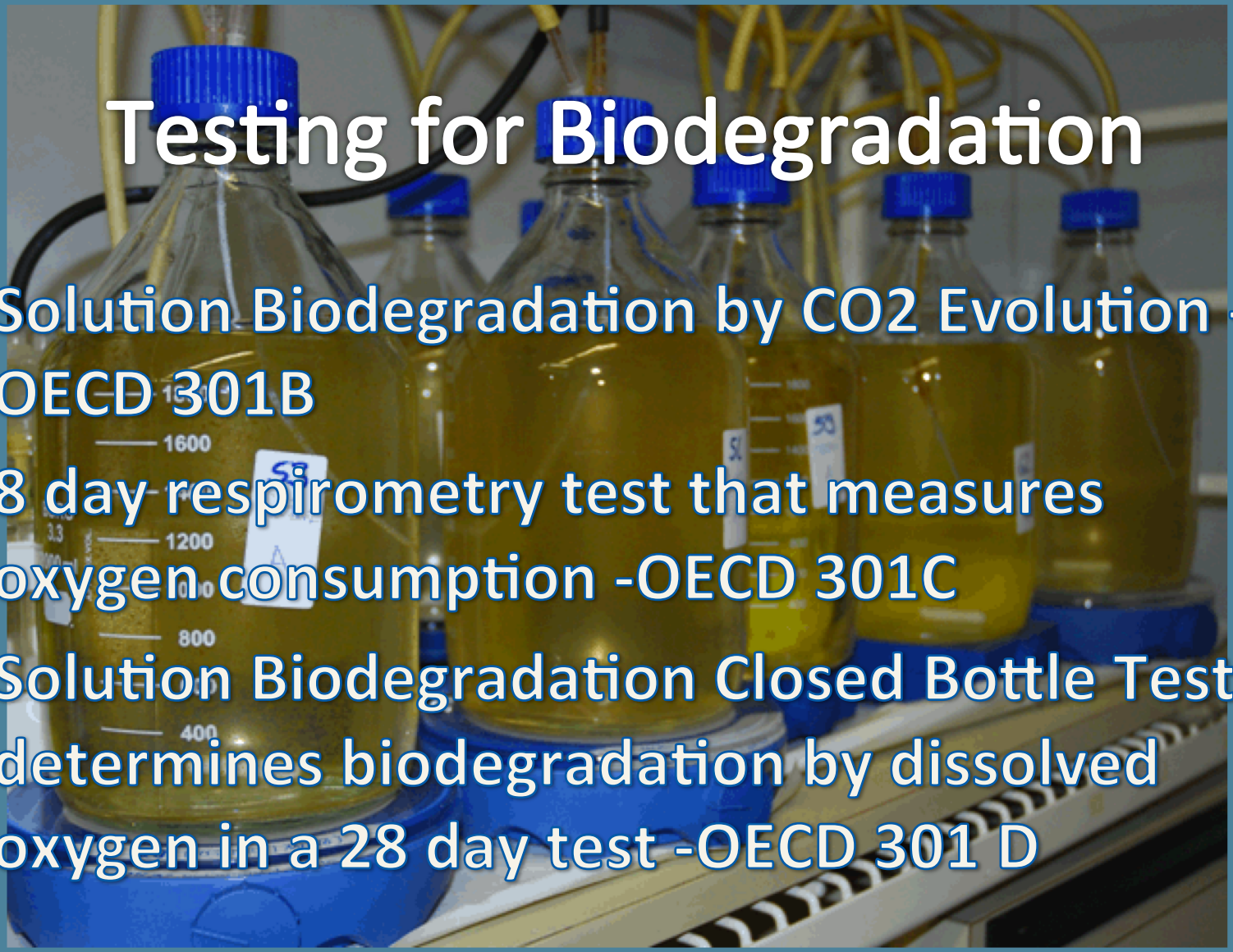
X= C-Halogen
Halogen
$C \equiv N$
COR_1
SO_3H
$N=N-R_2$
NO_2

- High intrinsic toxicity of compound
- Unfavorable environmental conditions for micro-organisms
  - high salt, temperature, color scheme
- Lack of nutrients

Journal of Biotechnology 51 (1996) 287-295
Water Science and Technology Vol 45 No 10 pp 9–18

Testing for Biodegradation

- Solution Biodegradation by CO2 Evolution - OECD 301B
- 8 day respirometry test that measures oxygen consumption -OECD 301C
- Solution Biodegradation Closed Bottle Test determines biodegradation by dissolved oxygen in a 28 day test -OECD 301 D

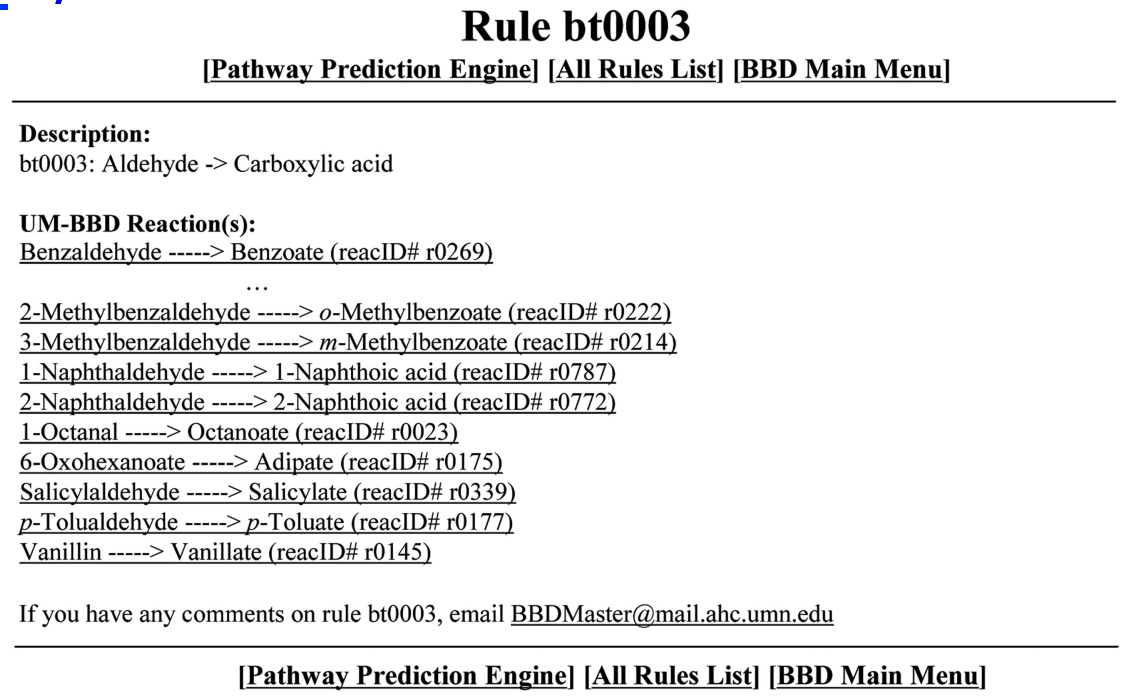


Ready biodegradability (OECD 301 B)

Determination of CO_2 production - test substance, mineral medium, activated sludge - air supply (CO_2 free) - 28 days

Predicting Biodegradation Metabolism Databases

- University of Minnesota Biocatalysis/ Biodegradation Database
  - http://umbbd.ethz.ch/



Rule bt0003

[Pathway Prediction Engine] [All Rules List] [BBD Main Menu]

Description:
bt0003: Aldehyde -> Carboxylic acid

UM-BBD Reaction(s):
Benzaldehyde -----> Benzoate (reacID# r0269)
…
2-Methylbenzaldehyde -----> *o*-Methylbenzoate (reacID# r0222)
3-Methylbenzaldehyde -----> *m*-Methylbenzoate (reacID# r0214)
1-Naphthaldehyde -----> 1-Naphthoic acid (reacID# r0787)
2-Naphthaldehyde -----> 2-Naphthoic acid (reacID# r0772)
1-Octanal -----> Octanoate (reacID# r0023)
6-Oxohexanoate -----> Adipate (reacID# r0175)
Salicylaldehyde -----> Salicylate (reacID# r0339)
p-Tolualdehyde -----> *p*-Toluate (reacID# r0177)
Vanillin -----> Vanillate (reacID# r0145)

If you have any comments on rule bt0003, email BBDMaster@mail.ahc.umn.edu

[Pathway Prediction Engine] [All Rules List] [BBD Main Menu]

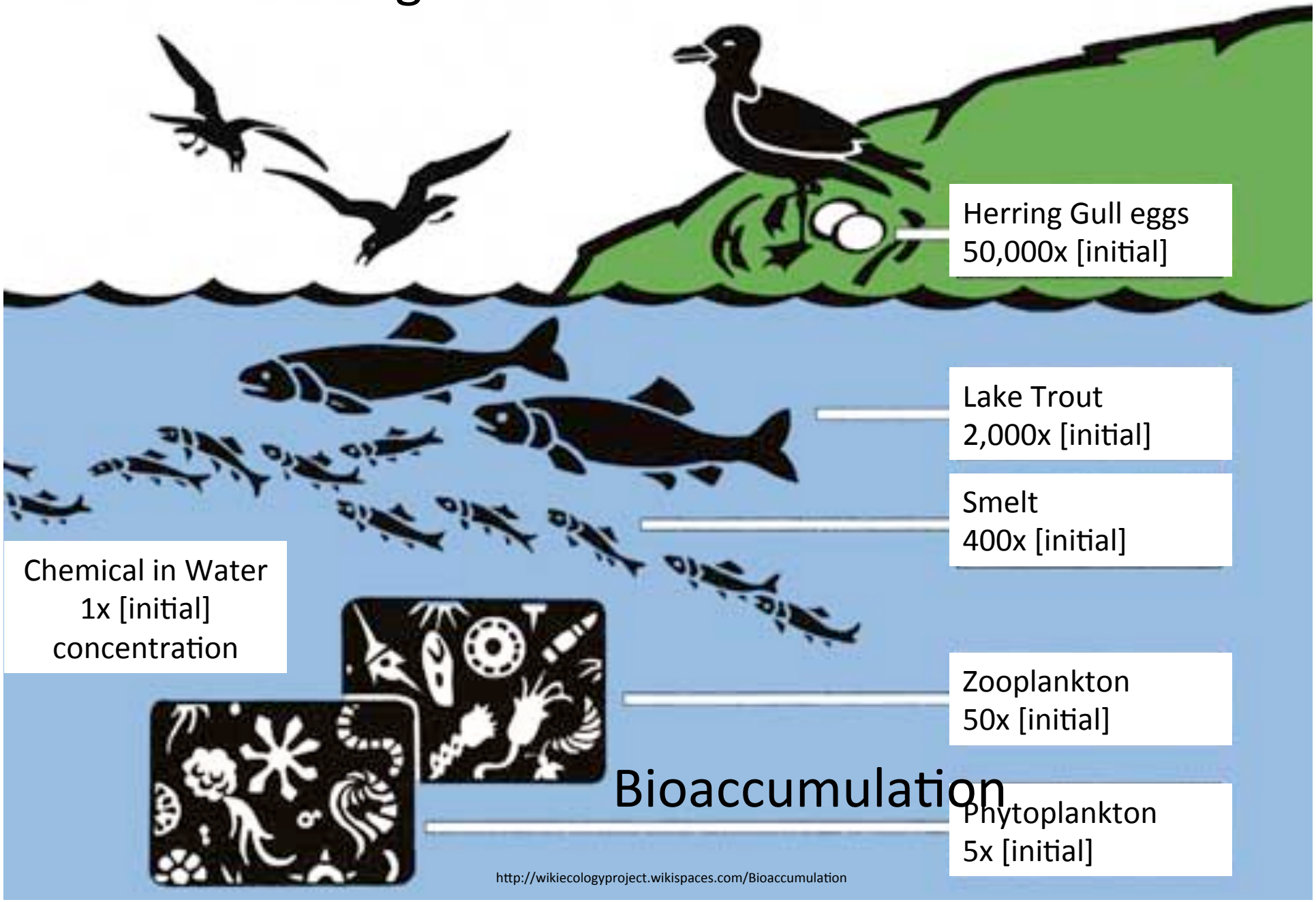
- PBT profiler
  - http://www.pbtprofiler.net/

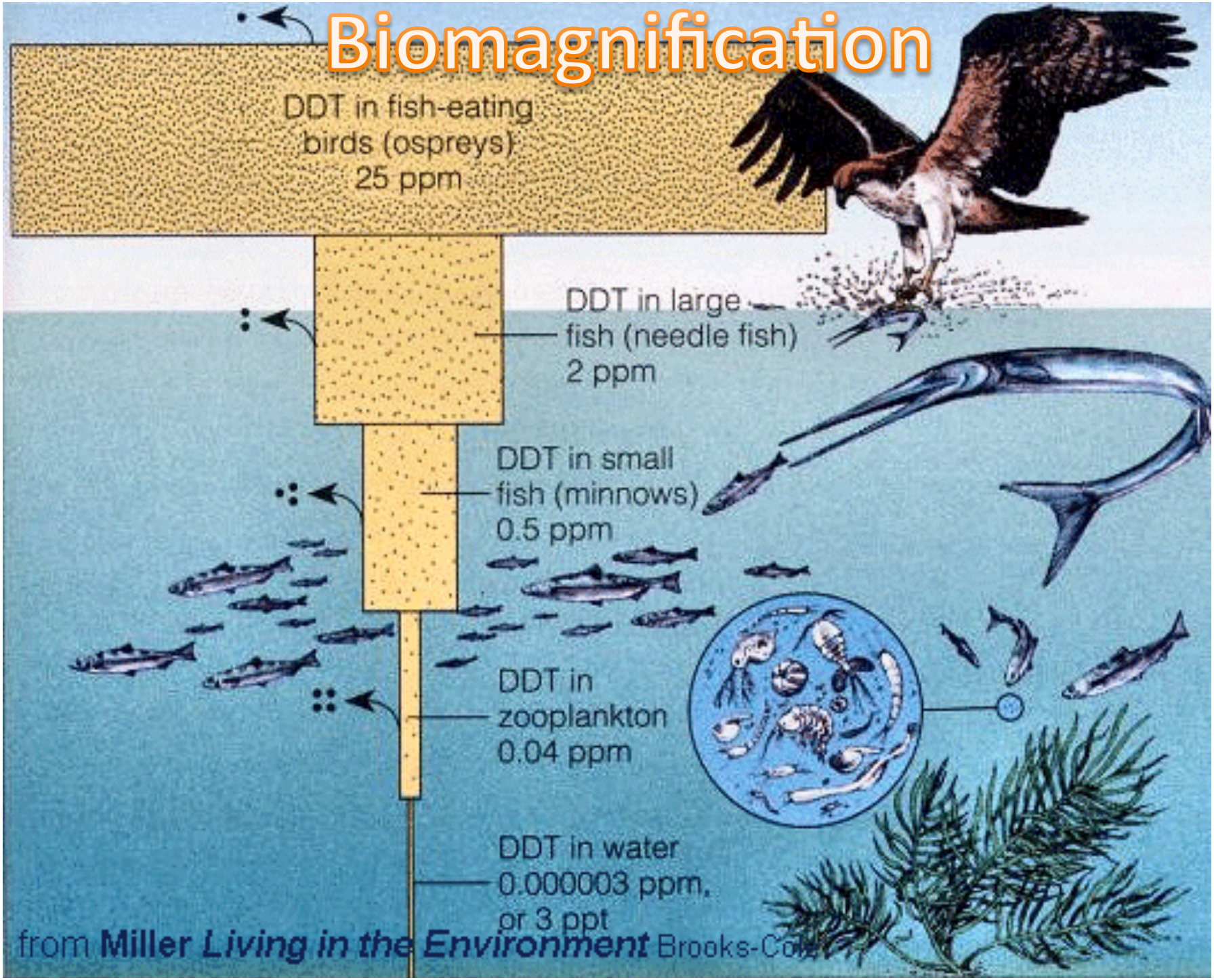
J. Chem. Inf. Comput. Sci. **2003,** 43, 1051-1057.

Bioaccumulation

- Bioconcentration -the process by which there is a net accumulation of a chemical directly from water into aquatic organisms resulting from simultaneous uptake (e.g., by gill or epithelial tissue) and elimination.
- Bioaccumulation -the accumulation of chemicals in the tissue of organisms through any route, including respiration, ingestion, or direct contact with contaminated water, sediment, and pore water in the sediment.
  - Bioaccumulation Factor (BAF) The ratio of the contaminant in an organism to the concentration in the ambient environment at a steady state, where the organism can take in the contaminant through ingestion with its food as well as through direct content.
- *Biomagnification*-Result of the process of bioaccumulation and biotransfer by which tissue concentrations of chemicals in organisms at one trophic level exceed tissue concentrations in organisms at the next lower trophic level in a food chain
- Trophic transfer -the movement of contaminants from one trophic level, i.e., prey, to another trophic level, i.e., predators

Biomagnification




Biomagnification
DDT in fish-eating birds (ospreys) 25 ppm
DDT in large fish (needle fish) 2 ppm
DDT in small fish (minnows) 0.5 ppm
DDT in zooplankton 0.04 ppm
DDT in water 0.000003 ppm, or 3 ppt
from Miller Living in the Environment Brooks-Cole

Structural factors bioaccumulation

- High partition coefficient between octanol and water (K_{ow}) – usually expressed as log K_{ow}

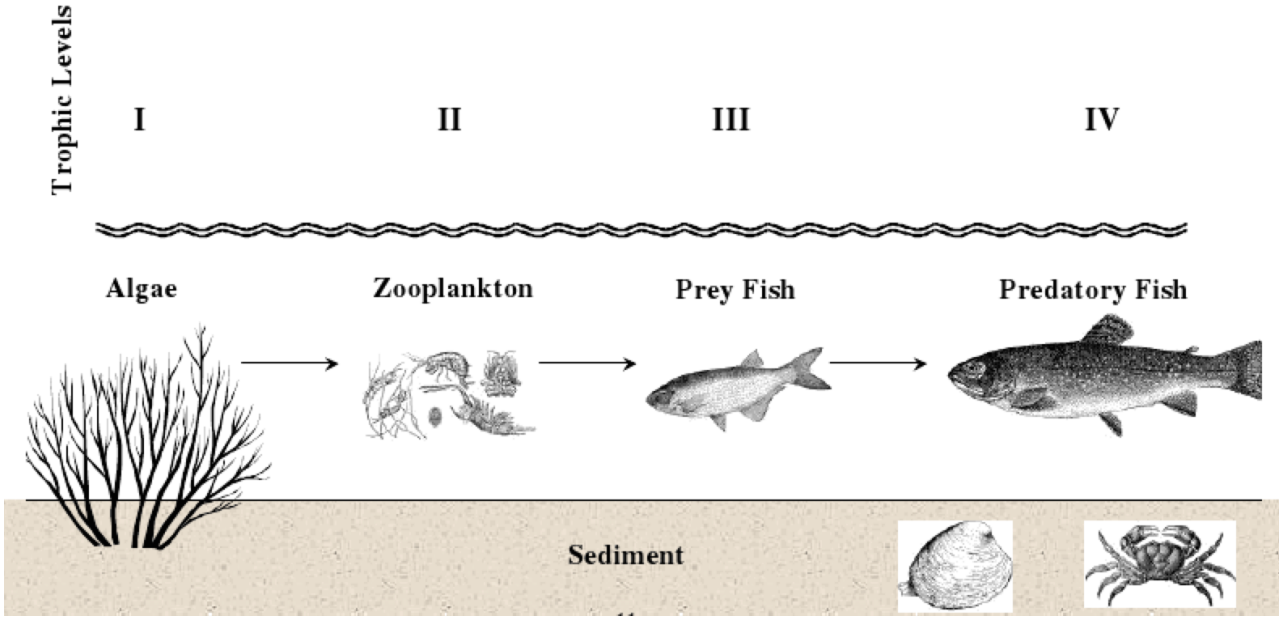
  Log Kow is the logarithm of the ratio of a chemical's concentration n-octanol to its equilibrium concentration in water contacting the n-octanol.

  Bioaccumulation generally increases as chemicals between log K_{ow} =1 – 6

  – Water Solubility
- Polarity
- Ionization
- Molecular Weight
- metals, such as mercury, that can form organo-metallic complexes
- Organismal Metabolism

Measuring Bioaccumulation

- Experimental – expensive rarely done
  - Field Studies & Mesocosms – measure concentration in each organism



Prediction:
Chemicals with log $K_{OW} \geq 5$ are regarded as potentially bioaccumulative in aquatic organisms.
Terrestial : log $K_{OW} \geq 2$ and log $K_{OA} \geq 6$.

http://dx.doi.org/10.1016/j.envint.2010.03.010,

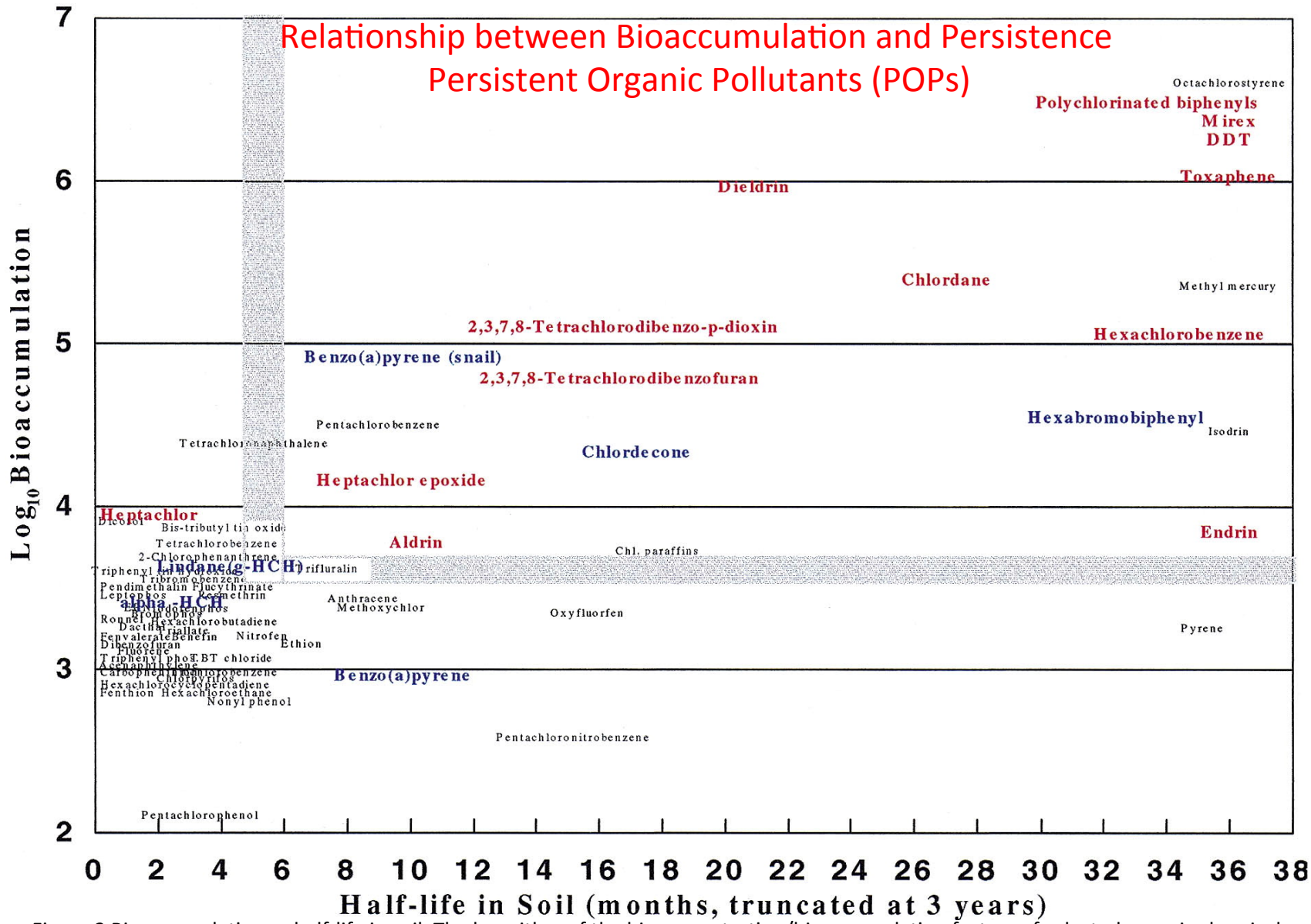
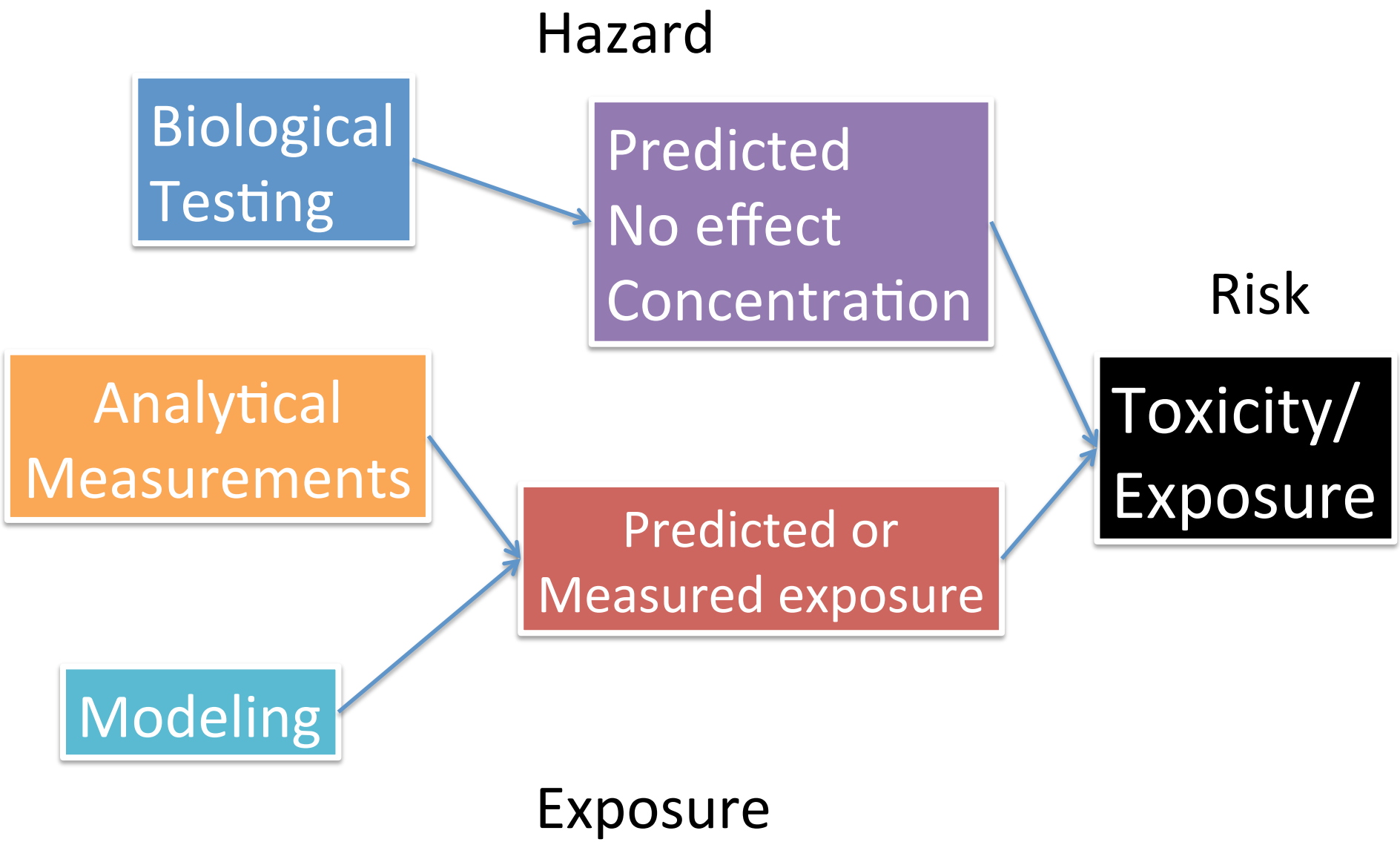


Figure 2 Bioaccumulation vs half-life in soil. The logarithm of the bioconcentration/bioaccumulation factors of selected organic chemicals versus their estimated half-lives in soil is graphed. UNEP POPs are displayed in red; additional substances included in the UNECE−LRTAP POPs protocol are displayed in blue. The shaded lines represent the UNECE−LRTAP and NAAEC−CEC guidance criteria.

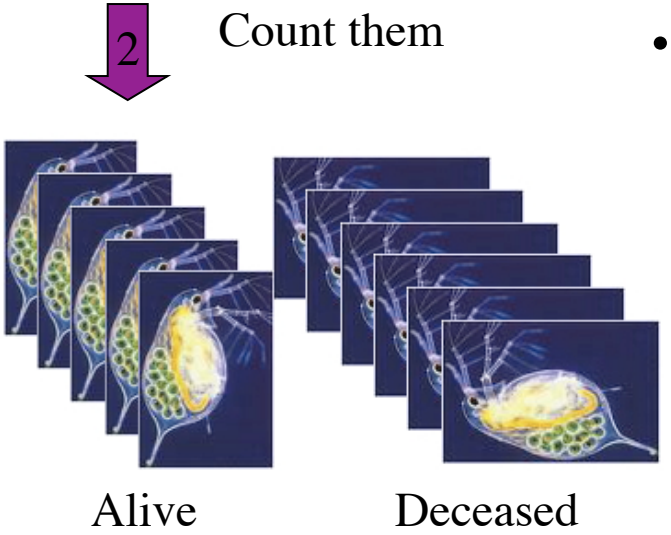
Published in: Bruce D. Rodan; Published in: David W. Pennington; Published in: Noelle Eckley; Published in: Robert S. Boethling; *Environ. Sci. Technol.* **1999,** 33, 3482-3488.DOI: 10.1021/es980060t

EcoToxicity testing History

- 1930's - some of the first uses of aquatic organisms for testing to determine the causes of observed fish kills
- 1945 - some of the first methods for conducting toxicity tests were published
- 1962 -Rachel Carson – Silent Spring - – Recognition of environmental toxicology as unique from human toxicology
- 1972 Clean Water Act
- 1984 - Standardized biological methods introduced to measure water quality developed quickly after the US EPA initiated a national policy in to control toxic substances based on a water quality approach.
- 1995 WET test methods -National Pollutant Discharge Elimination System - The issuance of permits for effluent discharges into surface waters was subsequently tied to **whole effluent testing** using standardized toxicity tests.
- 2006 – Mechanism based assessment – adverse outcome pathways - research area


Hazard
Biological Testing
Predicted No effect Concentration
Risk
Analytical Measurements
Toxicity/ Exposure
Predicted or Measured exposure
Modeling
Exposure

Current Approaches



- Laboratory Ecotox
  - Sentinel Species
  - Kill 'em and count 'em
  - No mechanistic insight
  - Doesn't identify primary toxicant in mixture
  - Sublethal toxicity evades current tests
  - Limited predictive possibilities
  - Expensive and time consuming

- Field Ecotox
  - Bioassessment - count them (did they die?) - biodiversity
  - Analytic Approaches - $$, specific assays, need to know possibilities, no link to biologic effect

Trophic Level Test Organisms

Sensitive species – representative of trophic level

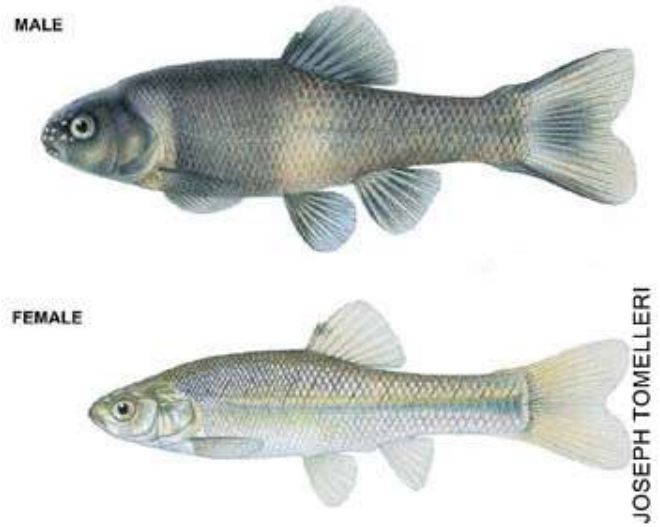
- Freshwater aquatic organisms
  - Primary Producer - Algae
  - Primary Consumer - Crustacean
  - Secondary Consumer - Fish
  - Tertiary Consumer – Bigger Fish
  - Sediment Organisms - Crustacean

Daphnia Magna

Toxicity Test Organisms

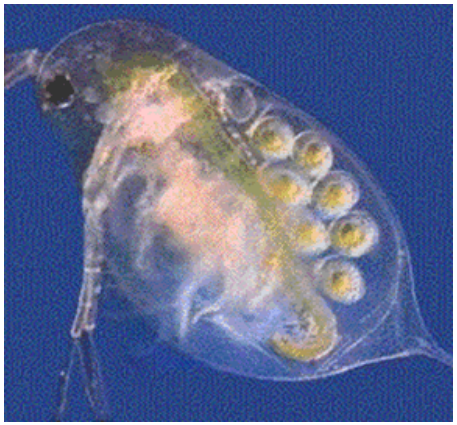
- Use of test species based on
  - Lab hardiness
  - Common
  - Known life cycle
  - Cheap
  - Short-lived

Fathead minnow



Hyallela

Daphnia



Acute vs. Chronic Toxicity Tests

- Acute Toxicity test
  - Drop dead testing – death or immobilization
  - Time = 2 days (invertebrates) to 4 d. (fish)
  - LD_{50} (Lethal dose 50 - dose where 50% buggers dead)
  - EC_{50} (effective concentration 50 – 50% endpoint other than death)
  - NOEC – No Observable Effect Concentration
    - Highest concentration not signficantly different from control
  - LOEC – Lowest Observable Effect Concentration
    - Lowest test concentration that is significantly different from control
  - quick, relatively cheap (but still ~$700-1,200 per test)

- Chronic Toxicity test
  - Sublethal
  - Time = 7d. to 18 months
  - Growth
  - Reproduction
    - brood size (Ceriodaphnia dubia can have 2-3 broods in seven days)
    - Hatching success
  - Usually more sensitive

Ecotox Database

Crustaceans; Standard Test Species										
Daphnia pulex Water Flea	D/ M/	FW LAB	1	BCF 153		ACC/ RSDE	A 0.04 ug/L		15337	View Details
Daphnia pulex Water Flea	FD/ M/	FW LAB	1	BCF 203		ACC/ RSDE	A 0.008 ug/L		15337	View Details
Daphnia pulex Water Flea	S M/	FW LAB	1	BCF 225		ACC/ RSDE	A 0.04 ug/L		15337	View Details
Daphnia magna Water Flea		FW	1	EC0		BEH EQUL	F 718000 ug/L		707	View Details
Daphnia magna Water Flea		FW	1	EC100		BEH EQUL	F 1400000 ug/L		707	View Details
Ceriodaphnia dubia Water Flea	S U	FW LAB	2	EC50	INC	ITX IMBL	F 130 (97-179) umol/L		18991	View Details
Daphnia magna Water Flea		FW	1	EC50		BEH EQUL	F 1020000 ug/L		707	View Details
Daphnia magna Water Flea	S M	FW LAB	1	EC50	INC	ITX IMBL	A 18000 (14810-21900) ug/L		16968	View Details
Daphnia magna Water Flea	S M	FW LAB	1	EC50		ITX IMBL	A 18000 ug/L		13142	View Details

http://cfpub.epa.gov/ecotox/report.cfm?type=short

Back to Report

***** AQUATIC TEST #: 401386 *****

CHEMICAL

	GRADE	PURITY	FORM.	RADIOLABEL	CAS #
TEST	**NR**	**NR**	**NR**	**NR**	**71432**

NAME: **Benzene**
COMMENT: **NR**

TEST CONDITIONS

STUDY TYPE:	**NR**
MEDIA:	**FW**
LOCATION:	**NR**
CONTROL:	**NR**
EXPOSURE TYPE:	**NR**
APPLICATION FREQ.:	**NR NR**
EXPOSURE DUR.:	**24 hour(s)**
STAND DUR. (D):	**1 day(s)**

SPECIES

SPECIES # (NAME):	**5 Daphnia magna (Water Flea)**
AGE:	**NR NR**
LIFE STAGE:	**not reported, unknown**
COMMENT:	**NR**

PUBLICATION

REFERENCE #:	**707 Bringmann,G., and R. Kuehn, 1982**

TEST CONCENTRATION

CHEM ANAL. METHOD: **not reported**
UNIT OF MEASURE: **mg/L** miligram per liter

TYPE	VALUE	RANGE			STANDARD CONC (ug/L)	ION
F	**718**	**NR**	TO	**NR**	718000 ug/L	NR

EFFECT RESULTS

EFFECT:	**Behavior**
TREND:	**NR**
RESPONSE SITE:	**NR**
% EFFECT:	**NR**

EFFECT MEASUREMENT:
Equilibrium

ENDPOINT

ENDPOINT:	**Effective concentration to 0% of test organisms**
ENDPOINT ASSIGN.:	**P**
SIGNIFICANCE:	**NA**
LEVEL:	**NA**
BCF Value **(F)**:	**NR (NR to NR)**
BCF Value **()**:	

EE Comment:

Ecotoxicity Structure Activity Relationships

- ECOSAR: http://www.epa.gov/opptintr/newchems/tools/21ecosar.htm]
- Empirical relationships between structure and toxicity in different organisms.

PBT summary

- Persistence –somewhat tractable to to predict/identify chemical characteristics which are likely to increase/decrease persistence
- Bioaccumulation - Relatively reasonable models for bioaccumulation
- Toxicity – Empirical data driven – mechanistic understanding lacking – many sub-lethal endpoints are not assessed – e.g. endocrine disruption.