Ecotoxicology

Lecture 3

Biotic phase transfer, fate in natural environment and risk assessment

Bioconcentration

- Originally referred to the amount of chemical residue accumulated by an organism by absorption (i.e., through the integument) or other routes of entry (including food ingestion)
- Results in increased concentration of a contaminant relative to the environmental matrix

Bioaccumulation

- Uptake of contaminants via bioconcentration as well as by food ingestion
- Most appropriately used if cannot distinguish between the two mechanisms of uptake

Expression of Bioconcentration & Bioaccumulation Potential

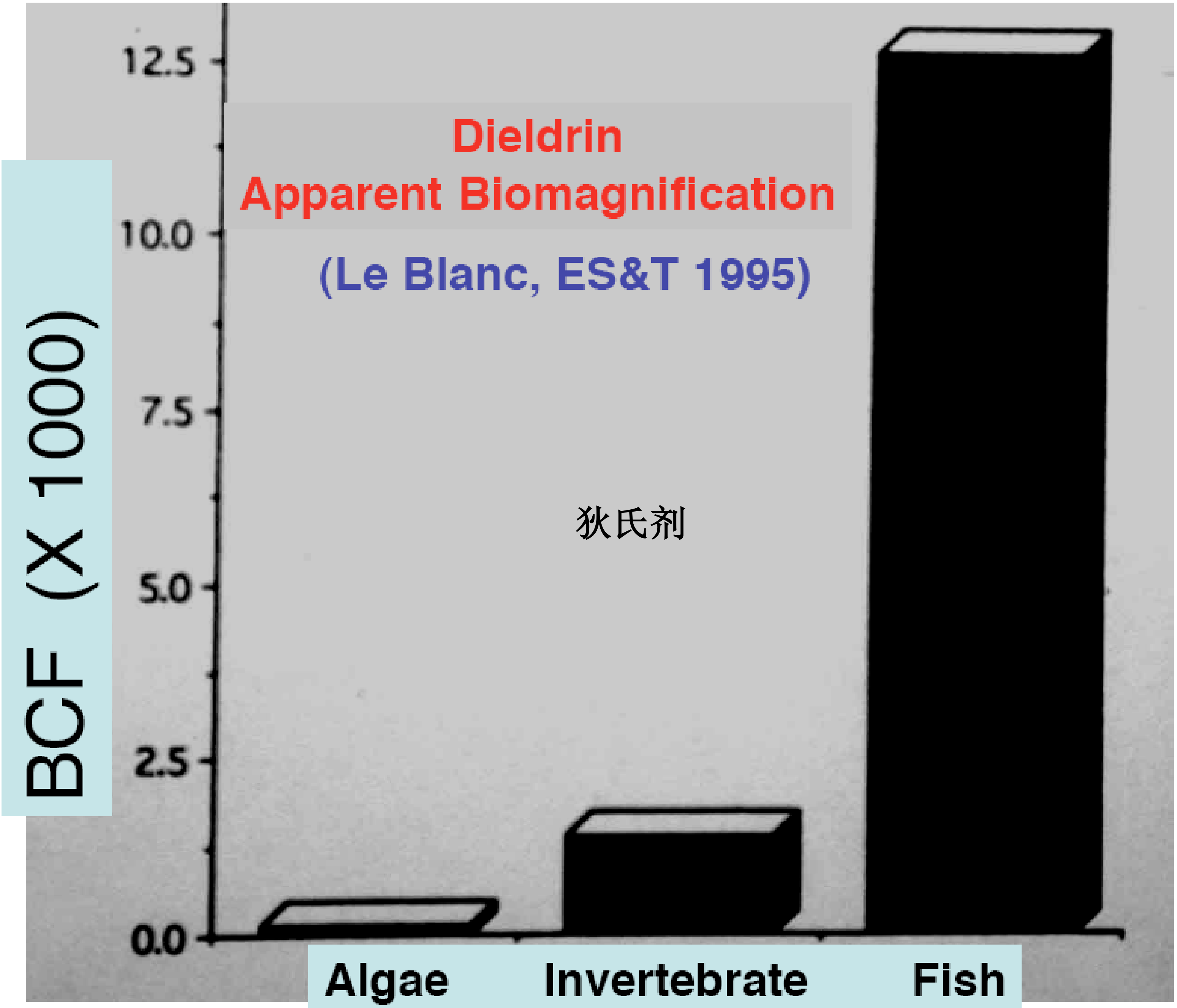
- Ratio of concentration of contaminant in the tissue (or whole body or biochemical compartment, like lipids) relative to the concentration of contaminant in environmental phase (or matrix)

$$\text{BCF or BAF} = \frac{[C]_{organism}}{[C]_{phase}}$$

Dazed & Confused

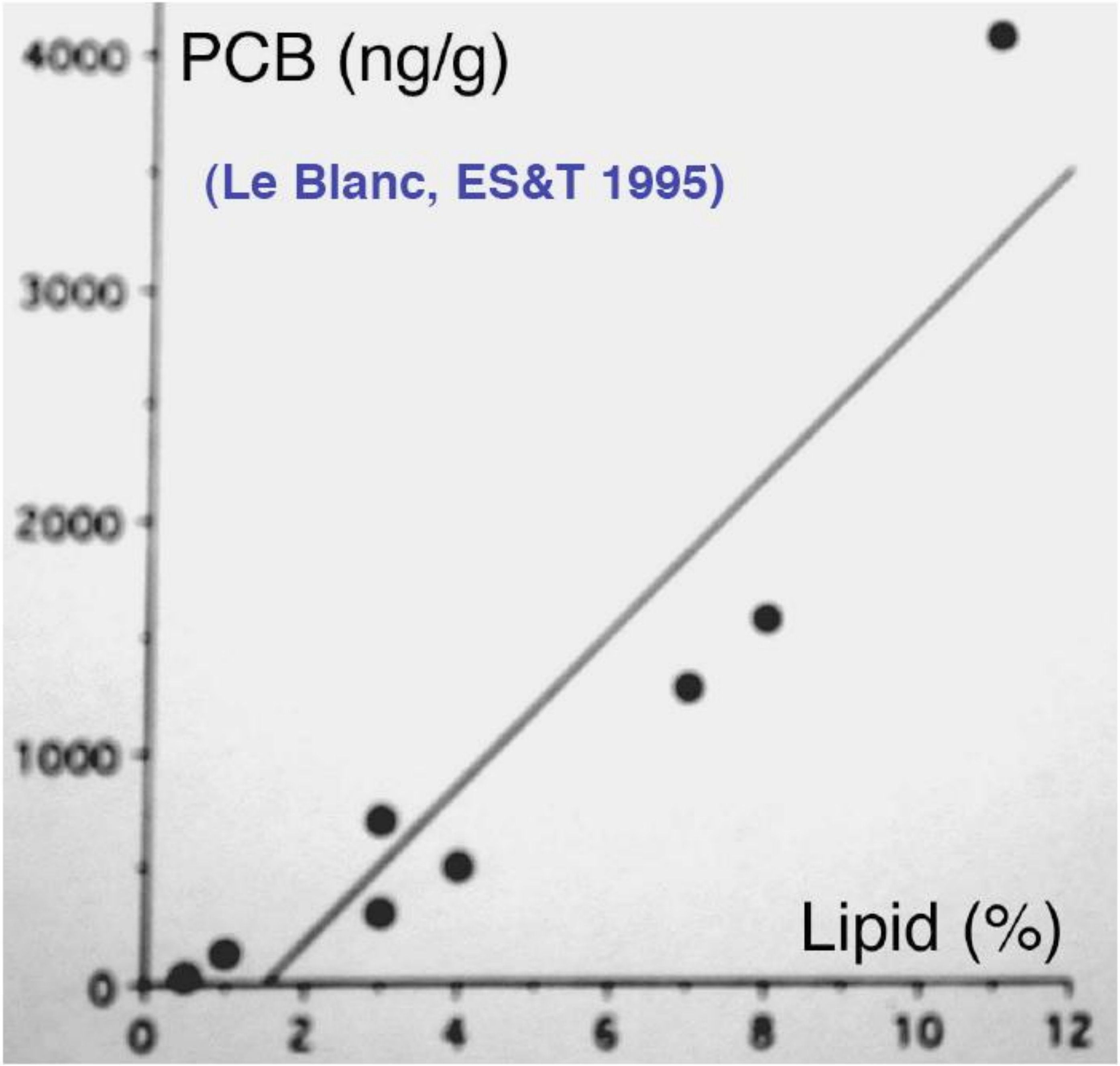
易混淆点

- Probably as a result of early work with DDT, compounded by the myths surrounding Carson's Silent Spring, the term biomagnification seems commonly thought to be occurring
- Biomagnification should be used only when contaminants have bioaccumulated and experiments have proven increasingly higher concentrations in tissues as trophic level increases
  - i.e., food chain magnification


Dieldrin
Apparent Biomagnification
(Le Blanc, ES&T 1995)
狄氏剂
BCF (X 1000)
12.5
10.0
7.5
5.0
2.5
0.0
Algae
Invertebrate
Fish

Biomagnification De-Mythified

- Experiments show that actually few substances actually biomagnify
- The so-called food chain effect has a low probability of occurrence, and differences in lipid content among organisms at different trophic levels can account for differences in BCF or BAF


PCB (ng/g)
(Le Blanc, ES&T 1995)
Lipid (%)
4000
3000
2000
1000
0
0
2
4
6
8
10
12

Measuring the BCF

- BCF (or BAF) should only be determined when the ‘system’ is at equilibrium (or for field measurements, at least in an empirical steady state)

Mechanistic Considerations That Influence Bioconcentration Potential

- Waxy layers on invertebrate cuticle & plant leaves
  表皮
- Mucilagnious layers on plant roots
  (植物的)黏液
- Lipid bilayer of cell membranes
- Possible movement along junctions between cells into interstitial spaces
  空隙的

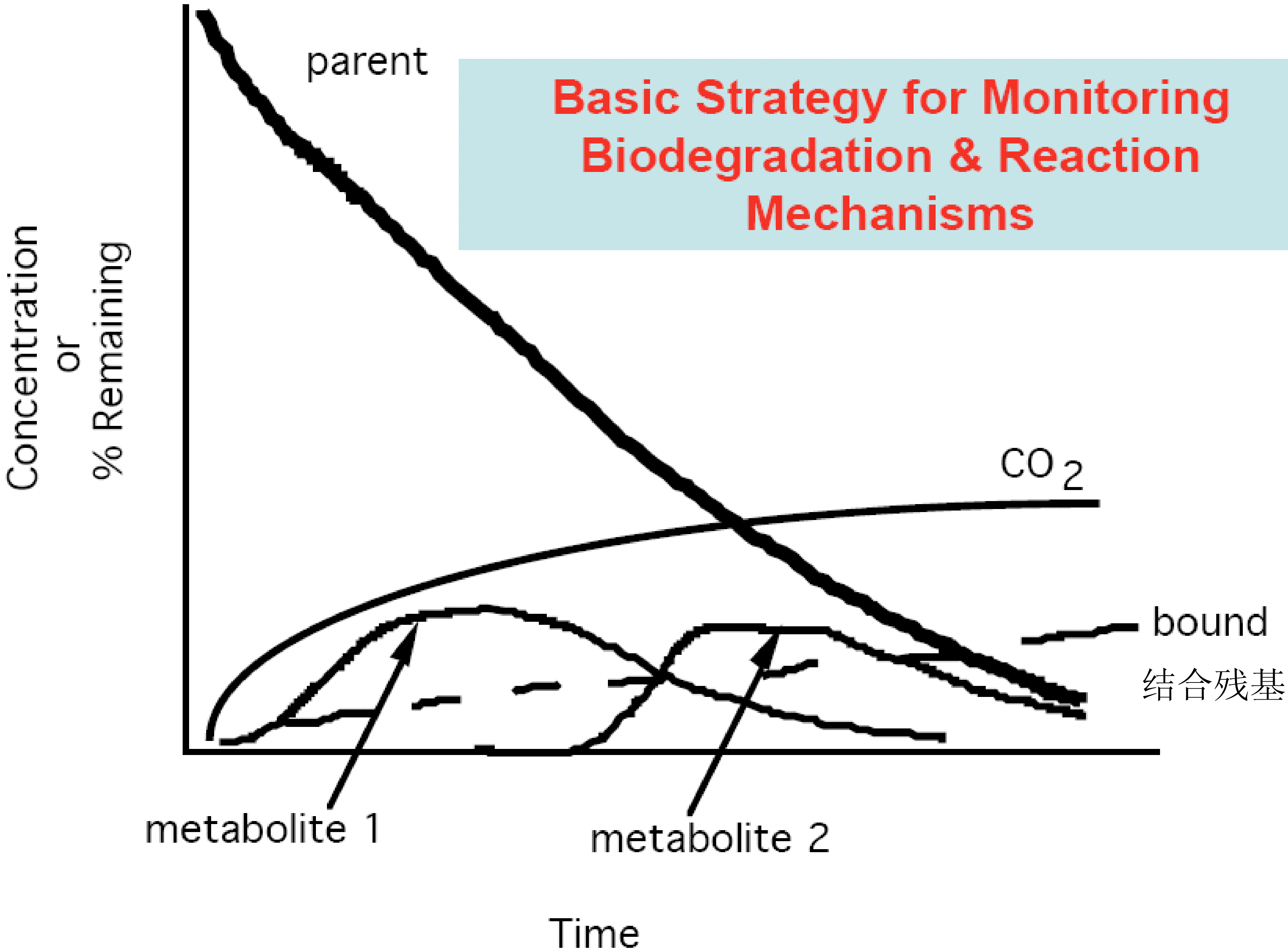
Definitions

- Degradation
  - Decrease in concentration of a contaminant due to nonreversible alteration of chemical structure
- Mineralization
  - Biologically mediated degradation of chemical resulting in release of carbon dioxide
- Persistence
  - Longevity of a contaminant residue in a medium or phase
- Detoxification
  - Degradation resulting in loss of toxicity or biological activity

Reaction Mechanisms

- The processes by which a chemical is degraded
- Divided into two basic mechanisms
  - Phase I (biologically or nonbiologically mediated)
    - ✓ Hydrolysis 水解
    - ✓ Oxidation
    - ✓ Reduction
  - Phase II (biologically mediated)
    - ✓ Conjugation 配合

Basic Strategy for Monitoring Biodegradation & Reaction Mechanisms



Reaction Kinetics

Rate Law=a mathematical function or differential equation describing the turnover rate of a compound as a function of the concentration

Power Rate Law 幂

$$Rate = \frac{-dC}{dT} = kC^n$$

First Order when n = 1 一级反应动力学

$$\frac{d[C]_t}{dt} = -k[C]_0$$

Differential eq.

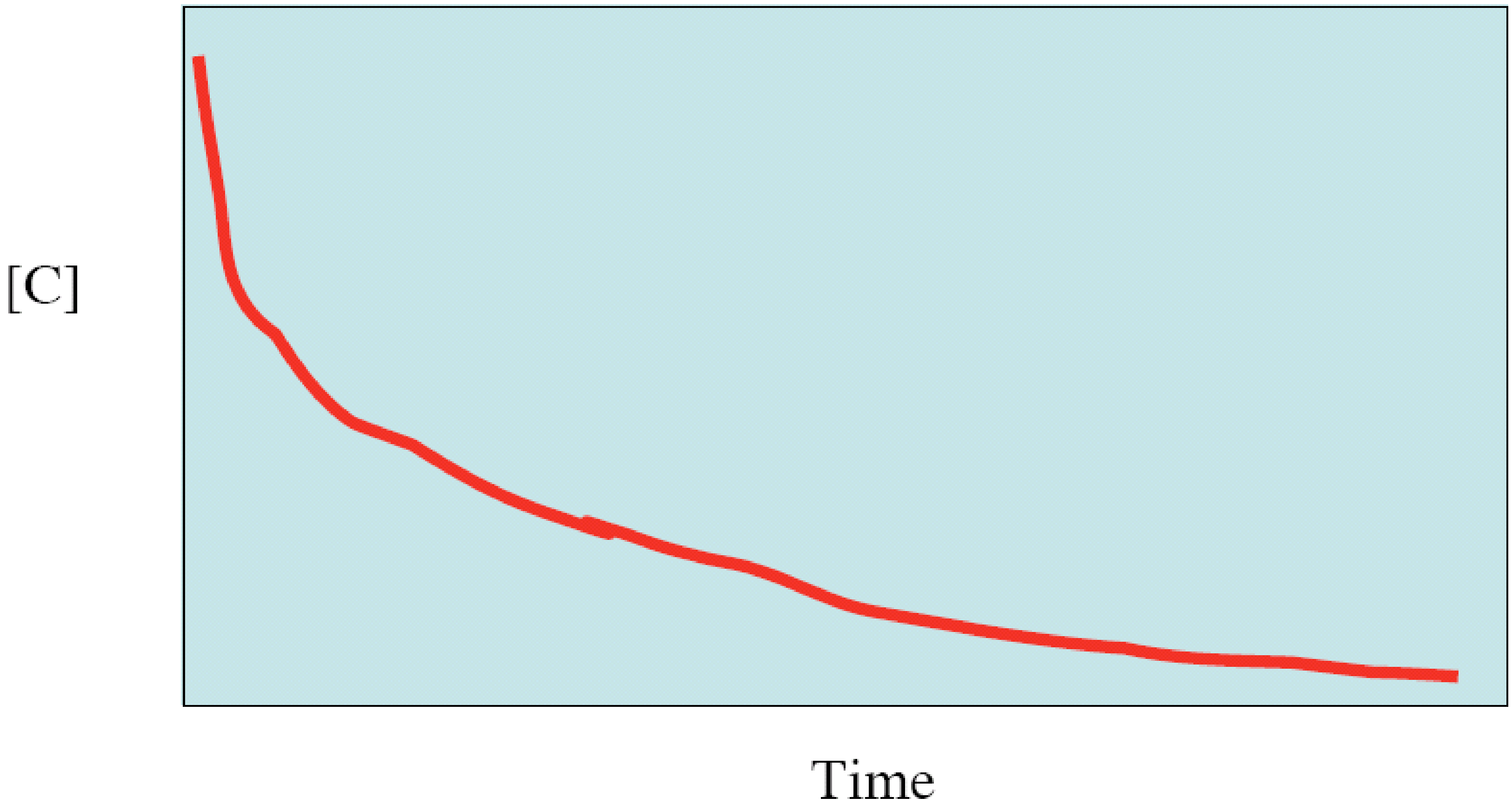
or

LnCt=LnC0-KT

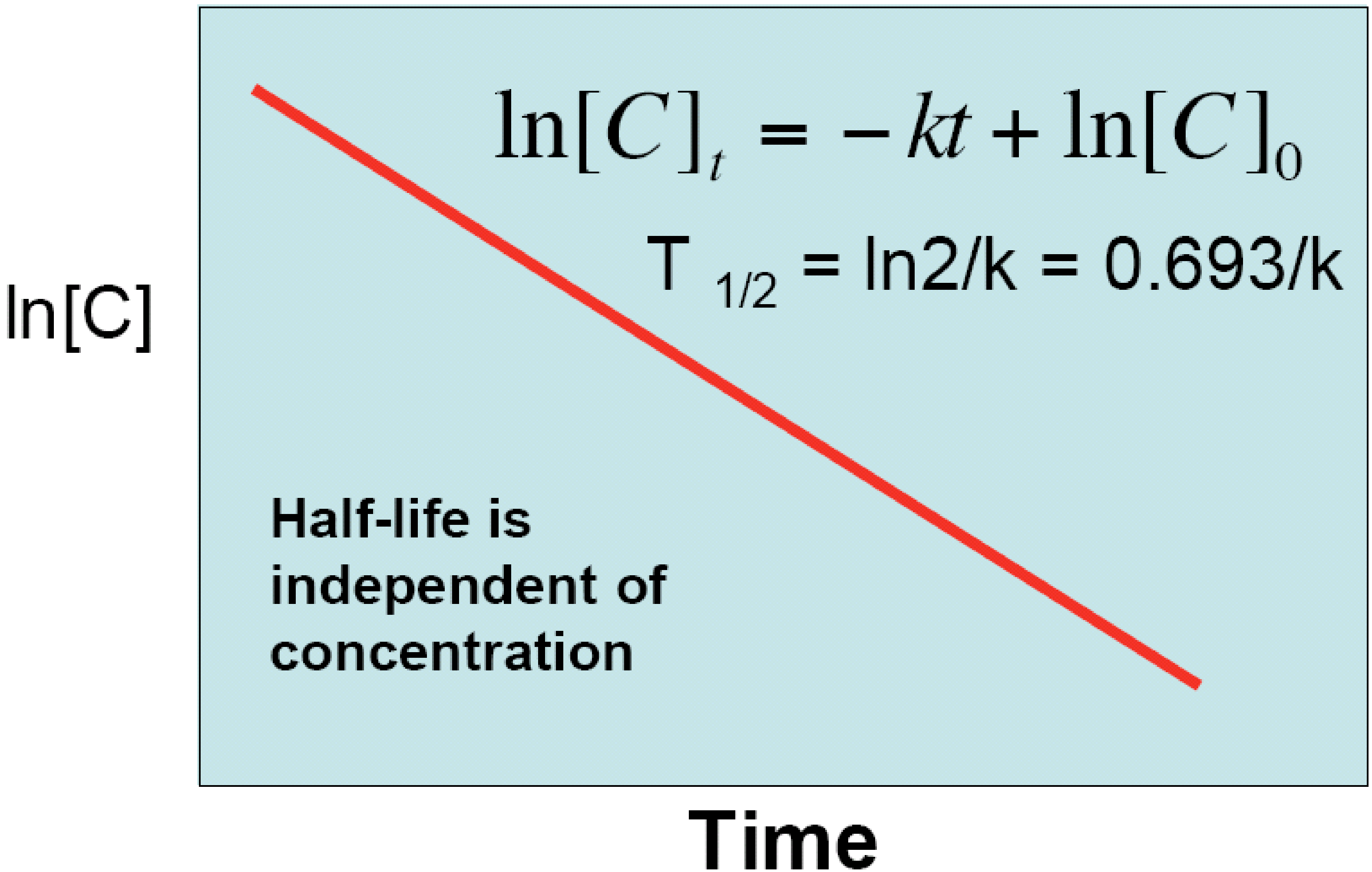
$$[C]_t = [C]_0 \bullet e^{-kt}$$

Integrated eq.

First Order Characterized by Exponential Decrease in Concentration Over Time

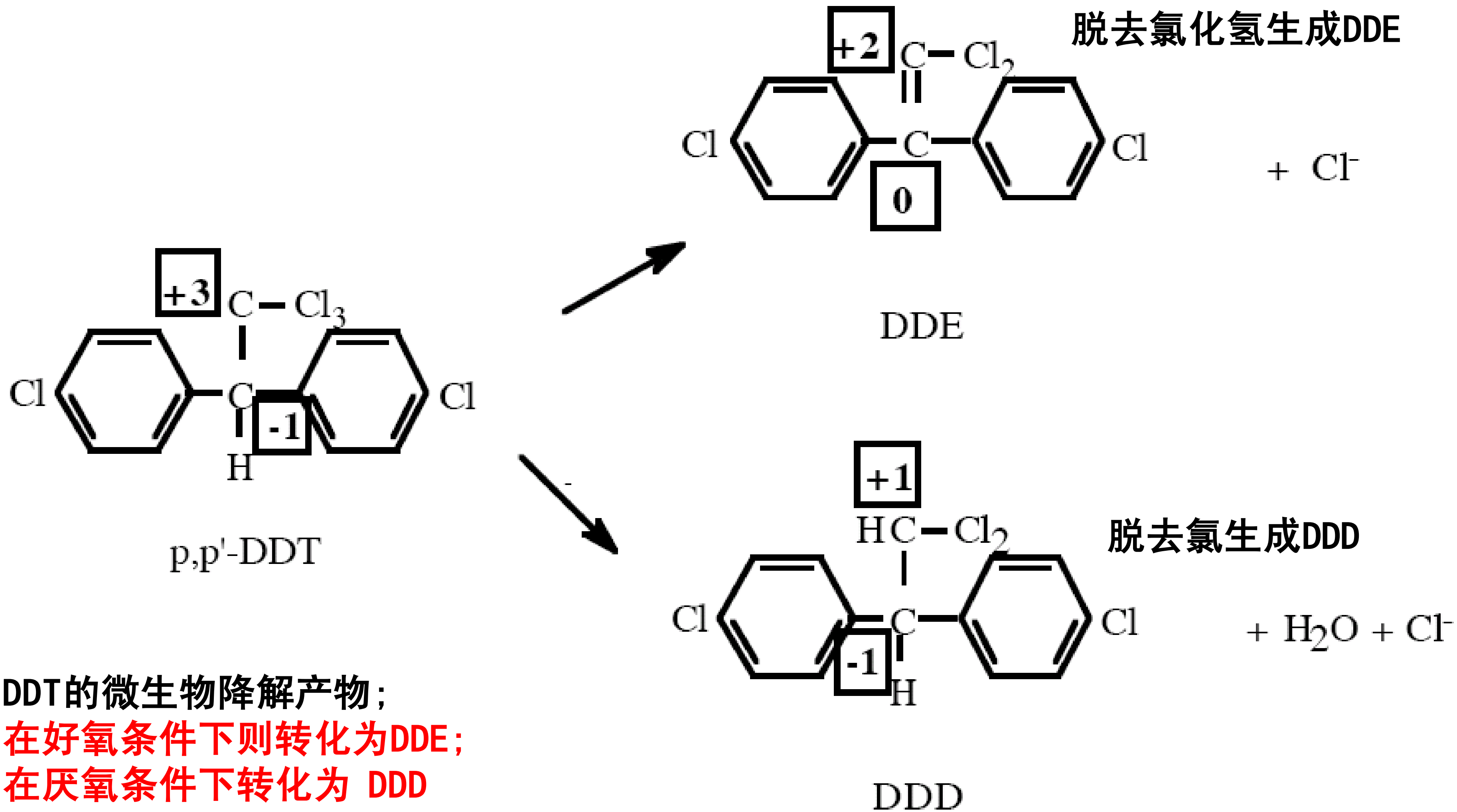


Linearization of First-Order Function



Reductions

- Transfer of electrons to acceptor molecule (REDOX rxs.)



DDT的微生物降解产物；

在好氧条件下则转化为DDE；

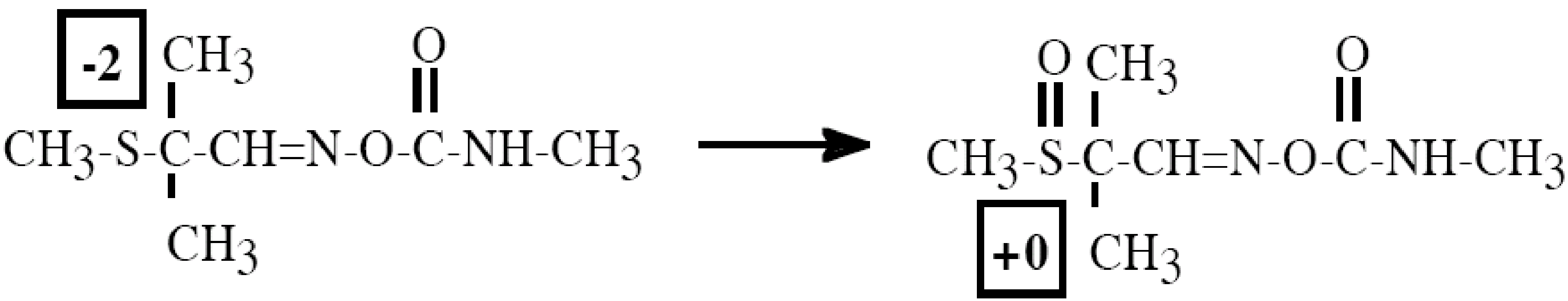
在厌氧条件下转化为 DDD

DDT degradation

- DDT也可被还原脱氯而生成DDD，DDD不如DDT或DDE稳定，而且是动物和环境中降解途径的第一步。
- DDD脱去氯化氢，生成DDMU[化学名称：2，2-双-(对氯苯基)-1-氯乙烯]，
- 再还原成DDMS[化学名称：2，2-双-(对氯苯基)-1-氯乙烷]，
- 再脱去氯化氢而生成DDNU[化学名称：2，2-双-(对氯苯基)-乙烷]，
- 最终氧化DDA[化学名称：双-(对氯苯基)乙酸]。此化合物在水中溶解度比DDT大，而且是高等动物和人体摄入及贮存的DDT的最终排泄产物。

Oxidations

- Removal of electrons from carbon or heteroatom 杂环原子



aldicarb (Temik)

aldicarb sulfoxide 亚砜

碳醛：一种晶体化合物，$C_7H_{14}N_2O_2S$，
作为如棉花、土豆和甜菜等作物的除虫剂而用于农业中

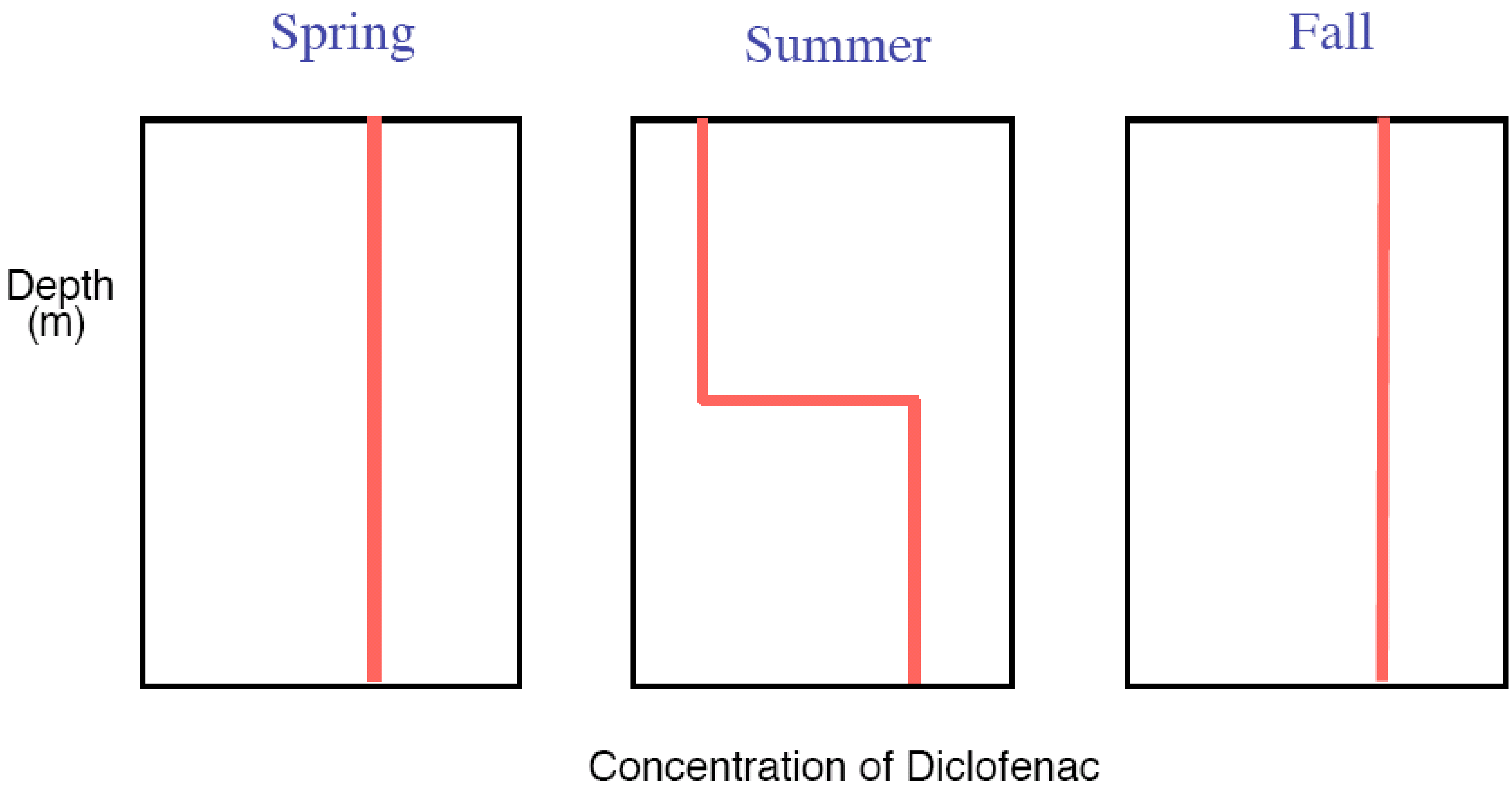
Photolysis 光解作用

Bond	Bond Energy ($kJ\ mol^{-1}$)	Wavelength (nm)
O-H	465	257
H-H	436	274
C-H	415	288
N-H	390	307
C-O	360	332
C-C	348	344
C-Cl	339	353
Cl-Cl	243	492
Br-Br	193	630
O-O	146	820

Whether a reactions will take place depends on the probability that a given compound absorbs a specific wavelength of light or on the probability that the excited molecular species undergoes a particular reaction.

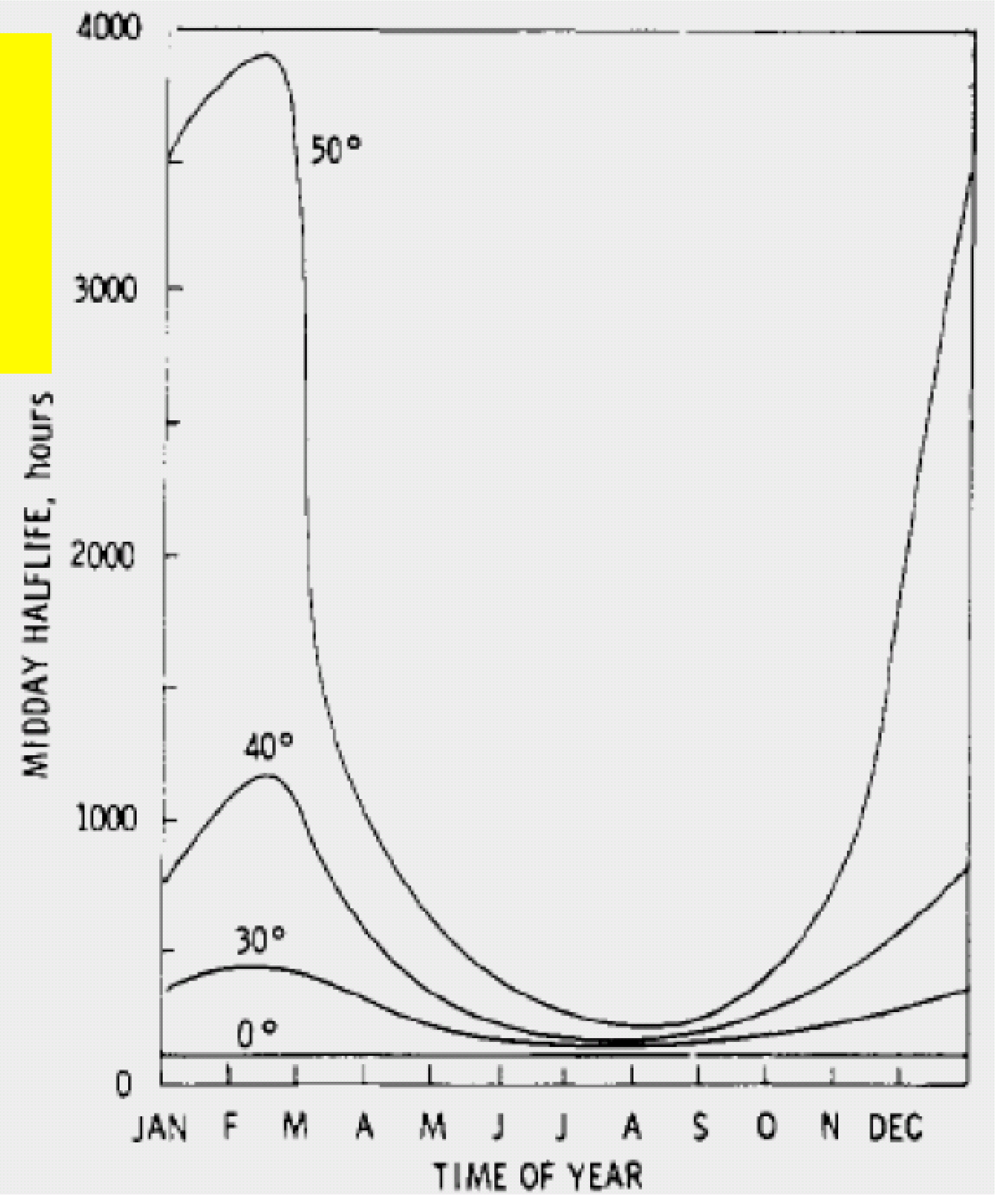
Distribution of Diclofenac, a pharmaceutical, in a lake

二氯苯胺苯乙酸钠,Diclofenac,解热镇痛抗炎药



Thermal Influence on Potential for Photoloysis:
Lake Turnover & Stratification

Dependence of 2,4-D Butoxyethyl Ester Photolysis Half-Life on Season & Northern Latitude



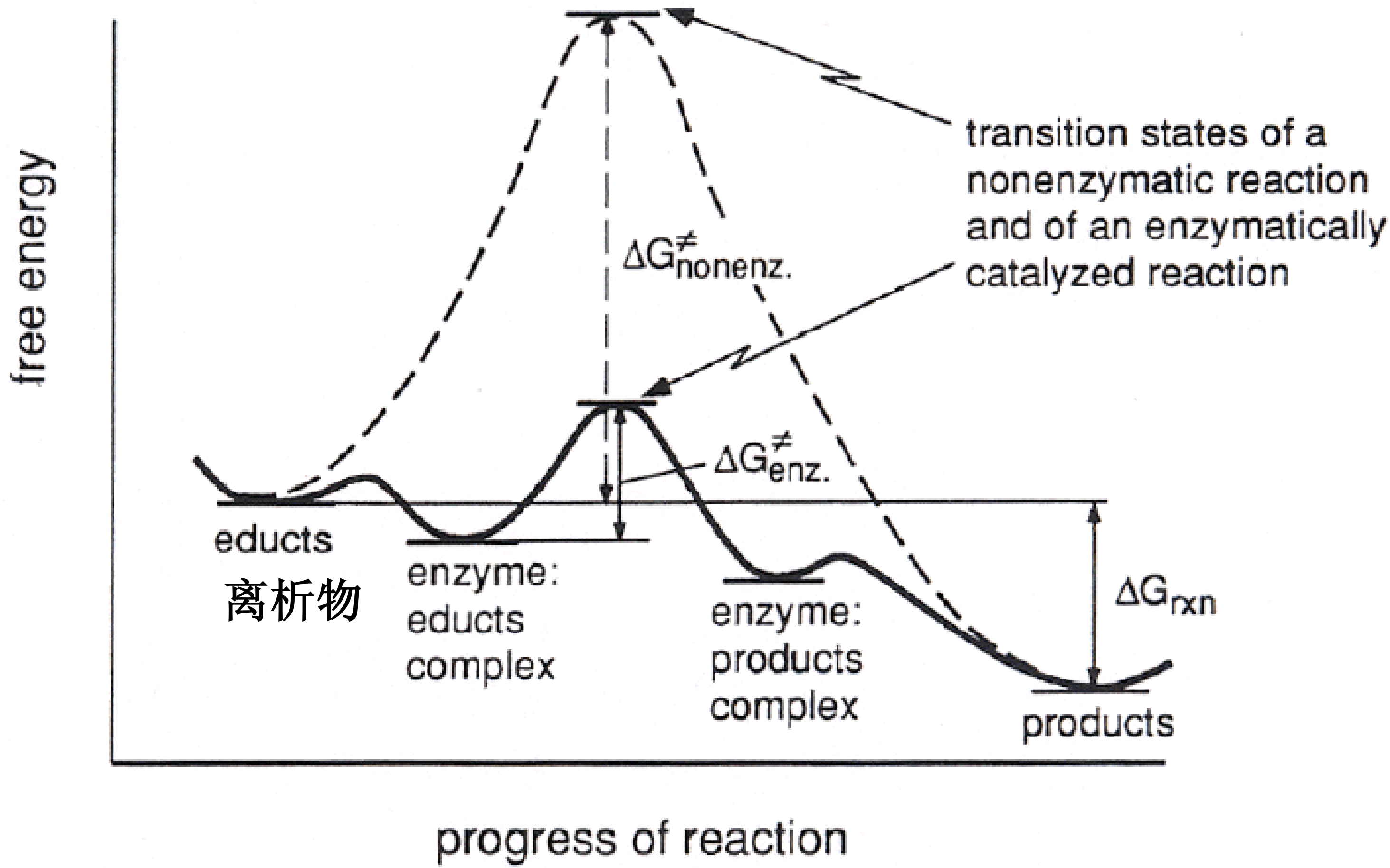
Copied from Zepp et al. 1975

Biochemical Ecology of Biodegradation

- End products represent
  - Mineralizations
  - Transformations
- Biochemical reactions involve catalysis by enzymes

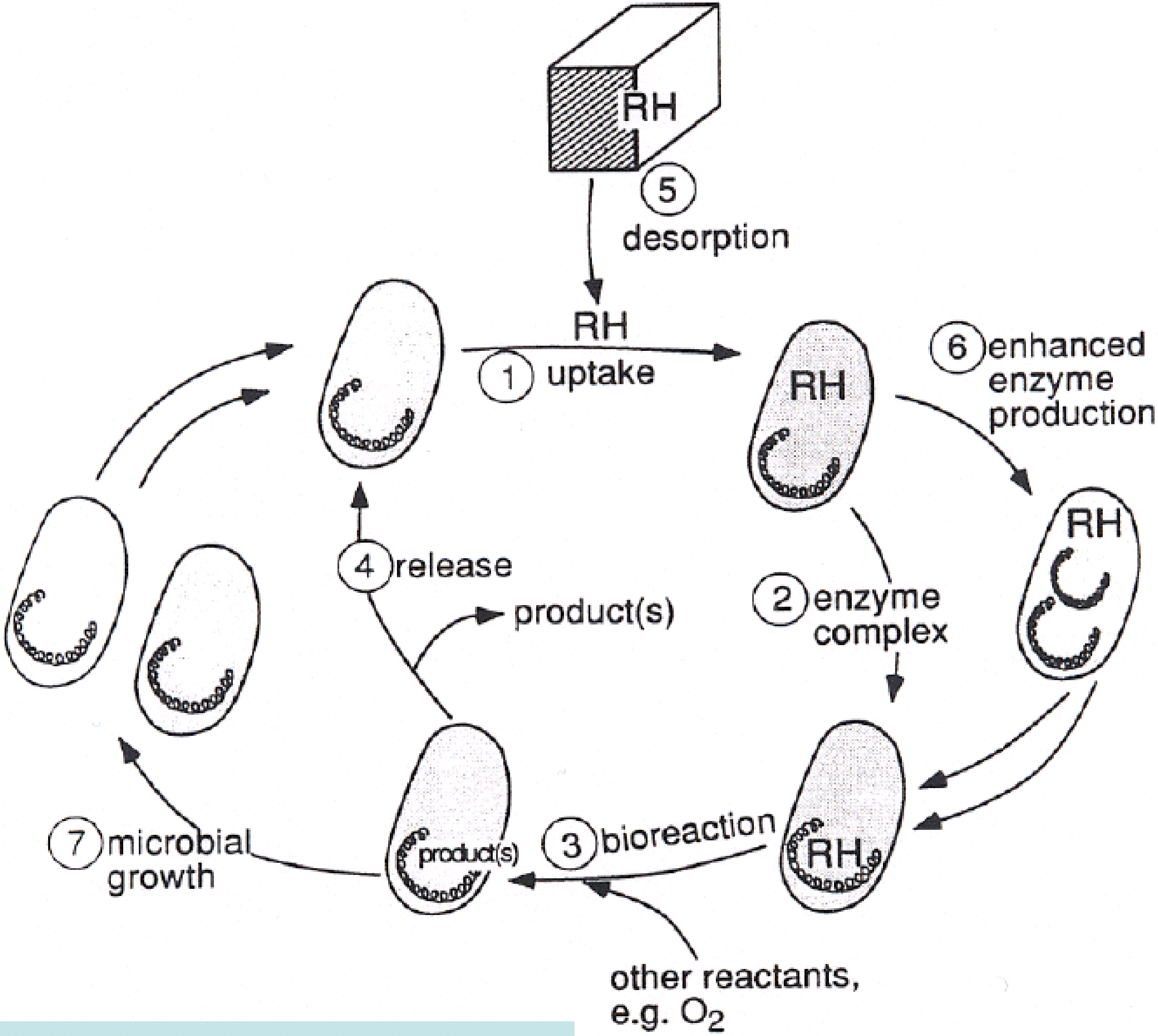
Biodegradation

- Catalysis by enzymes



(Copied from Schwarzenbach et al. 1993)

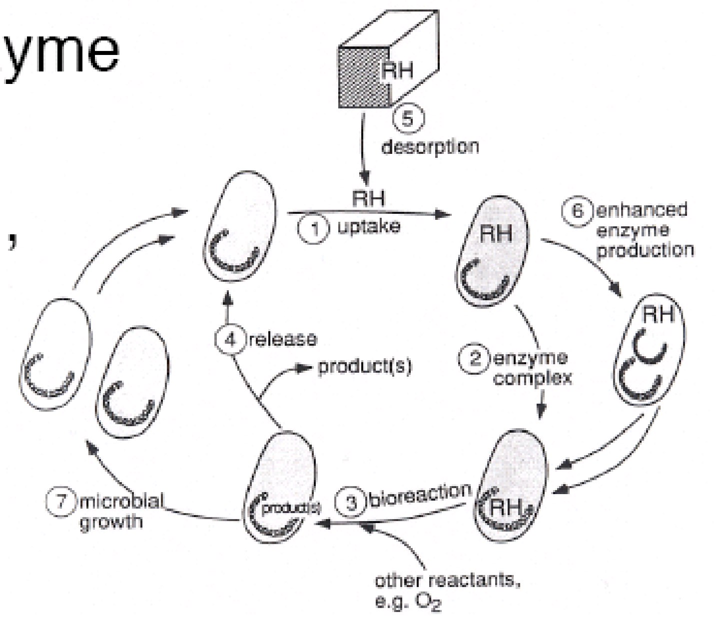
Conceptualization of Biodegradation



(Copied from Schwarzenbach et al. 1993)

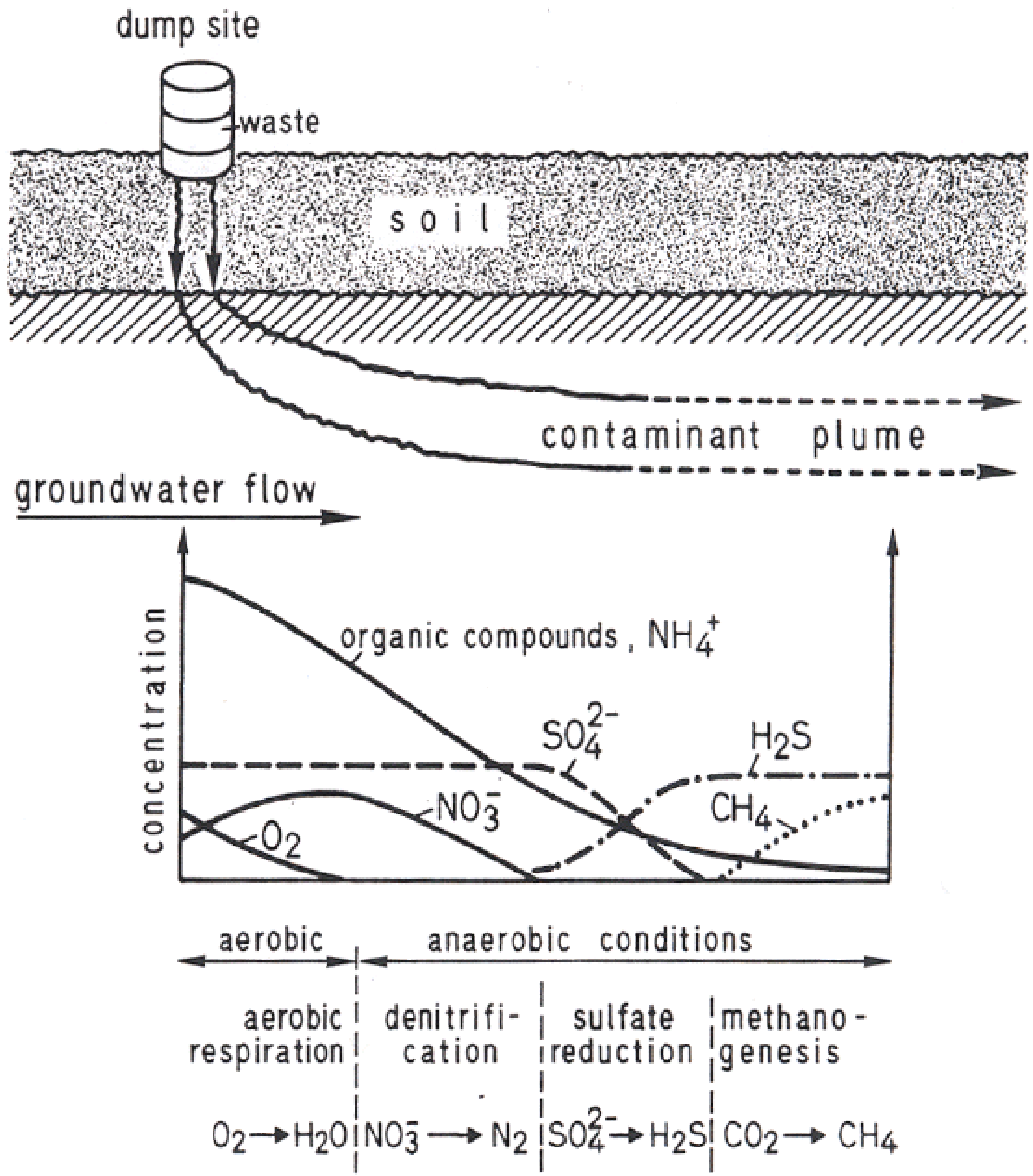
Conceptualization of Biodegradation

1. Bacterial cell containing enzymes takes up chemical
2. Chemical binds to suitable enzyme
3. Enzyme-chemical complex reacts, producing transformation products
4. Products released from enzyme
5. Sorption in soil may influence processes above
6. Production of new or additional enzyme capacity (induction, activation)
7. Growth of total microbial population, and thus biodegradation capacity



Anaerobic Biodegradation

- Alternative electron acceptors (ie., alternative to O_2)
  - Methanogenesis (CO_2; methane)
  - Sulfate Reduction (SO_4; hydrogen sulfate)
  - Denitrification (Nitrate; N_2)

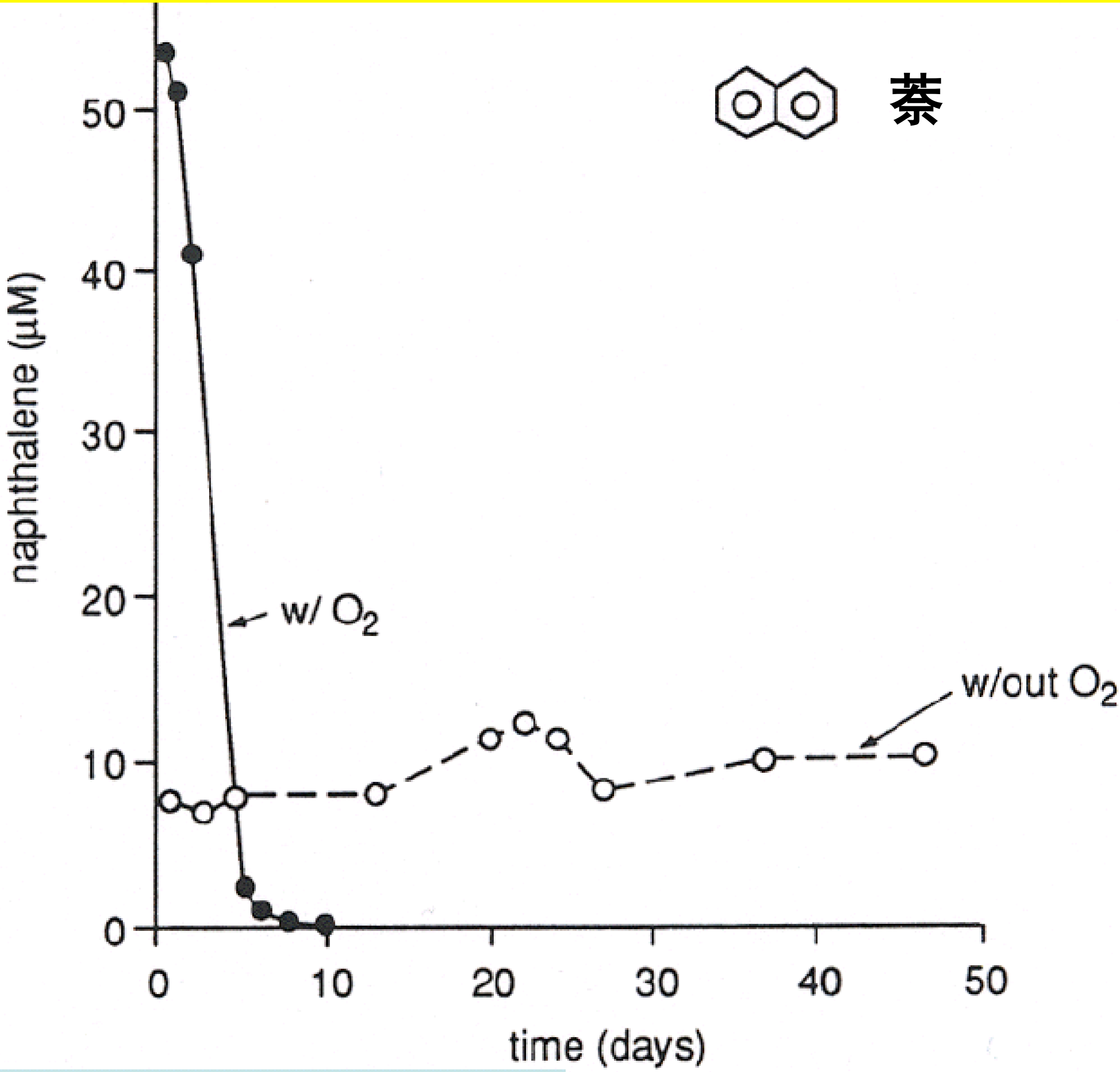


(Copied from Schwarzenbach et al. 1993)

Factors Influencing Degradation

- Concentration of chemical
- Temperature
- Moisture
- Sunlight
- Soil type and characteristics (texture, pH, OC)
- Nutrients
- Product formulation ingredients
- Other chemicals and previous exposures
- Aging of residues

Effect of Oxygen Concentration on Naphthalene Biodegradation



(Copied from Schwarzenbach et al. 1993)

Toxicity Testing
Dose-response curve

and

Environmental Risk Assessment

Dimensions of the Toxic Chemical Problem

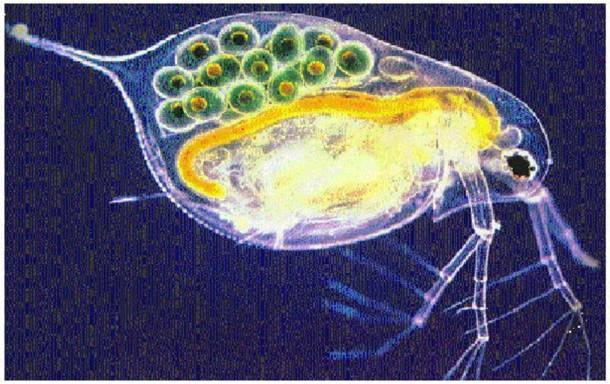
• Chemical entities		4-10 million
• Developed annually		~6000
• In commerce		~65,000
• In common use		~6,000
• Regulated	water	129
	air	25

Toxicology – Historical Perspective

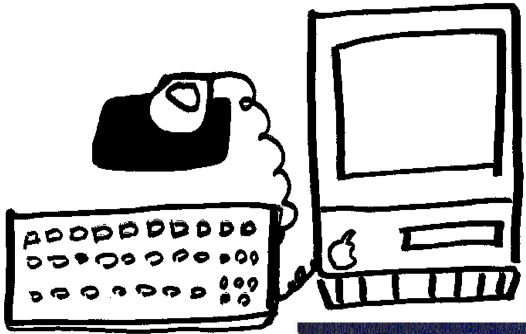
- Human (Mammalian) toxicology

- White Rat

- Water Flea



- Computer simulation



Toxicity testing

Simultaneous chemical detection and biological effects

Acute toxicity test

- **Short time frame exposure (96h)**
- **“kill ‘em and count ‘em”**

Chronic toxicity test

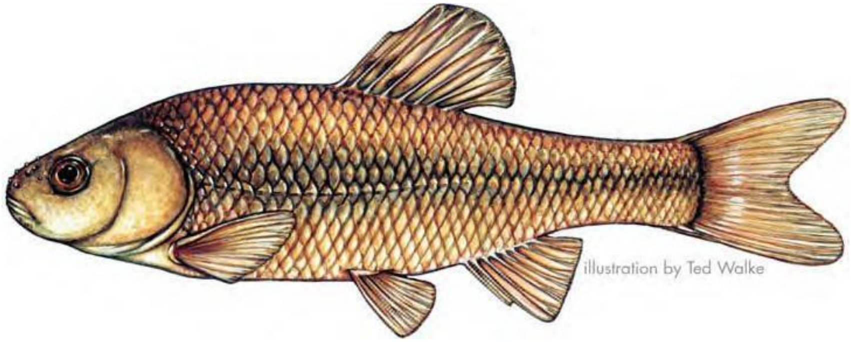
- **Longer time frame exposure (1 week to 3 years)**
- **reproduction, physiology, behavior, biochemistry**
- **More ecologically relevant**

Chronic toxicity testing

Reproduction

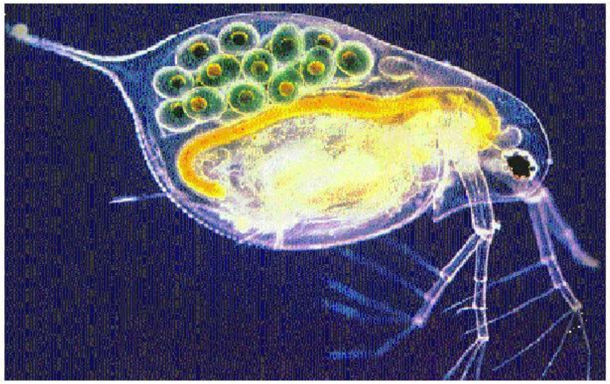
Fish – life cycle at least 3 to 6 months

(fathead minnow)



Invertebrates – complete life cycle in 3 days

(water flea - *Ceriodaphnia dubia*)



Toxicity Test Methods

Test variable	Acute Fish, Macinvert, Amphib.
Organism(s)	Various
Age of test organisms	early life stage, uniform size
Experimental design	
No. per chamber	varies, no overcrowding
Vessel type/size	3x largest length and height
No. of replicates	3 minimum
Feeding regime	Daily to support normal funct.
Test duration	96 h (48 h for daphnids/midges)
Physical/chemical variables	
Temperature	varies by organism (12-25 C)
Light	16 h L/8 h D, 15-30 min trans
DO	60-100%
Endpoint	Death, immobilization

Endpoints

Toxicology

- Survival
- Growth
- Reproduction
- Behavior (avoidance)

Ecology

- Abundance
- Diversity
- Biomass
- Processing rate

Mechanism of Toxicity
Targets and Effects

- Cell membranes
- Enzymes
- Lipids
- Protein synthesis
- Microsomes（微粒体）
- Regulatory processes (hormones)
- Carbohydrate metabolism

What is the purpose of bioassays?

- Rank hazards
- Set discharge limits → regulate hazards
- Predict environmental consequences
- Protect important species
  - Reason why rainbow trout tested (commercially and recreationally important)
  - Reason why Zn, Cl standards based on toxicity to rainbow trout even if stream has none

Criteria for Selecting Test Organisms

- Broad range of sensitivities
- Widely available and abundant
- Indigenous or representative
- Recreationally, commercially, or ecologically important
- Laboratory tolerant
- Adequate background information

Ecotoxicological testing

Single species → Multi-species → Mesocosm

LOEC = lowest observable test concentration

The lowest test concentration that is significantly different from control

NOEC = no observable effect concentration

The highest test concentration that is *not* significantly different from control

MATC = geometric mean of NOEC and LOEC

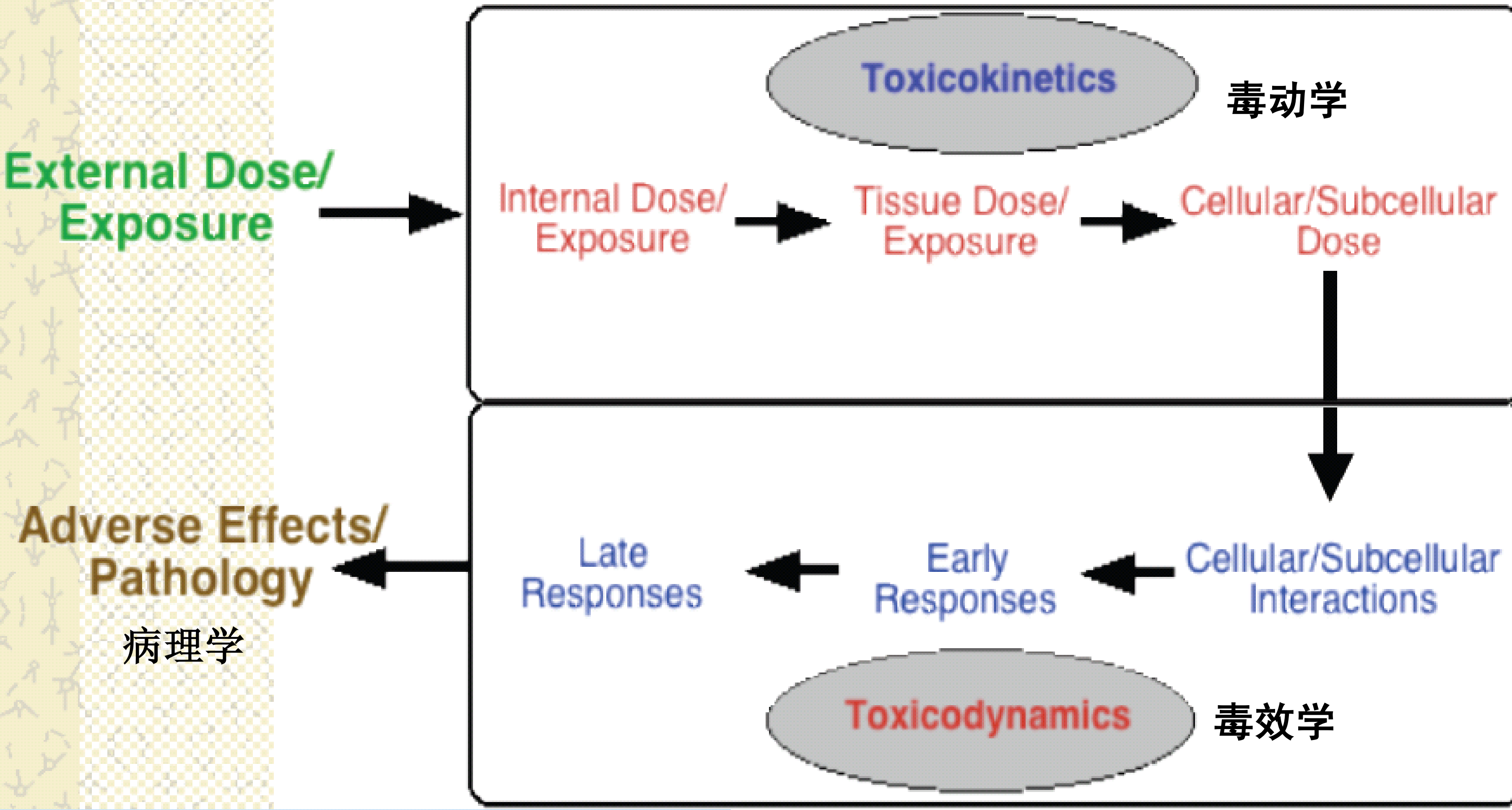
- Often referred to as the chronic value

- MATC = $\sqrt{NOEC * LOEC}$

Example problem

Dose	% alive
0 mg/L (control)	100
1	100
3	90
10	30
30	20
100	0

Conceptual Model of Relationship

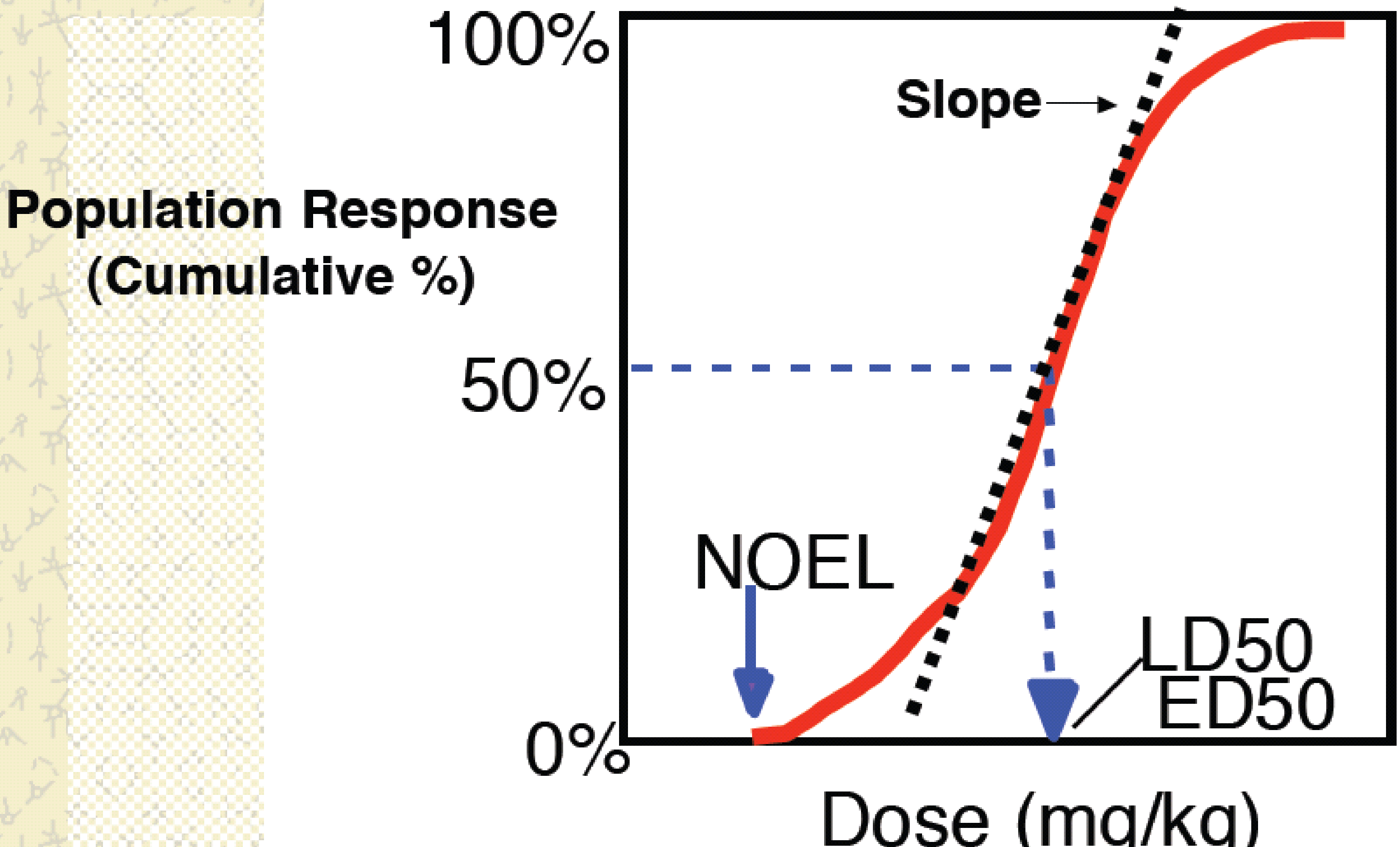


Redrawn from Heinrich-Hirsch et al. 2001

Measuring Toxicity

- Determination of relationship between dose or concentration of substance and response of test organism
- Must determine toxicological endpoint
  - Death
  - Development/Reproductive Effects
  - Weight Loss
  - Neurological function
  - Endocrine function
  - Enzyme inhibition

Cumulative Proportion Responding



Risk Management Devices

- "Acceptable" Margins of Exposure (MOE)
  - Hedging your bets with safety factors
  - "Codified" as numerical standards
    - MCLs (Maximum Contaminant Levels)
    - Ambient Water Quality Criteria for Protection of Aquatic Organisms
    - Reference Doses (RfDs)
    - Population Adjusted Doses (PADs)
    - Levels of Concern (LOCs)

An “Acceptable” Margin of Exposure (MOE)

Pacific Oyster LC50

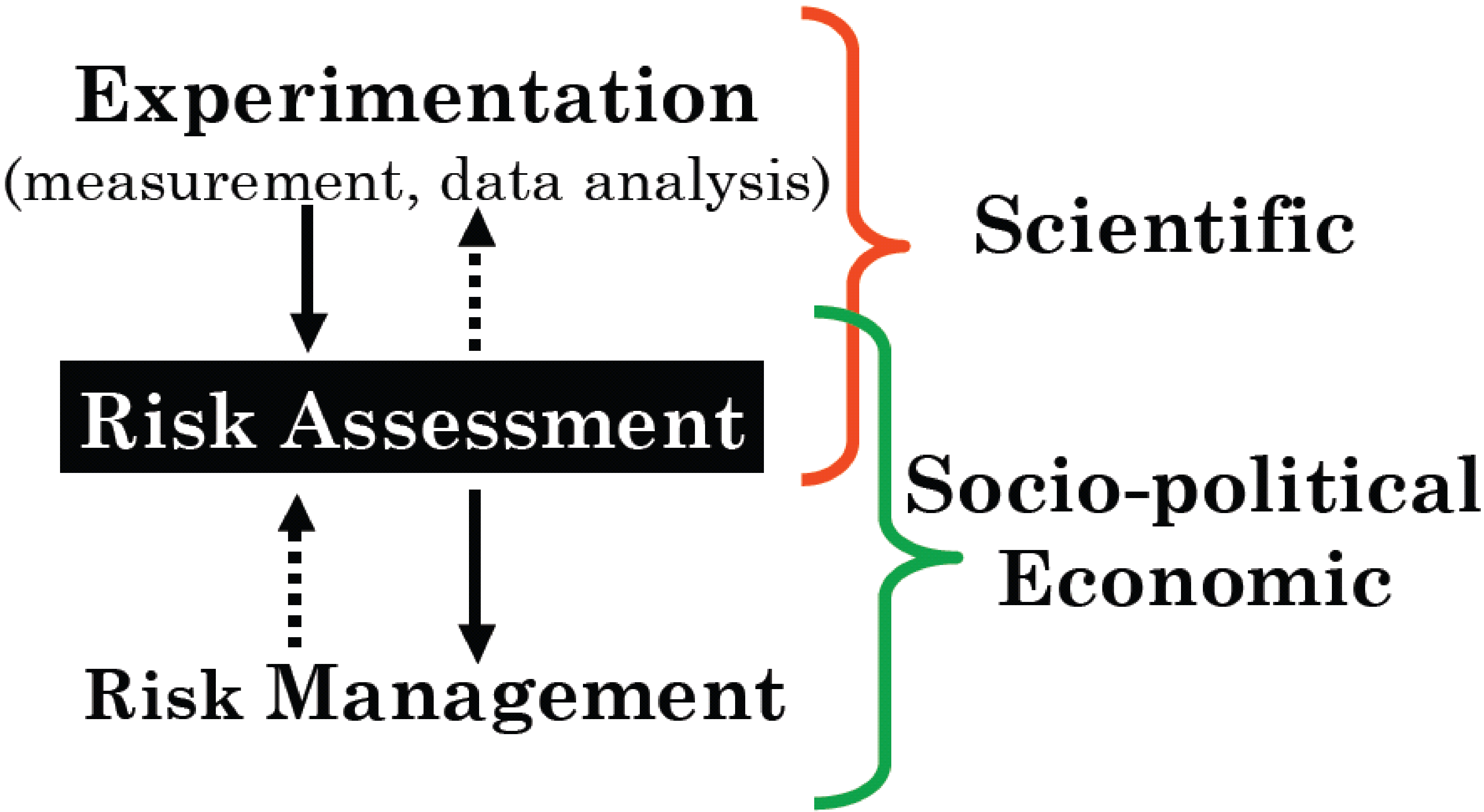
Glyphosate Concentration Lethal to
草甘膦50% of Exposed Oysters

3,988 μg/g (ppm), sediment

If MOE = 100

Glyphosate in mudflats
Must be < 40 ppm

Don't Be Confused By the Process-- Know Where You Are



Dose-Response Assessment

- After the hazard is identified,
  - i.e., the toxicological endpoint of concern is chosen
- Then, the next step is to determine how the magnitude of the response varies with increasing concentrations or doses.
- Generation of NOAEL's for most sensitive toxicological hazard
  - Relevant for threshold responses (anything but genotoxic or tumorigenic effects)
- Generation of slope factor for tumorigenic responses in chronic assays (2-yr rodent assays)

Risk Characterization

- Part Science
  - Divide the dose **observed** to cause no effect by the exposure level
  - State the ratio (the MOE)
    - MOE = NOAEL (mg/kg/day) ÷ exposure (mg/kg/day)
- Part Risk Management
  - Divide the estimated level of exposure by the dose **believed** to be “safe” (Exposure/RfD)
  - Determine if the ratio is **acceptable or not**

Risk Characterization
MOE vs. RfD

Margin of Exposure (MOE) =

$$\frac{\text{NOAEL (mg/kg/day)}}{\text{Exposure (mg/kg/day)}} \geq 100 \text{ (EPA not concerned)}$$

$$\text{Reference Dose (RfD)} = \frac{\text{NOAEL}}{100}$$

Risk =(Exposure/RfD) x 100

if < 100, EPA not concerned

Child Sensitivity Is Considered

- If fetal and newborn rats are more sensitive at a given dose than adult rats, then up to an extra 10-fold safety factor may be applied to the RfD
- The RfD divided by this FQPA Safety Factor is called the
  - Population Adjusted Dose (PAD)

$$\frac{\text{NOEL}}{100} = \text{Reference Dose (RfD)}$$

$$\frac{\text{RfD}}{10} = \text{Population Adjusted Dose (PAD)}$$

Thank you for your attentions!