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Principles of Toxicology

The Study of Poisons

WATER BIOLOGY
PHC 6937; Section 4858

Andrew S. Kane, Ph.D.
Environmental Health Program
College of Public Health & Health Professions
KANE@UFL.EDU

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"The problem with toxicology is not the practicing toxicologists, but chemists who can detect, precisely, toxicologically insignificant amounts of chemicals"

Rene Truhaut, University of Paris (1909-1994)


THE SCIENTIFIC COMMUNITY IS DIVIDED.
SOME SAY THIS STUFF IS DANGEROUS, SOME SAY IT ISN'T.

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Sources of Environmental Chemicals

Air Emissions
- Industrial Processes
- Incinerators
- Gasoline and diesel exhaust
- Spraying of agricultural chemicals

Water Discharges
- Industrial effluents
- Sewage effluent

Non-Point Sources
- Surface run-off from roads and agricultural land
- Leachate from dump-sites
- Accidental spills

Household Chemical Use

• Aquatic animals as environmental sentinels

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Toxicology……

- Is the study of the harmful effects of chemicals and physical agents on living organisms
- Examines adverse effects ranging from acute to long-term
- Is used to assess the probability of hazards caused by adverse effects
- Is used to predict effects on individuals, populations and ecosystems

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An interdisciplinary field…

Descriptive Toxicology: The science of toxicity testing to provide information for safety evaluation and regulatory requirements.

Mechanistic Toxicology: Identification and understanding cellular, biochemical & molecular basis by which chemicals exert toxic effects.

Regulatory Toxicology: Determination of risk based on descriptive and mechanistic studies, and developing safety regulations.

Federal agencies: FDA (FDCA- Federal Food, Drug & Cosmetic Act)

EPA (FIFRA-Federal Insecticide, Fungicide and Rodenticide Act)

EPA (TSCA-Toxic Substance Control Act)

EPA (CERCLA- Comprehensive Env Response, Compensation, & Liability Act); Superfund

DOL (OSHA-Occupational Safety and Health Administration)

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An interdisciplinary field…

Clinical Toxicology: Diagnosis and treatment of poisoning; evaluation of methods of detection and intoxication, mechanism of action in humans (human tox, pharmaceutical tox) and animals (veterinary tox). Integrates toxicology, clinical medicine, clinical biochemistry/pharmacology.

Occupational Toxicology: Combines occupational medicine and occupational hygeine.

Environmental Toxicology: Integrates toxicology with sub-disciplines such as ecology, wildlife and aquatic biology, environmental chemistry.

Approach

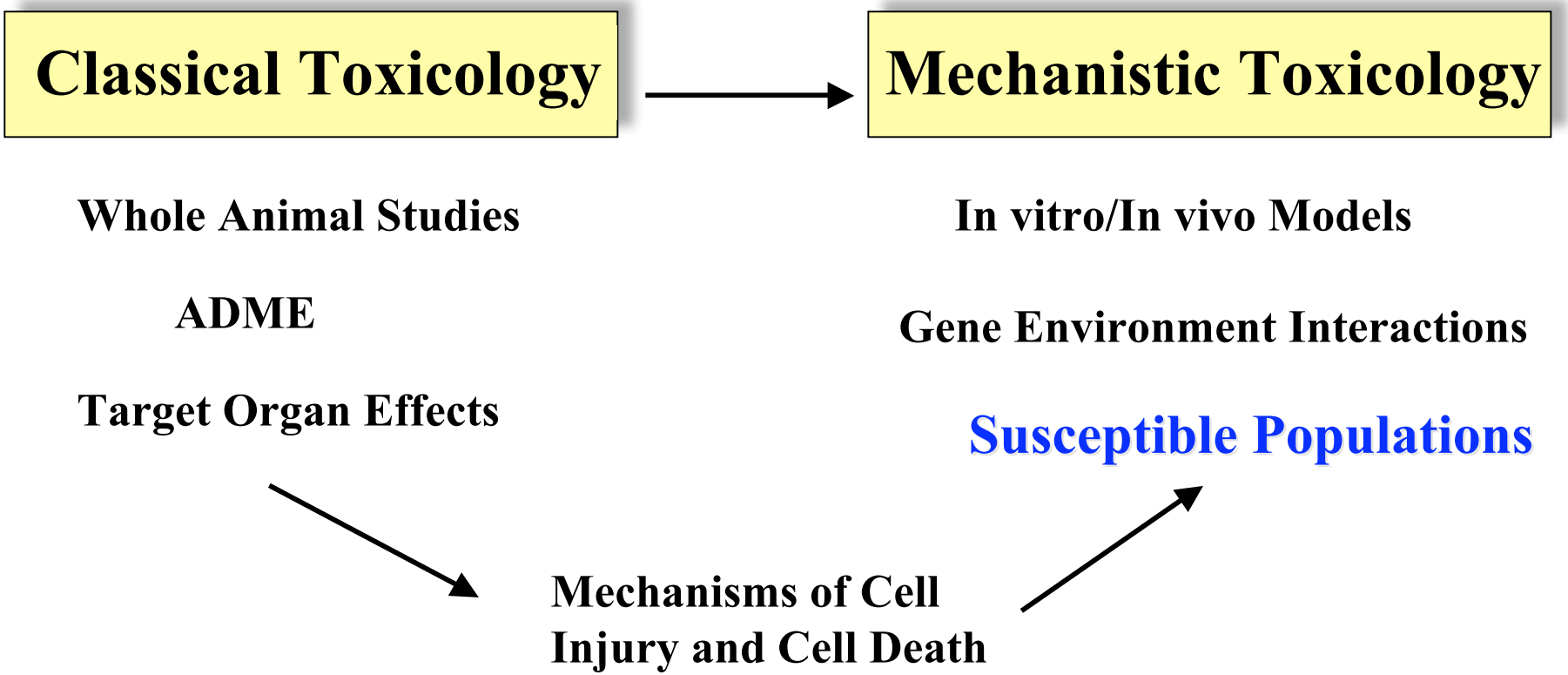
Classical Toxicology
Mechanistic Toxicology
Whole Animal Studies
ADME
Target Organ Effects
In vitro/In vivo Models
Gene Environment Interactions
Susceptible Populations
Mechanisms of Cell
Injury and Cell Death

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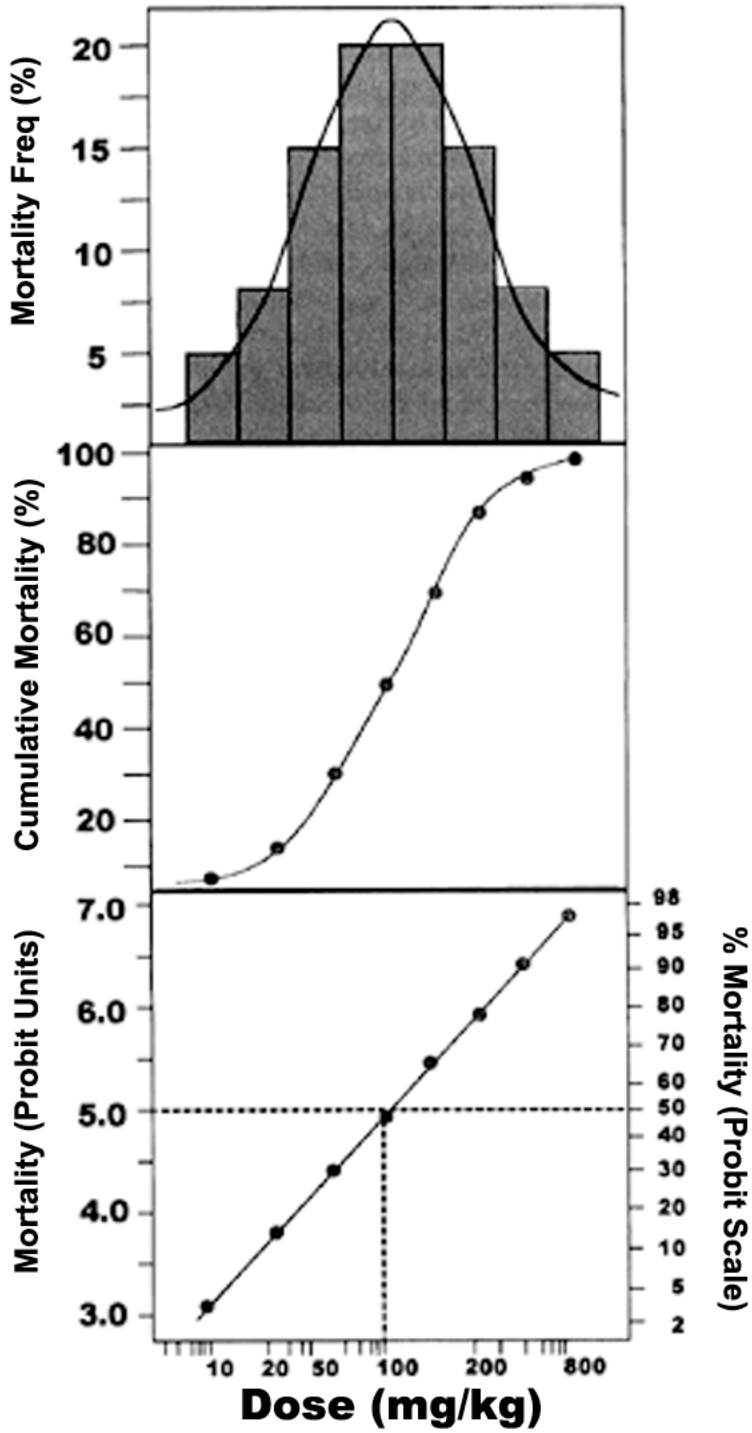
Diagram of quantal dose-response relationships



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Relative Toxicity

Approximate acute LD50s for selected chemical agents

AGENT	LD_{50}, mg/kg*
Ethyl alcohol	10,000
Sodium chloride	4,000
Ferrous sulfate	1,500
Morphine sulfate	900
Phenobarbital sodium	150
Picrotoxin	5
Strychnine sulfate	2
Nicotine	1
d-Tubocurarine	0.5
Hemicholinium-3	0.2
Tetrodotoxin	0.10
Dioxin (TCDD)	0.001
Botulinum toxin	0.00001

*LD_{50} is the dosage (mg/kg body weight) causing death in 50 percent of exposed animals.

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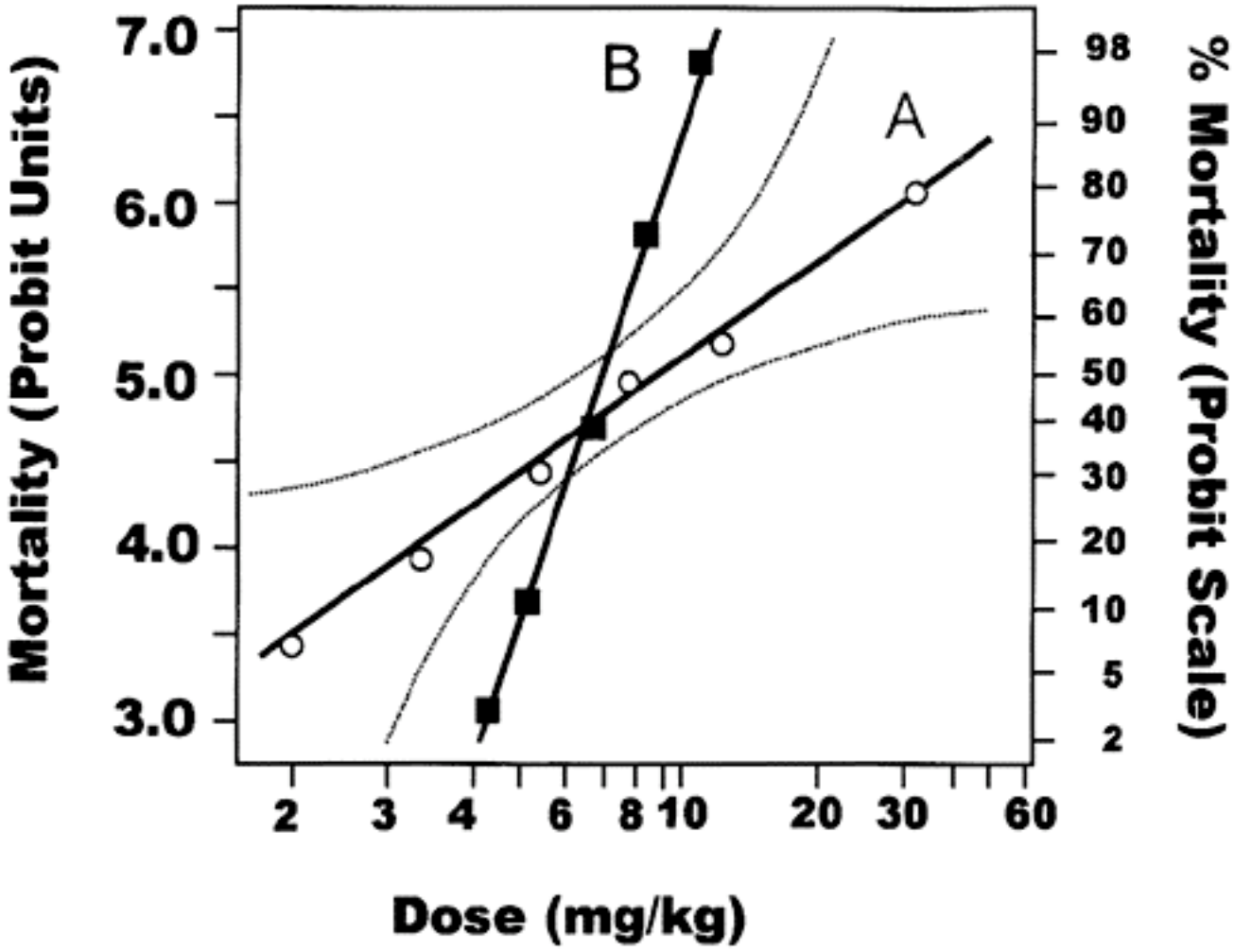
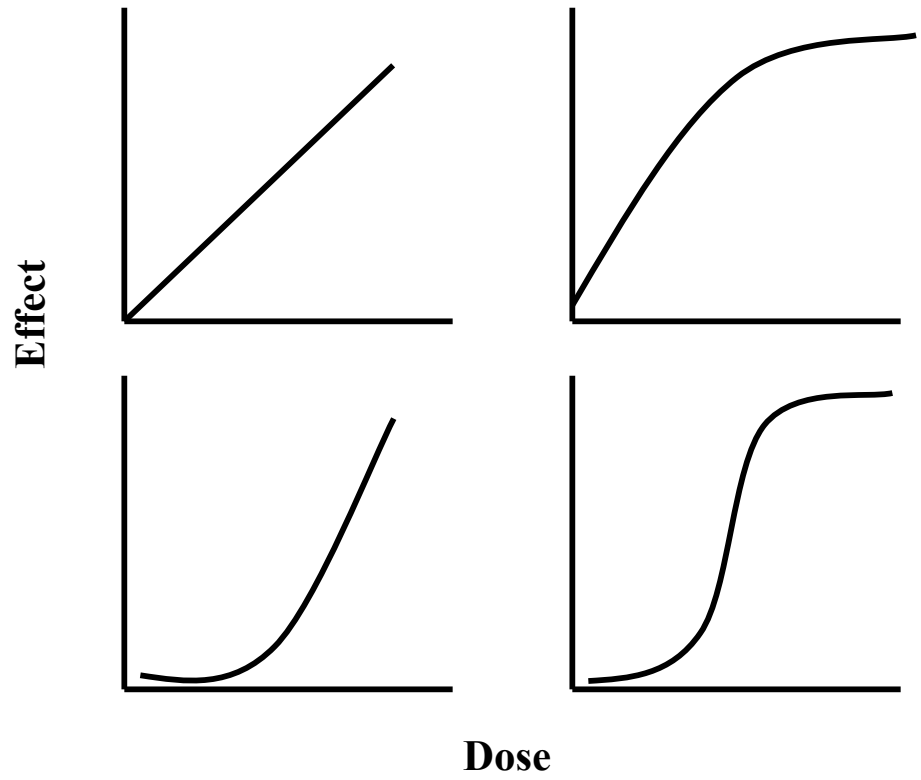
Comparison of dose-response relationship for two different chemicals plotted on a log dose-probit scale



“All substances are poisons: there is none which is not a poison. The right dose differentiates a poison and a remedy.”

Paracelsus (1493-1541)



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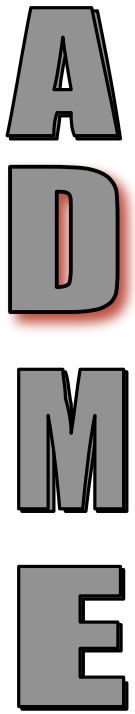
Classical Toxicology



Absorption

Distribution to tissues

Metabolism

Excretion

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Dose vs Exposure

Dose: Amount of chemical an organism is exposed to per unit of body weight (mg/kg b.wt)

Exposure: Concentration of a chemical in either the air or water through which the exposure occurs

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Exposure concentrations

Concentrations in liquids or solids:

ppt = parts per thousand (g/L; ‰; PSU); easily confused

ppm = parts per million (µg/mL = mg/L or µg/g = mg/kg)

ppb = parts per billion (ng/mL = µg/L or ng/g = µg/kg)

Concentrations in air:

mg vapor/m^3 =molecular weight (ppm)/24.45

ppm = ug/m^3

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Primary Routes of Exposure

Gastrointestinal

Respiratory

Dermal (skin)

There are tremendous differences in the absorption of compounds depending on the route of exposure due to physiological differences between these organs.

Great differences between various species.

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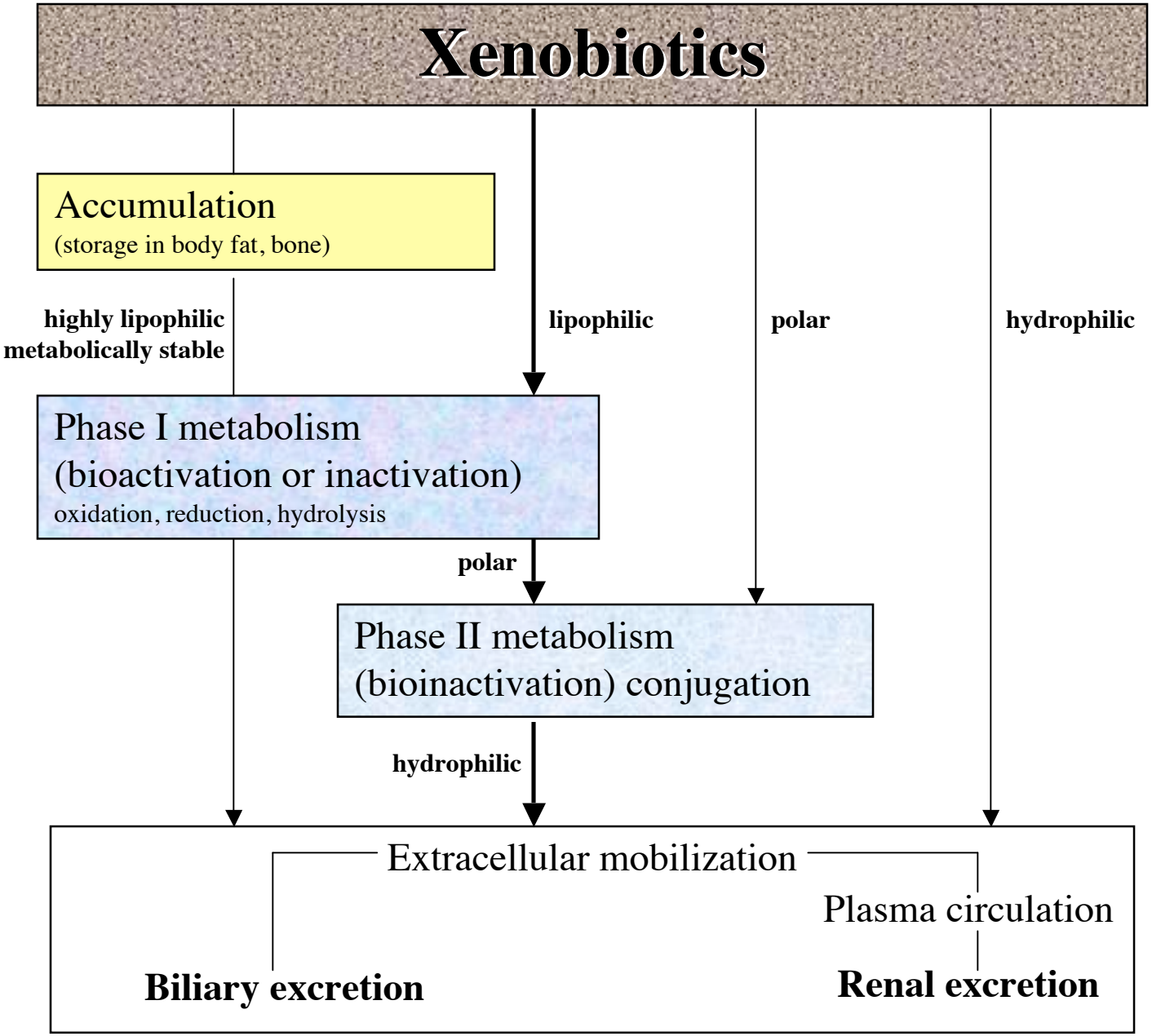
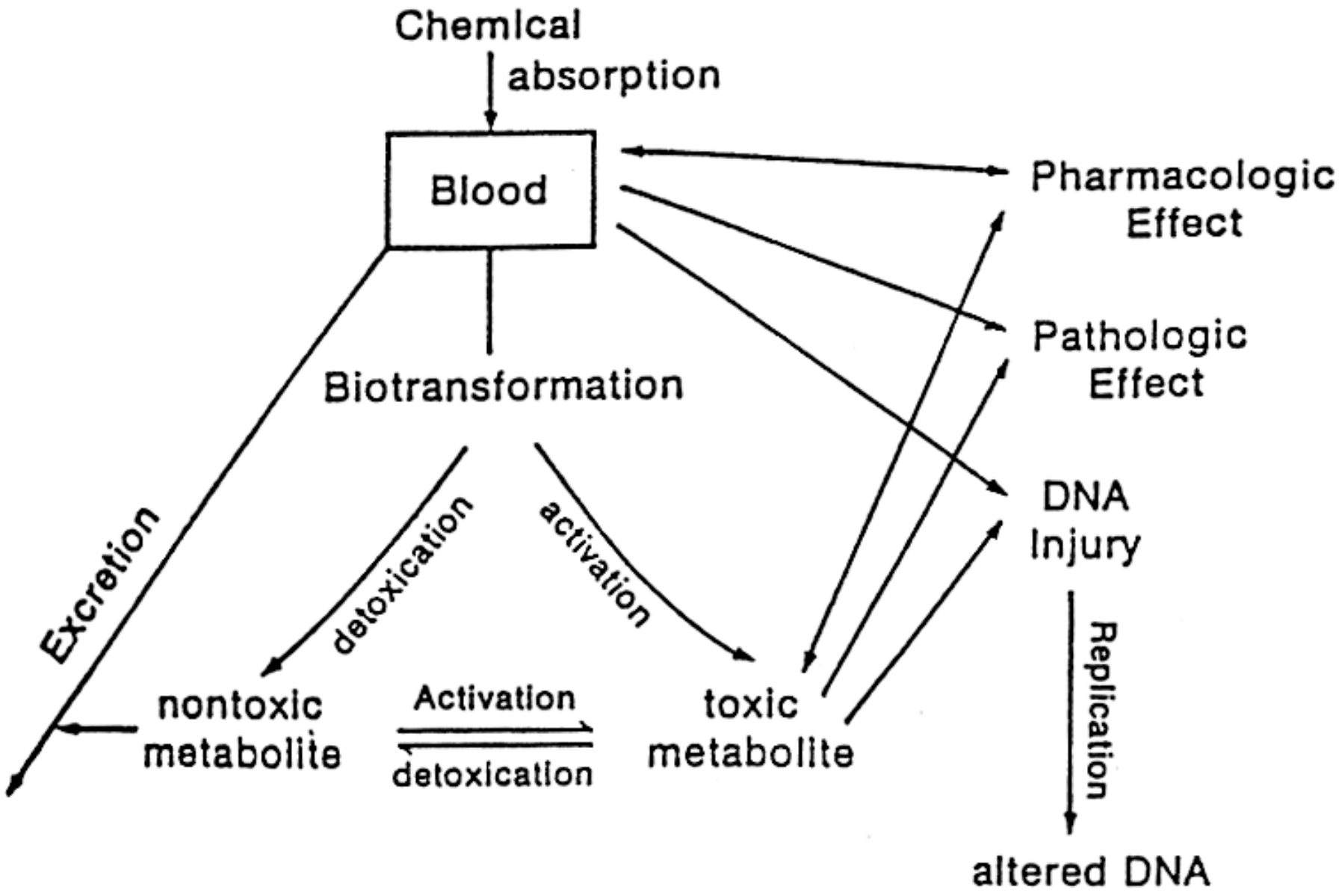
Metabolism

Metabolites: conversion products of substances, often mediated by enzyme reactions.

Bioactivation (activation): production of metabolites that are more toxic than the parent substance.

Detoxication: production of metabolites that are less toxic than the parent substance.


Xenobiotics
Accumulation
(storage in body fat, bone)
highly lipophilic
metabolically stable
lipophilic
polar
hydrophilic
Phase I metabolism
(bioactivation or inactivation)
oxidation, reduction, hydrolysis
polar
Phase II metabolism
(bioinactivation) conjugation
hydrophilic
Extracellular mobilization
Plasma circulation
Biliary excretion
Renal excretion

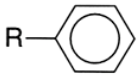
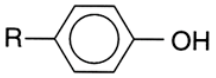
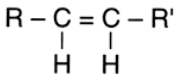
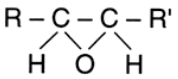
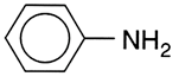
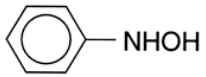
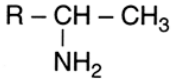
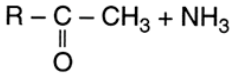
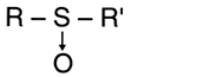
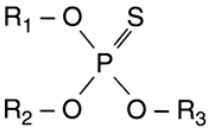
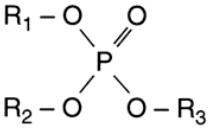
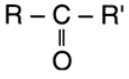
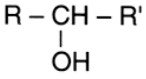
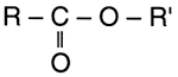
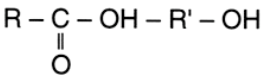

Chemical
absorption
Blood
Pharmacologic
Effect
Pathologic
Effect
Biotransformation
DNA
Injury
Excretion
detoxication
activation
Replication
nontoxic
metabolite
Activation
detoxication
toxic
metabolite
altered DNA

Overview of possible types of phase I biotransformation reactions

Oxidation reactions:
Loss of electrons, often addition of **O** to replace **H**

Reduction reactions:
Gain of electrons, often addition of **H** to replace **O**

Hydrolysis reactions:
Water interacts with substrate such that O_2 makes bond

Type of reaction	*Substrate*	*Metabolite(s)*
A. oxidations		
I mixed-function oxidase-dependent reactions		
aromatic hydroxylation		
aliphatic hydroxylation	$R-CH_3$	$R-CH_2OH$
epoxidation		
N-hydroxylation		
O-dealkylation	$R-O-CH_3$	$ROH + CH_2O$
N-dealkylation	$R-NHCH_3$	$R-NH_2 + CH_2O$
S-dealkylation	$R-S-CH_3$	$R-SH + CH_2O$
deamination		
S-oxidation	$R-S-R'$	
dechlorination	CCl_4	$[CCl_3^{\bullet}] \rightarrow CHCl_3$
oxidative desulfuration		
II amine oxidation	$R-CH_2-NH_2$	$R-CHO + NH_3$
III dehydrogenation	CH_3-CH_2-OH	CH_3CHO CH_3COOH
B. reductions		
azoreduction	$R-N=N-R'$	$R-NH_2 + R'-NH_2$
nitroreduction	$R-NO_2$	$R-NH_2$
carbonyl reduction		
C. hydrolyses		
esters		
amides	$R-CONH_2$	$R-COOH + NH_3$

Phase II Conjugation Reactions

Some important phase II reactions, with their intracellular location and endogenous substrates

Phase II reaction	*Location*	*Endogenous substrate*
glucuronidation	endoplasmic reticulum	steroids thyroxine catecholamines bilirubin
sulfation	cytosol	steroids carbohydrates
acetylation	cytosol	serotonin
methylation	cytosol and endoplasmic reticulum	biogenic amines
glutathione conjugation	cytosol and endoplasmic reticulum	metabolites of arachidonic acid

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Routes of Elimination

Biliary

Renal

Fecal

Respiratory

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Pharmacokinetic Parameters

Volume of distribution: V_d = Total dose/[blood]

The apparent volume of distribution (Vd) is the volume of fluid which the drug would occupy if it were evenly distributed through that volume at the concentration measured in the plasma (central compartment).

Vd is a convenient method for describing how well a drug is removed from the plasma and distributed to the tissues. However, it doesn't provide any specific information about where the drug is or whether it is concentrated in a particular organ. A large volume of distribution implies wide distribution, or extensive tissue binding, or both. Conversely, ionized drugs that are trapped in plasma, will have small volumes of distribution.

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Pharmacokinetic Parameters

Half-life:

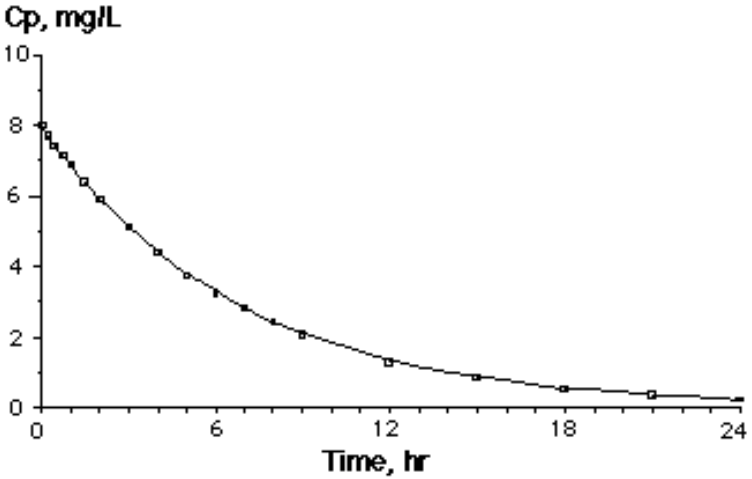
The half-life ($t_{1/2}$) is the time taken for the xenobiotic concentration to decline by 50%. It is related to the rate constant by the following:

$$t_{1/2} = 0.693/ k_{el}$$

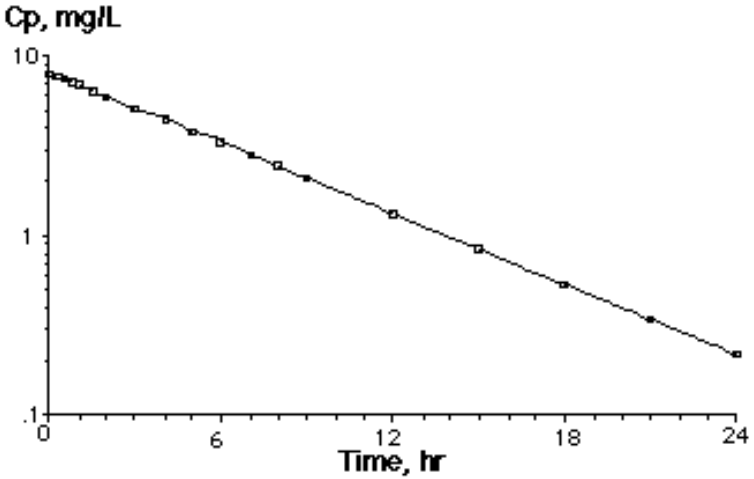
50 % of the xenobiotic is lost in 1.00 half-life
90 % of the xenobiotic is lost in 3.32 half-lives
95 % of the xenobiotic is lost in 4.32 half-lives
99 % of the xenobiotic is lost in 6.64 half-lives

Pharmacokinetic Parameters

Elimination rate constant: k_{el} = 2.303 x slope



The serum level curve observed from a xenobiotic eliminated by a first order process.

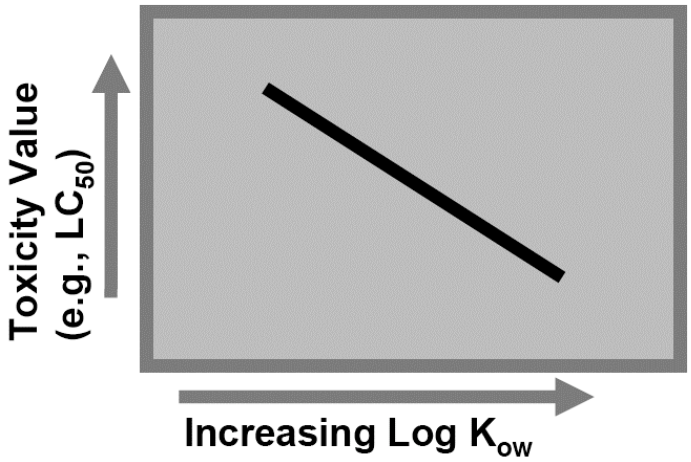


A plot of this same data using a log scale on the y-axis results in a straight line.

Pharmacokinetic Parameters

Octanol Water Partition Coefficient (K_{ow})

- Ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature.
- Predict solubility
- Predict bioaccumulation



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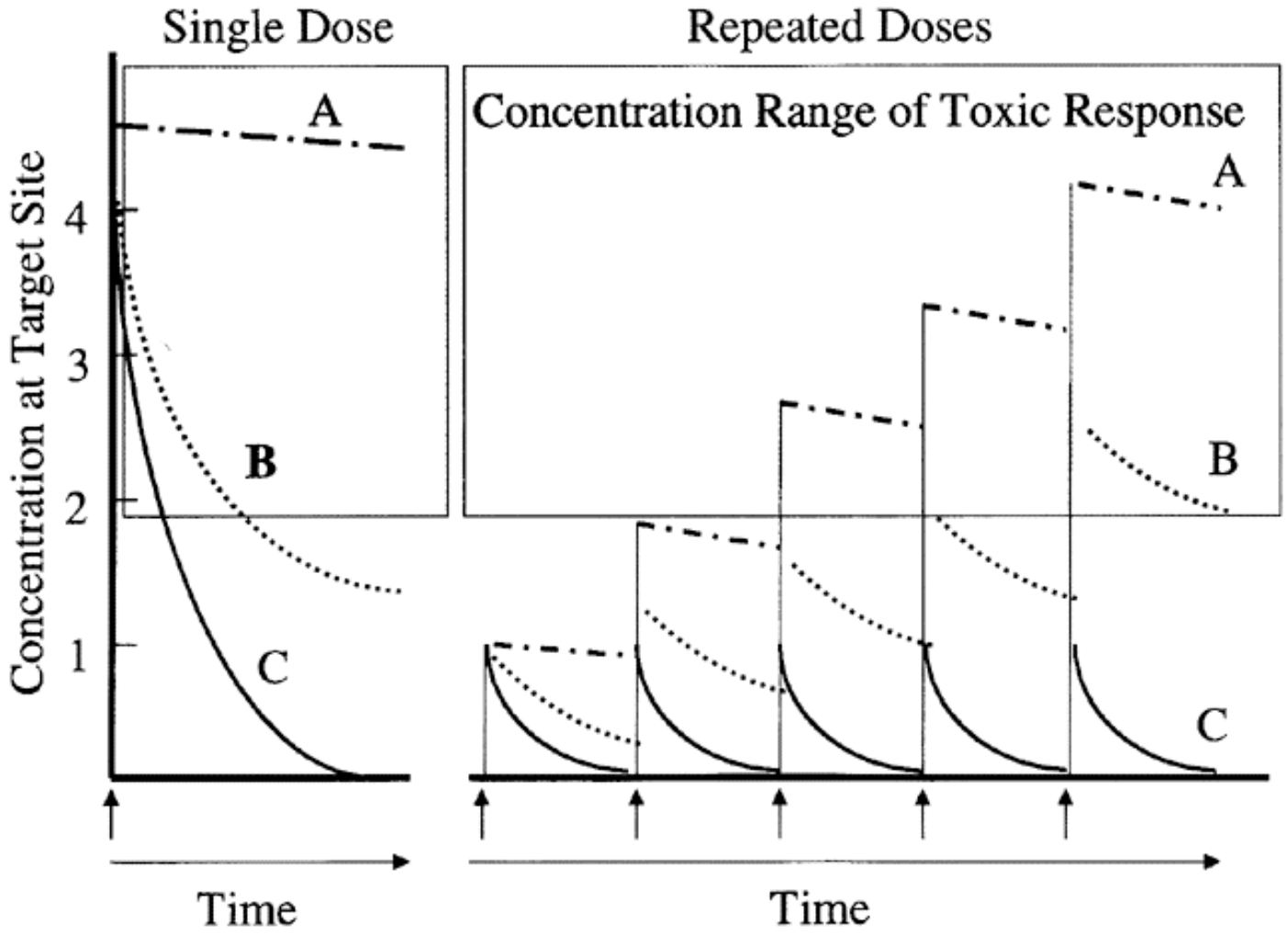
Bioaccumulation

- Accumulation of substances, such as pesticides or other organic chemicals in an organism or part of an organism.
- Biological sequestering through respiration, diet, epidermal (skin) contact.
- Results in the organism having a higher concentration of the substance than the concentration in the surrounding environment.
- Amount depends on the rate of uptake, the mode of uptake, how quickly the substance is eliminated, transformation of the substance, the lipid content of the organism, the K_{ow} of the substance, and environmental factors, and other biological and physical factors.
- General rule: the more hydrophobic a substance is the more likely it is to bioaccumulate in organisms. Exceptions (e.g. methylmercury).
- Bioconcentration refers only to the uptake of substances into the organism from water alone. Bioaccumlation is the more general term because it includes all means of uptake into the organism.

Pharmacokinetic Parameters

Relationship between dose and concentration at the target site under different conditions of dose frequency and elimination rate



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Haber's Law

For many compounds…

The toxic effect of a substance is determined by the product of the concentration and the duration of the exposure

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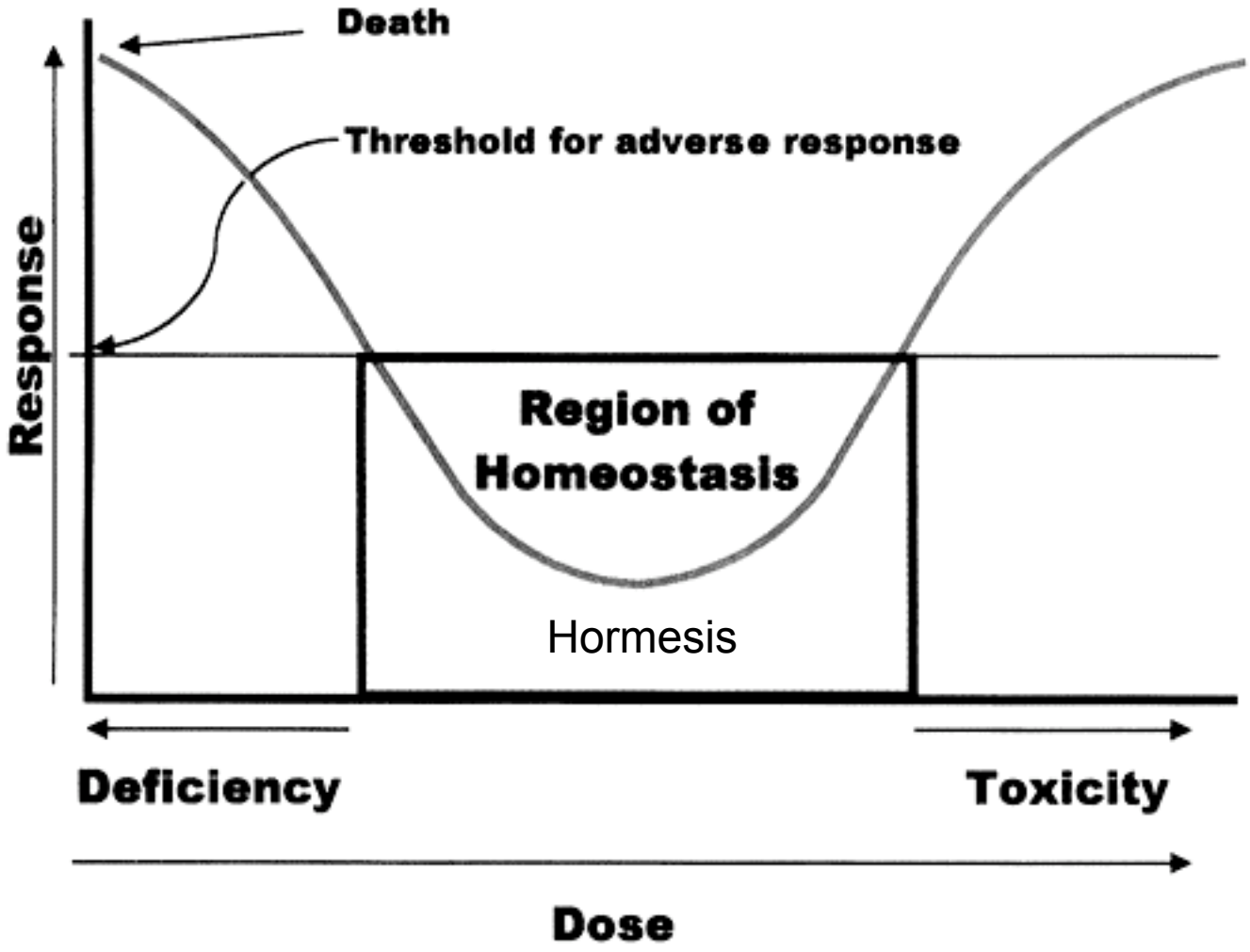
Dose-response relationship for representative essential substances, such as vitamins or trace elements (e.g., Cr, Co, Se)



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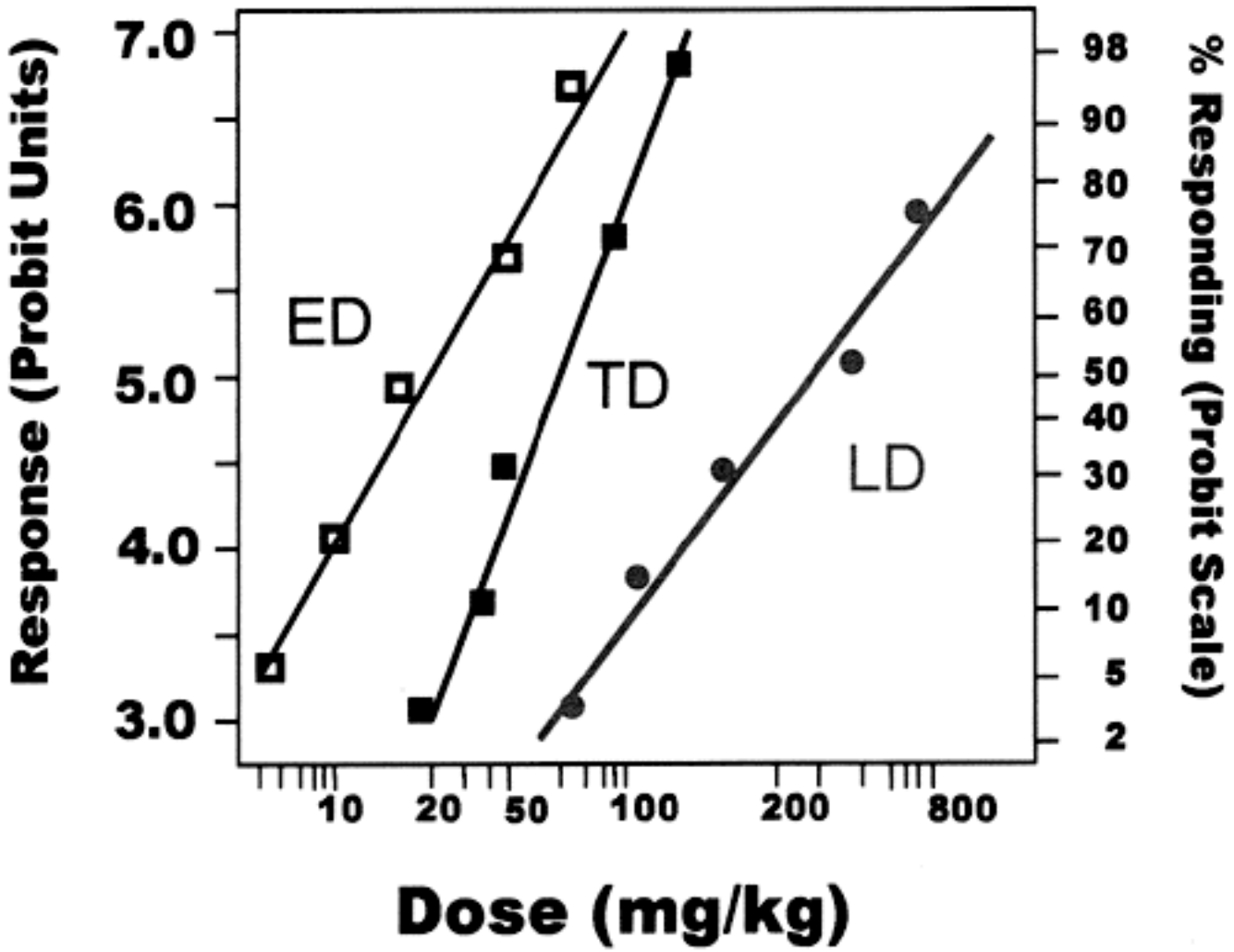
Effective, toxic and lethal dosages



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Acute vs Chronic Toxicity

- **Acute effects do not predict chronic effects**
- **Doses causing chronic effects may not cause acute or sub-acute effects**
- **In human and veterinary arenas chronic effects of a chemical exposure may manifest themselves as a common disease and go unnoticed**
- **SARs and K_{ow} predictors**

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Chemical Interactions

Additive: **2+3=5** (2 OPs - cholinesterase inhibition)

Synergistic: **2+2=20** (CCl_4 + EtOH)

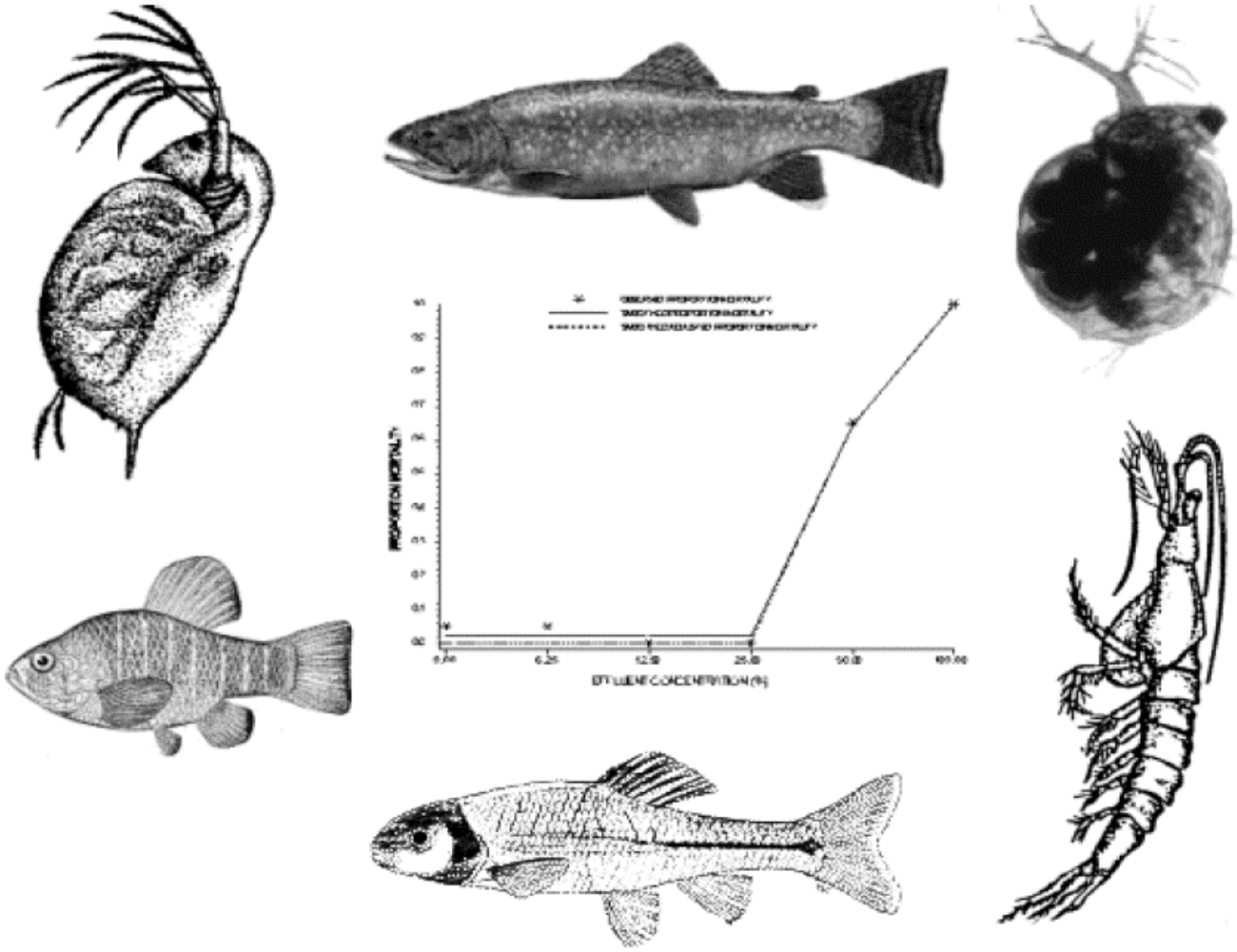
Potentiation: **0+2=10** (isopropanol + CCl_4)

Antagonism: **4+6=8; 4+0=1**

- **Functional antagonism:** 2 chemicals counterbalance each other by producing opposite effects on the same physiologic function (eg epinephrine + diazepam).
- **Chemical antagonism** (inactivation): chemical rxn between 2 compounds that produces a less toxic product (eg chelators and metals).
- **Dispositional antagonism:** alters A,D,M or E to that conc or duration at target site is diminished (eg ipacac, charcoal, diuretics, SKF-525A or piperonyl butoxide).
- **Receptor anatagonists** (blockers): clinical trtmt by competitive binding to same receptor (eg atropine and OPs to block cholinesterase receptors; tamoxifen as an anti-estrogen to lower risk of breast cancer).

United States
Environmental Protection
Agency

Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms



United States Environmental Protection Agency

Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms

Freshwater:

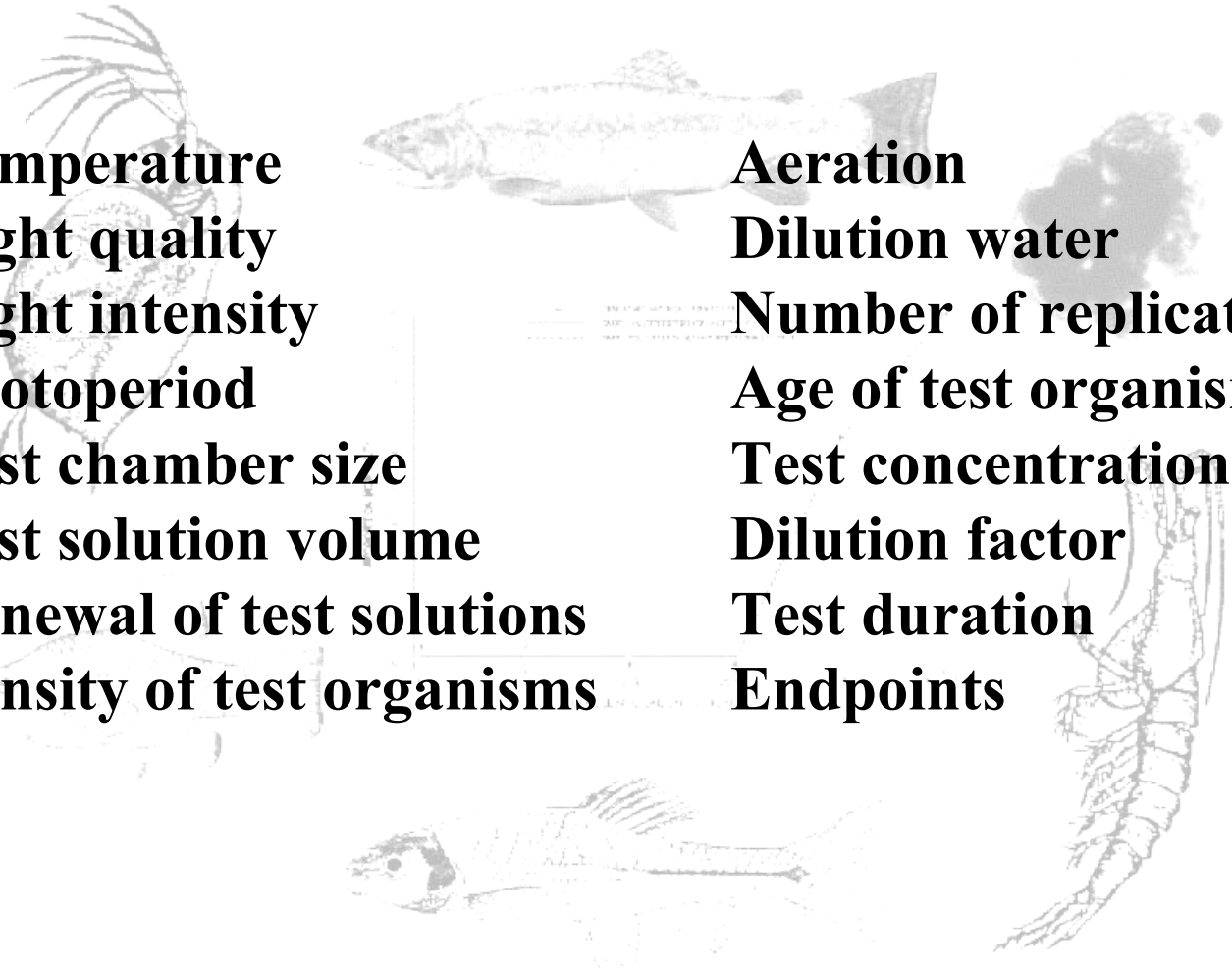
Ceriodaphnia dubia (daphnid)
Daphnia pulex and D. magna (daphnids)
Pimephales promelas (fathead minnow)
Oncorhyncus mykiss (rainbow trout)

Estuarine & Marine:

Mysidopsis bahia (mysid)
Cyprinodon variegatus (daphnids)
Menidia beryllina, M. menidia & M. peninsulae
(inland, Atlantic & tidewater silversides)

United States Environmental Protection Agency

Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms



Temperature
Light quality
Light intensity
Photoperiod
Test chamber size
Test solution volume
Renewal of test solutions
Density of test organisms

Aeration
Dilution water
Number of replicates
Age of test organisms
Test concentrations
Dilution factor
Test duration
Endpoints