

# **FACTORS AFFECTING TOXICITY**

## **i) Physical/Chemical Properties of the Environment**

- **Salinity: Freshwater (<0.5% salinity) vs. estuarine/coastal (0.5-30) vs. oceanic (>30%)**
- **Tidal flushing**
- **Dilution capacity and rates**
- **Flushed vs. stagnant**
- **Aerated vs. anoxic**
- **Conservative vs. non conservative constituents (O<sub>2</sub>, H<sub>2</sub>S, CO<sub>2</sub>, nitrate, phosphate, amino acids, fulvates, humates, metals, particulates, excreta, organisms etc)**
- **Physical/Chemical Buffering**

i) Physical/Chemical Properties of the Environment (cont)

- **Photolysis**
- **pH and acid base phenomena**
- **Complexation**
- **Dissolution/precipitation**
- **Oxidation/reduction**
- **Adsorption/desorption**
- **Hydrolysis**

## ii) Factors Affecting Environmental Concentrations of Contaminants

- **Duration of the inputs**
- **Concentrations and total loading of chemical entering the system**
- **Chronic vs. acute**
- **Continuous discharge/seepage vs. accidental spills/dumping**
- **Mobility and distribution (phase effects)**
- **Physico-chemical form  
(dissolved/absorbed/bioaccumulated)**

## ii) Factors Affecting Environmental Concentrations of Contaminants (continued)

- **Chemical and Biological Reactivity**
- **Stability of the chemical form (thermodynamics vs kinetics)**
- **Persistence (biophysical half-life)**
- **Hydrophobicity and hydrophilicity**

### iii) Organismal Parameters

- **Point of impact (critical/lethal or non-critical/sublethal)**
- **Sensitivity**
  - species dependant
  - lifestage dependant (egg/larvae/adult)
- **Susceptibility**
  - Life style and behavior
  - Accumulated in the trophic web
  - Bioconcentrated and amplified
  - Mode of accumulation (dietary/cutaneous)

#### iv) Chemical Interaction with the Biota.

- **Non-selective (narcosis) vs. selective action**
- **Species (physico-chemical) specificity**
- **Predictive structure / activity relationships**
  - SAR's (structure activity relationships),
  - QSAR's (Quantitative SAR)
  - QSRP's (QS property R)

## v) Mode of Toxicity and Biological Effects

- **Acute vs. chronic**
- **Lethal vs. sublethal**
- **Reversible vs. irreversible**
- **Local vs. systemic**
- **Sequential vs. simultaneous**
- **Immediate vs. delayed**
  - **teratogenic (developmental affects)**
  - **mutagenic (causes genetic mutation)**
  - **carcinogenic (causes cancer)**

## vi) Biological Detoxification Mechanisms

- **Behavioral**
- **Exclusion**
- **Elimination**
- **Biotransformation**

## vi) Interaction between toxins

- **Additive (1+1=2)**
- **Synergistic (1+1=5)**
- **Potentiation (0+1=5)**
- **Antagonism (3+2=2 or 4+0=2)**
  - **Functional antagonism (elicit opposite counteracting responses)**
  - **Chemical (one neutralizes the other through chemical interaction)**
  - **Dispositional (biochem/physiology changed to reduce exposure)**
  - **Receptor (competitive or non-competitive binding to a target site)**

# **HAZARD EVALUATION AND RISK ASSESSMENT**

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- **Risks to Ecosystem and Human Health**
- **Law and Legislation**
- **Compliance and Enforcement**
- **Accountability**
  - **point sources/vs. seepage/discharge;**
  - **anthropogenic vs geological**
- **Remediation**
- **Causal demonstration of Toxicity and Effect**

# **TOXICITY TEST AND METHODS**

## **TOXICITY TEST AND METHODS**

- **Establishing dose-response relationships**
- **Establishing sensitivity and variability to toxicity**
- **Establishing cause effect relationships**
- **Establishing mechanisms of interaction**

## i) What criteria should be used for establishing a test

- **Accepted as scientifically sound**
- **Reproducible within and between labs hence defined and standardized**
- **Cover a dynamic range that is ecologically realistic**
- **Quantifiable by mathematical and statistical modeling**
- **Economical and easy to conduct in even the most primitive setting**
- **Sensitive so that it can detect and measure effect**
- **Useful for risk assessment**

## i) What criteria should be used for establishing a test (cont)

- **Should have field predictive behavior**
  - Scalable controlled laboratory experiment that mimics environmental parameters
  - Mesocosm studies involving artificial ecosystems
    - Natural ecosystem studies
- **Requires appropriate negative, positive and carrier/vehicle controls**

## ii) Establishing Dose-Response Relationships

- Different organisms/lifestages and endpoints used.
- Most common parameter used is mortality (acute toxicity or LC50)
- LC50 is the concentration of toxin required to kill 50% of organisms over a specified time period
- Time periods normally 24 hour or 96 hours
- Death easy to detect and observe

## ii) Establishing Dose-Response Relationships (continued)

- **Quantal response (Y/N) so no measurements are required**
- **Statistics simpler than quantitative measure of toxicity that do not use death (EC50's)**
- **Death is unquestionably significant to the individual/ population and is a parameter that legal council can understand.**

### iii) Choice of Organism.

- **Should include a suite of organism with differing sensitivity (most sensitive species may be overprotective while resistant species underestimate the potential effect)**
- **Should be widely available and common to allow worldwide use**
- **Should be indigenous to the ecosystem under impact**
- **Ideally should be recreationally, commercially or ecologically important**
- **Husbandry should be easy including maintenance, rearing, culture and exposure**

### iii) Choice of Organism (continued).

- Background basic information on life history, physiology, genetics, biochemistry provides for the opportunity to elucidate mechanisms of toxic interaction.
- Isogenetic stocks ideal (*Neanthes arenaceodentata*) otherwise populations captured from non-impacted areas preferred. Look for acclimatized or resistant populations.
- Efforts to get away from vertebrate testing. Cell lines now available and bacterial tests becoming more common. QSAR's becoming more important due to modelling.
- Common species used: water flea (*Daphnia*), fathead minnow (*Pimephales promelas*), Sheepshead Minnow (*Cyprinodon variegates*), Rainbow trout (*Oncorhynchus mykiss*) and the mussel (*Mytilus edulis*)

#### iv) Exposure Systems.

- **Static test**

- degradation, adsorption, uptake, volatilization
- if organic may be decomposed with high BOD demand, O<sub>2</sub> must be monitored
- Metabolic waste build up which may be more toxic than material under test

- **Recirculation tests**

- **Static Renewal tests**

- **Flow through systems**

# Toxicity Test Endpoints:

- **No Observable Effect Concentration (NOEC)**

Maximum concentration that produces no statistical significant harmful effects

- **Lowest Observed Effect Concentration (LOEC) or Minimum Threshold Concentration (MTC)**

Lowest concentration that a statistically significant deleterious effect is observed

- **Maximum Acceptable Toxicant Concentration (MATC)**

Estimated threshold range defined at the upper level by the LOEC and at the lower level by the NOEC.

# Toxicity Test Endpoints (continued):

- **Median Lethal Concentration (LC50)**  
Concentration where 50% organisms die
- **Medium Effective Concentration (EC50)**  
Concentration where 50% organisms show measurable effect
  - **Application Factors  $AF = MATC/LC50$  (time independent value)**  
Relates chronic toxicity to acute toxicity and predicted by test to be a safe concentration.

# Duration of Exposure

## Time Dependent tests (tend to be acute tests)

Conducted for a prescribed time (24- 96 hour tests most common)

e.g. 24 hr-LC50 (Concentration required to kill 50% test organisms in 24 hrs)

## Time Independent Tests (tend to be chronic tests)

Indefinite prescribed period continued until

- toxic effect has ceased
- an incipient threshold effects concentration can be determined
- logistical reasons

# Acute Toxicity Test

Determine Short-term exposure to various concentrations

## Effect Criteria

- Fish (mortality)
- Invertebrates (immobility loss of equilibrium)
- Algae (growth)

Data Units (Concentration: mass or activity of toxicant. L-1)

## Reported Units

Median Lethal Concentration (LC50)

- Concentration where 50% organisms die

Medium Effective Concentration (EC50)

- Concentration where 50% organisms show measurable effect

# Chronic Toxicity Tests

## Evaluation of effects over long-term sublethal exposures

Full chronic toxicity test: exposed to at least 5 different concentrations over entire life cycle (egg to egg) and therefore test reproductive capacity and fecundity

Partial Chronic Toxicity test: over several most sensitive stages (reproduction and growth over initial part of life cycle)

### Data Units:

Maximum Acceptable Toxicant Concentration (MATC)

Estimated threshold range defined at the upper level by the LOEC and at the lower level by the NOEC.

Generally reported as NOEC<MATC<LOEC

# **Standardization of Testing:**

**Variety of methods to evaluate potential toxicity and hazard of materials to aquatic organisms**

- American Public Health Association (APHA)**
- U.S. Environmental Protection Agency (EPA)**
- American Society for Testing and Materials (ASTM)**
- International Standardization Organization (ISO)**
- Environment Canada and Organization for Economic Cooperation and Development (OECD)**

# **Advantages of Standardized Testing:**

- Allows selection of one or more uniform and useful test by a variety of laboratories**
- Allows development of standard operation procedure manual (SOP)**
- Facilitates comparison of data and results and thus increases usefulness of published data**
- Increases Accuracy of the data**

## **Advantages of Standardized Testing (continued):**

- **Allows replication of test**
- **Allows test to be easily initiated and conducted by a variety of personnel**
- **Legal advantage if procedures are accepted by the courts**
- **Useful in routine monitoring**

# Steps to Standardization of a Toxicity Testing Method

- Thorough understanding of the physical, chemical and biological factors that affect the test results
- Use of a Standard Test Species
- Selection of Specific Test Types Designed to Meet Specific Objectives
- Use of Reference Toxicants and Placebos
- Disease free “certified” test animals

## Effectiveness judged by 5 “R’s”

- relevance,
- reproducibility,
- reliability,
- robustness,
- repeatability