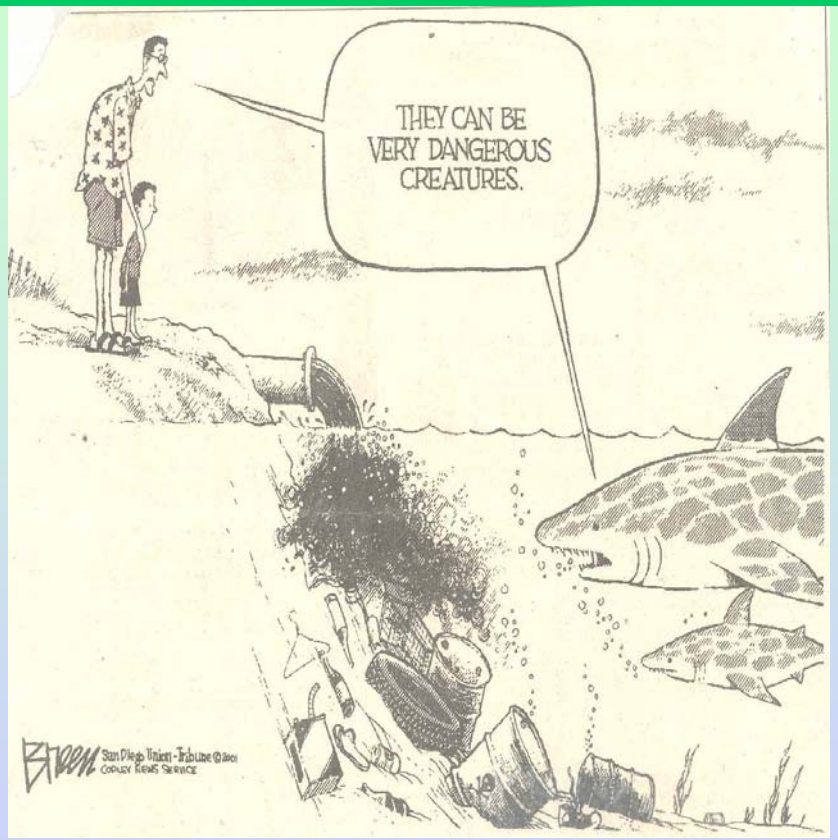
Toxic Effects to Aquatic Fauna



Jay Davis, U.S. Fish Wildlife Service
ECS 3119 – Pesticides and Fish & Wildlife Resources
NCTC – Shepherdstown, WV
September 13 – 17, 2010

Importance of Aquatic Habitats

- **85% of T/E listed species utilize wetlands and aquatic habitats**

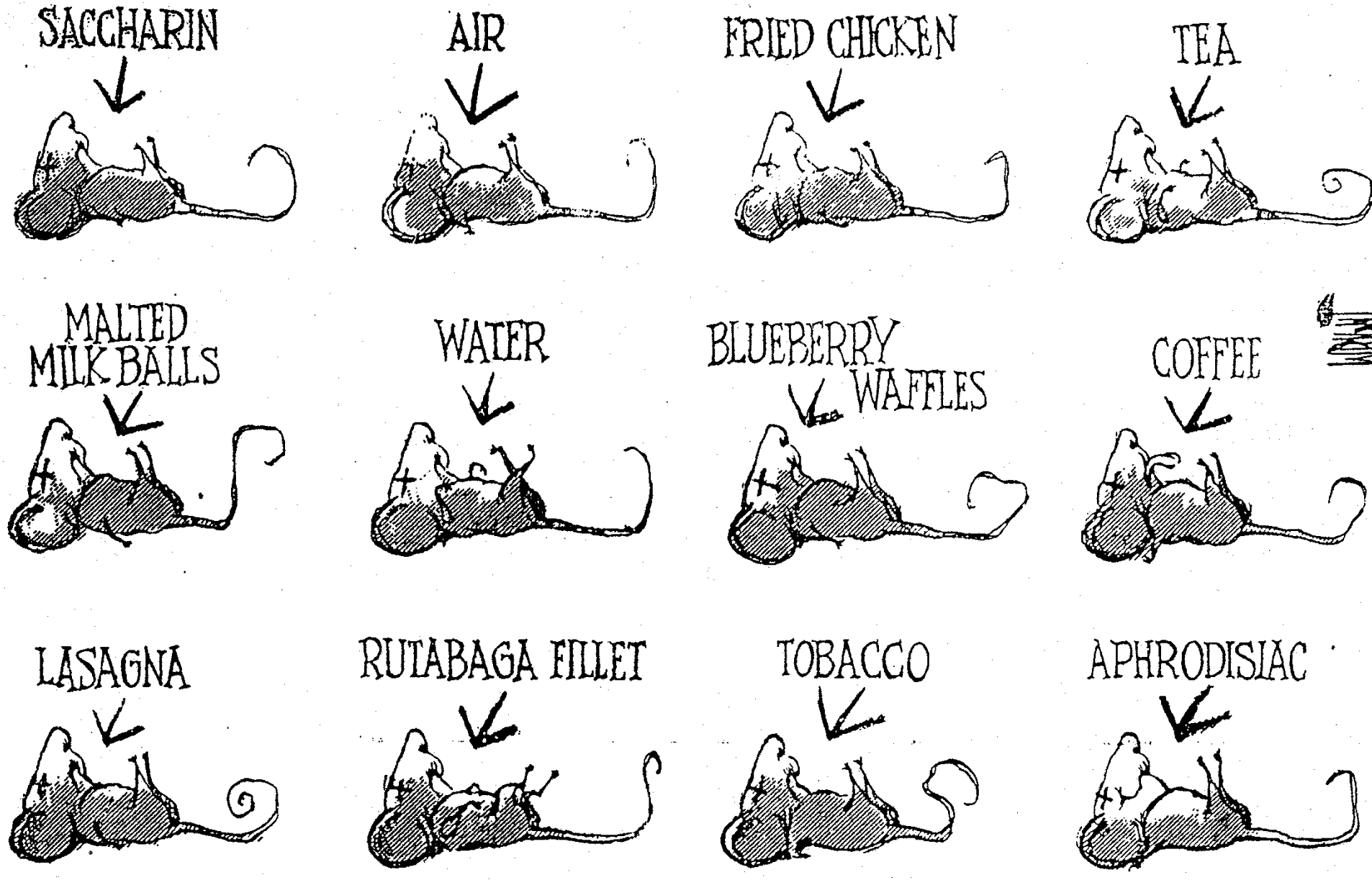
Toxicology:

The study of poisons

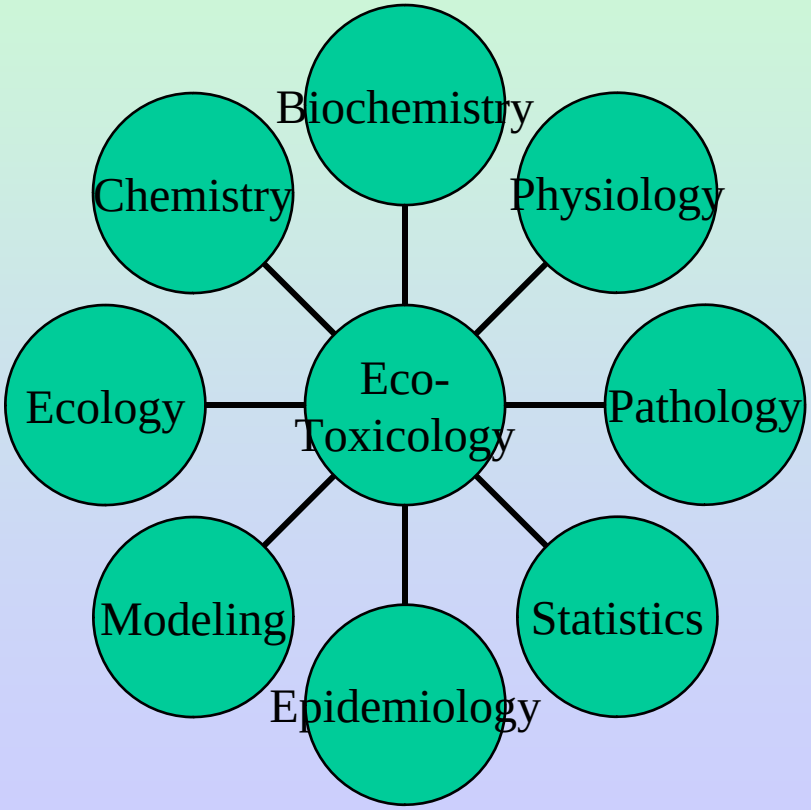
“The dose makes the poison”
Paracelsus (1493-1541)

Acute LD50's of some common chemicals (oral - rat)

Botulism toxin	0.000001 mg/kg
Ricin	0.001 mg/kg
Aflatoxin B1	9 mg/kg
Sodium Fluoride	180 mg/kg
Tylenol	338 mg/kg
Diazinon	350 mg/kg
Aspirin	1,500 mg/kg
Malathion	2,800 mg/kg
Table Salt	3,750 mg/kg
Ethanol	10,600 mg/kg


SACCHARIN
AIR
FRIED CHICKEN
TEA
MALTED MILK BALLS
WATER
BLUEBERRY WAFFLES
COFFEE
LASAGNA
RUTABAGA FILLET
TOBACCO
APHRODISIAC

***Ecotoxicology* – the study of poisons and their effects on living biological systems**



Interdisciplinary

***Toxicity* - the state of being poisoned**

…OR… a general term used to indicate adverse effects produced by a toxicant in organisms.

Toxicity is a function of:

- **quantity/dose (concentration for aquatic organisms)**
- **Composition or properties of the toxicant**
- **The length or duration of the exposure**

Toxicity can be -

- **Direct – toxic agent acts on the organisms**

- **Indirect – toxic agent causes changes in factors external to the organism (habitat change, decrease food source, etc.)**

Toxicity/Hazards Dependent upon:

- **Chemical & physical properties of pesticide**
- **Formulation**
- **Rate, type, and method of application**
- **Characteristics of receiving/test water**
- **Multiple chemical exposure**

Contaminant Exposure

The three primary routes of exposure for organisms are via:

- *inhalation*
- *ingestion*
- *dermal*

Routes of Exposure

1.) Inhalation - aquatic

a. Gills are particularly vulnerable to foreign chemicals because they are designed to maximize diffusion.

b. They are thin (2 to 4 µm) with a large surface area (2 to 10 times the surface area of the body).

Routes of Exposure

2). Ingestion

a. prey - contaminants can be ingested by the consumption of prey items (food chain transfer)

b. incidental - contaminants can be ingested in soil/sediment and water during normal foraging.

Routes of Exposure

3). Dermal (water concentration)

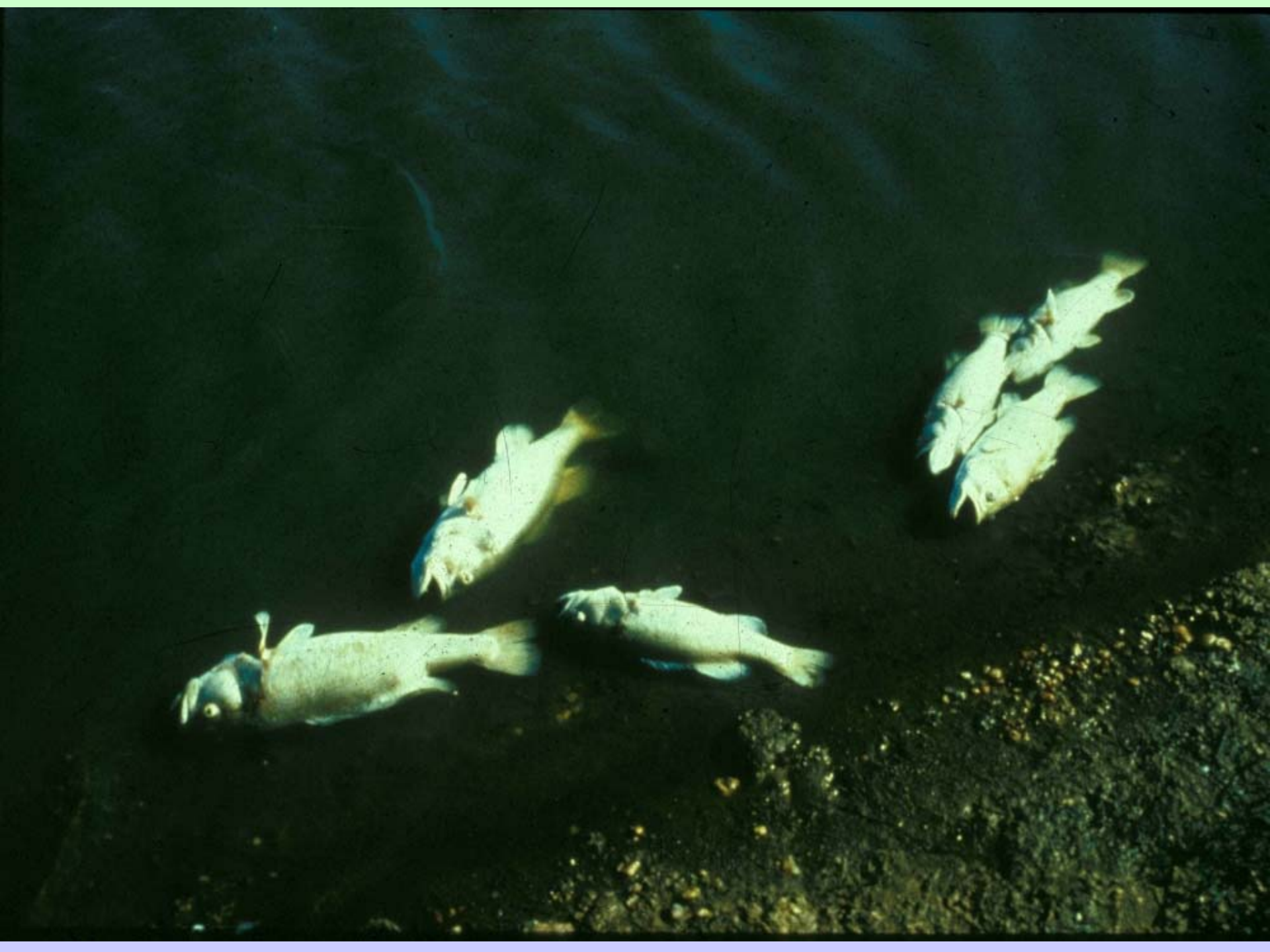
a. Largest organ of many organisms and therefore has significant exposure to potential toxicants especially for aquatic organisms which are in constant contact with their environment.

b. Scales and exoskeletons can serve as an effective barrier.

c. size dependent – small fish have a greater surface area to volume ratio than larger fish which increases pesticide absorption.

Duration of Exposure

- **Acute – adverse effects occur after one or only a few exposures over a short amount of time.**
- **Sub-chronic – adverse effects occur after a few to moderate number of exposures over an intermediate amount of time.**
- **Chronic – adverse effects occur after repeated exposures or continuous exposures over an extended amount of time.**



Exposure Types in General

- **Acute: Short term (96 hours or less)**
  - **Severe effects**
  - **Rapid response to toxicant**
  - **Mortality endpoint (e.g. LC_{50})**

- **Chronic: Long-term exposure (> 96 hours)**
  - **Mild effect**
  - **Gradual response to toxicant**
  - **Sublethal endpoints (growth and reproduction are most common)**
  - **Examples: Early Life Stage (ELS), partial and complete Life Cycle, and Bioaccumulation Tests.**

Toxicological Endpoints

- **Lethality**
- **Carcinogenicity**
- **Mutagenicity**
- **Neurotoxicity**
- **Immunotoxicity**
- **Target Organ**
- **Ecological Effects**
- **Growth**
- **Reproduction**
- **Physiological**
- **Biochemical**
- **Developmental**
- **Hormonal/Endocrine**
- **Behavioral**

Popular Aquatic Bioassay Critters

Freshwater

Fish

- Fathead minnow
- Rainbow trout
- Bluegill

Invertebrates

- Water fleas
- Amphipods
- Bivalves
- Chironomids
- Polychaetes

Algae and aquatic plants

Saltwater

Fish

- Sheepshead minnow
- Silversides
- Gambusia

Invertebrates

- Mysid & grass shrimp
- Amphipods
- Bivalves
- Copepods
- Polychaetes

Algae

Advantages of Aquatic Bioassays

- **Advantages:**
  - **Standardized methodologies (simplicity)**
  - **Generate a substantial amount of data in a relatively short period of time on many chemicals**
  - **Relatively low cost**
  - **Controlled conditions**
  - **Convenient to collect and/or culture test species**

Limitations of Aquatic Bioassays

- **Limitations:**
  - **Sensitivity of the species tested**
  - **Relatively insensitive endpoints tested**
  - **Need for interspecies extrapolation (few species tests)**
  - **Field conditions differ from laboratory (ecological significance?)**

Aquatic Toxicity Tests

Performed in laboratories (generally), under field scenarios (mesocosms and microcosms), or in "true" field conditions (in-situ)

- **Conducted under a range of exposure scenarios**
  - **Static/renewal/continuous flow**
- **Preliminary (range finding) or Definitive**
  - **Logarithmic versus geometric dilution series**
- **Utilize serial dilutions generally with 5 concentrations as well as controls**
  - **200, 100, 50, 25, 12.5 ppm, control & solvent control (if a carrier is needed)**

Chronic Toxicity Terminology

Chronic = Lethal or sublethal toxicity from prolonged or repeated exposure for more than 96 hours.

LOEC and NOEC

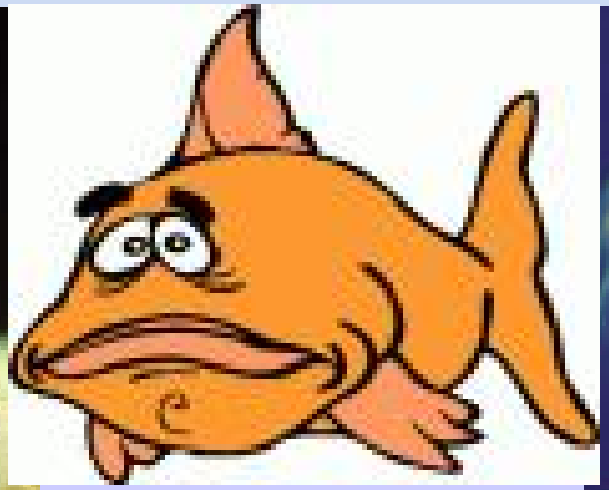
= Lowest Observed Effect Concentration and No Observed Effect Concentration

LOAEC and NOAEC

= Lowest Observed Adverse Effect Conc. and No Observed Adverse Effect Conc.

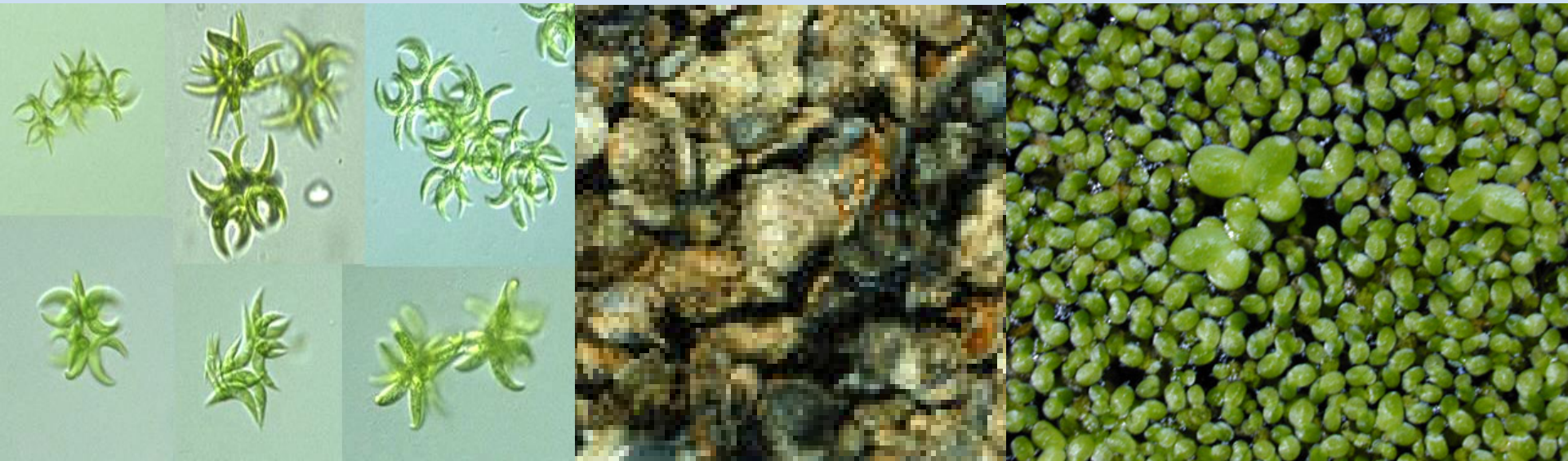
Acute Toxicity Terminology - EC_{50} or LC_{50}

The Concentration at which 50% of an exposed sample population dies (Lethal) or is immobilized (Effect) after a short term (48 hr or 96 hr) exposure.



Acute Toxicity Terminology - IC_{50} (Inhibition Concentration)

The concentration at which a 50% reduction occurs as compared to the controls after a short term exposure (i.e. growth, enzyme activities, etc.).



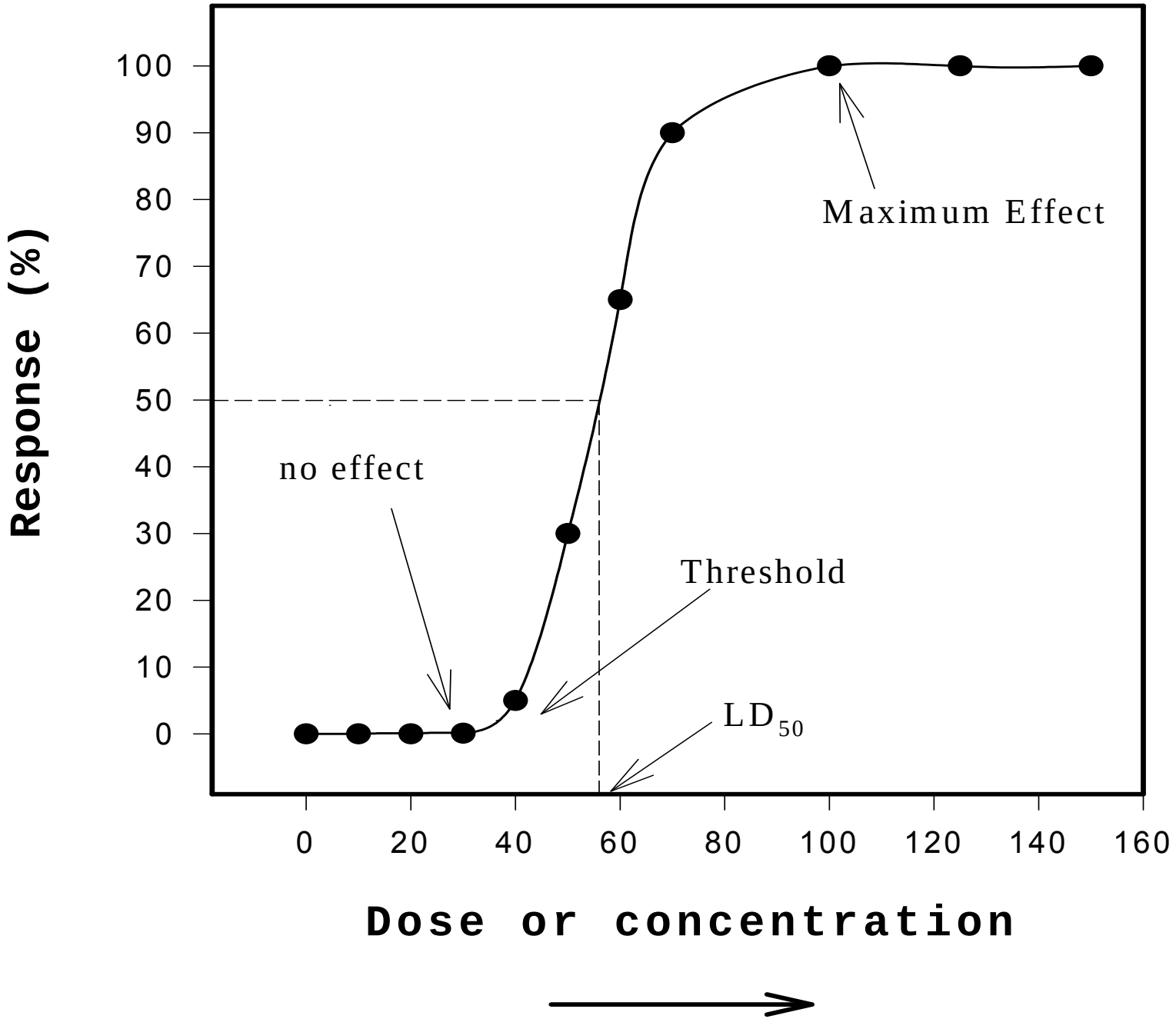
Common Units

- **Parts per million (ppm) = mg/L or mg/kg**
- **Parts per billion (ppb) = µg/L or µg/kg**

ACUTE-TOXICITY RATING SCALES (in ppm)

Relative Toxicity	**Aquatic EC or LC_{50} (mg/L)**
Super Toxic	< 0.01
Extremely Toxic	0.01 – 0.1
Highly Toxic	0.1 – 1
Moderately Toxic	1 – 10
Slightly Toxic	10 – 100
Practically Nontoxic	100 – 1000
Relatively Harmless	> 1000

USFWS Research Information Bulletin. 1984. No. 84-78

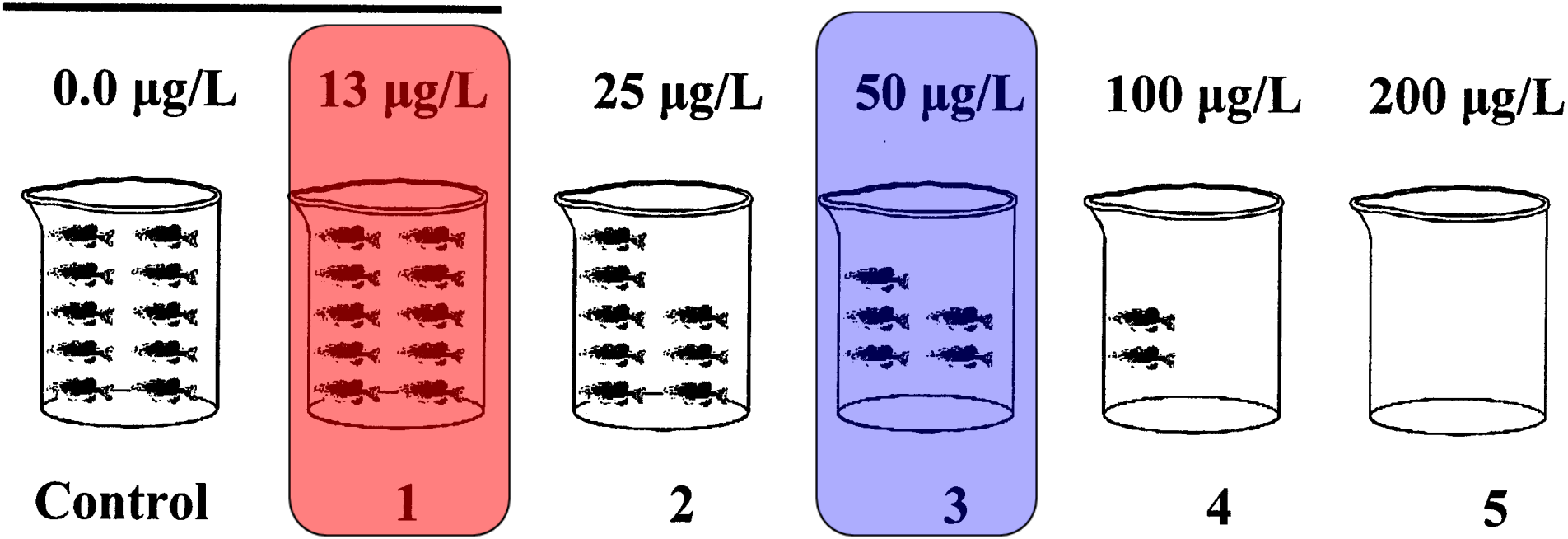

Response (%)
100
90
80
70
60
50
40
30
20
10
0
Maximum Effect
no effect
Threshold
LD50
0
20
40
60
80
100
120
140
160
Dose or concentration

REVIEW

ACUTE TOXICITY DATA

96-hour LC50 = 50 µg/L

Concentration:



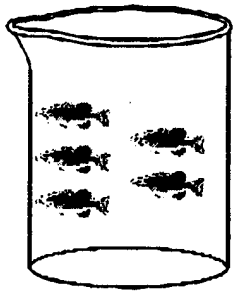
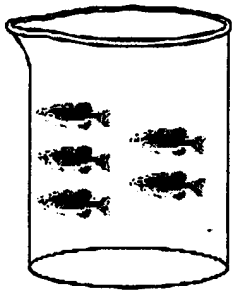
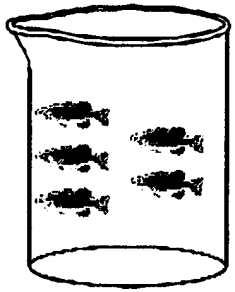
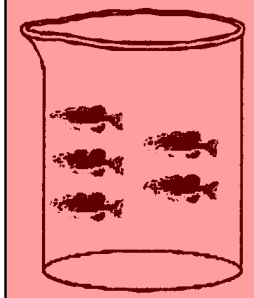
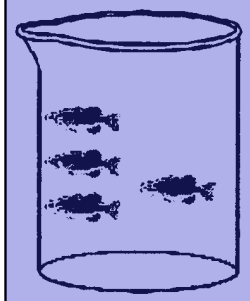
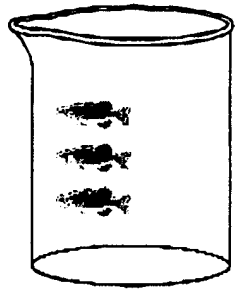
NOEC

REVIEW

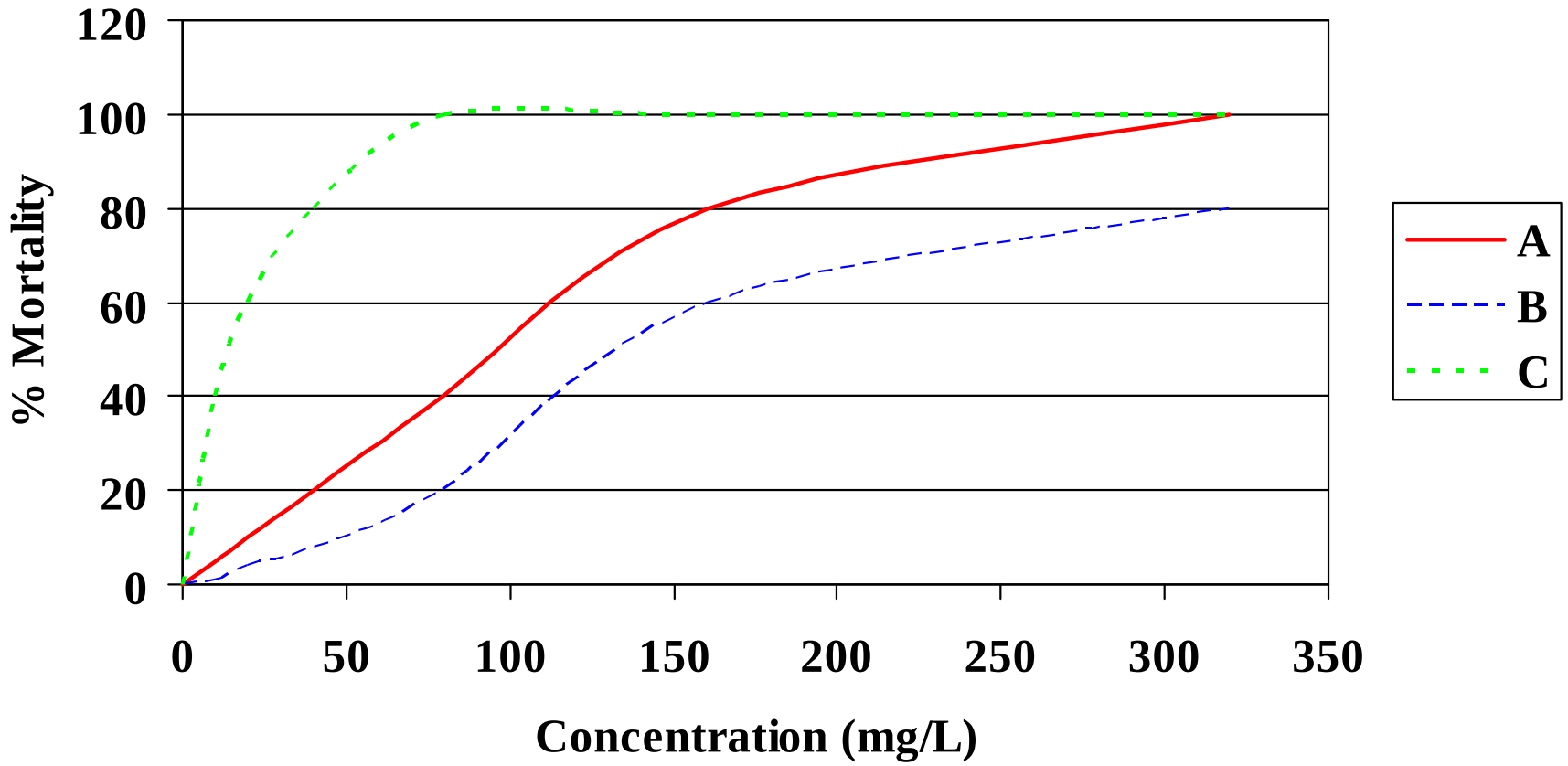
CHRONIC TOXICITY DATA

Fathead Minnow Early Life Stage Test
Growth Measured as Length

Concentration:

Control	0.0 µg/L	3.8 µg/L	7.5 µg/L	NOEC 15 µg/L	LOEC 30 µg/L	60 µg/L
						
Length:						
	40 mm	41 mm	38 mm	37 mm	25 mm	5 mm

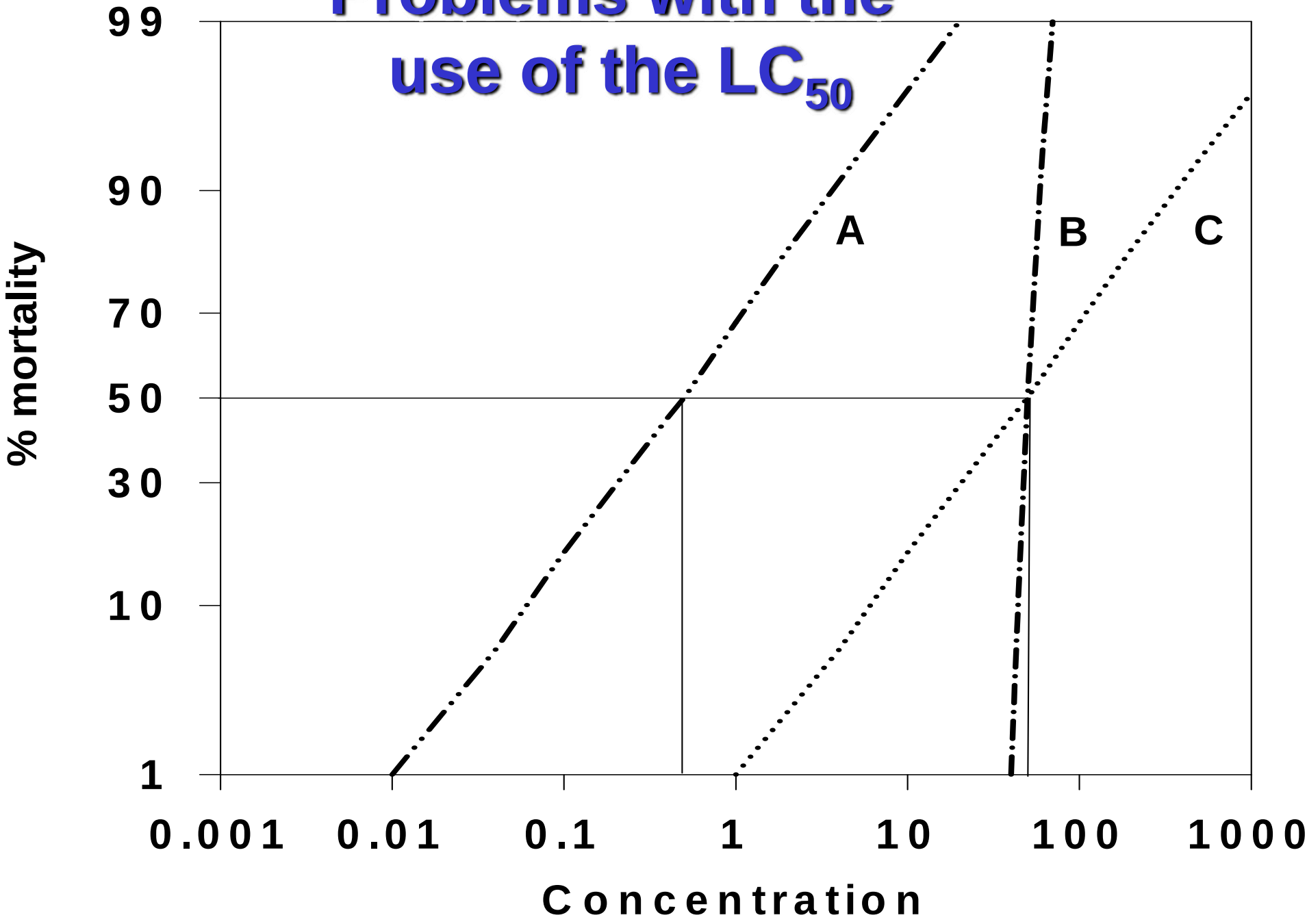
Dose Response Curve Review



Limitations of Dose-Response Data

- **Paucity of data**
- **Endpoint selection**
- **Limits of the dose-response model (extrapolation)**
- **Species sensitivity**

Problems with the use of the LC_{50}



Ecotoxicology Studies for U.S. Pesticide Registration

-Oral LD_{50} Duck

-Oral LD_{50} Quail

-Dietary LC_{50} Duck

-Dietary LC_{50} Quail

-Wild Mammal LC_{50}

-Duck Reproduction

-Quail Reproduction

-Acute LC_{50} Trout

-Acute LC_{50} Bluegill

-Acute LC_{50} Daphnia

-Acute LC_{50} Marine Fish

-Acute LC_{50} Marine Shrimp

-Oyster Shell Growth

-Fish Early Life Stage

-Invertebrate Life Cycle

-Fish Full Life Cycle

-Microcosms

-Mesocosms

-Nontarget Plants- Tier 1

-Nontarget Plants- Tier 2

-Field Testing- Tier 3

-Acute Honeybee

-Honeybee Foliage

-Field Testing

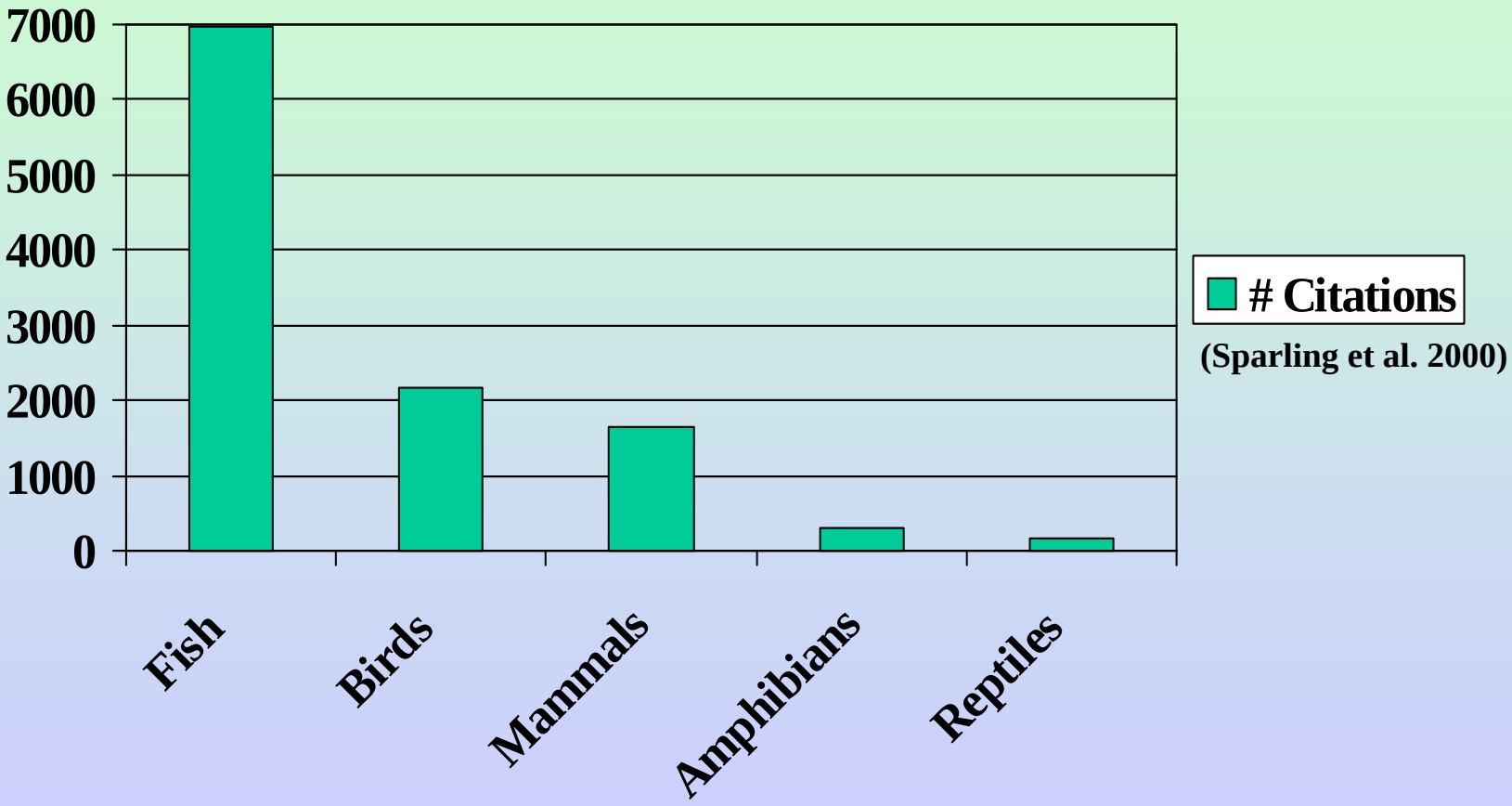
-Pollinators

Reptiles and Amphibians

- **Facts**
  - **There are approximately 4500 species of amphibians, and 6300 species of reptiles.**
  - **1972-1998 Wildlife Review and Sport Fisheries publications = 11,271**

    (Sparling et al. 2000)
    - **312 (2.7%) = amphibians**
    - **163 (1.4%) = reptiles**
  - **Amphibs & Herps are generally not included in assessments – surrogates are used.**

Research Citations



Toolbox – Sources of Toxicity Data

ECOTOX

acute toxicity;
chronic toxicity;
bioaccumulation;
sublethal effects;
chemical substance information;
supporting data
references to the original sources

http://www.epa.gov/ecotox/

Toolbox – Sources of Toxicity Data

USGS - ACUTE TOXICITY DATABASE

USGS Columbia Environmental Research Center (CERC).

4,901 acute toxicity tests toxicity tests conducted by CERC since 1965 with 410 chemicals and 66 species of aquatic animals

http://www.ecrc.cr.usgs.gov/acute.html

Biotic Factors Affecting Aquatic Results

- **Species**
- **Gender (reproductive status)**
- **Age (Life History Stage)**
- **Size**
- **Nutritional status**
- **Physiological condition of the organism**
- **Acclimation/holding time/transport**
- **Disease state**
- **Availability of “true” control or reference site**
- **Time of day (circadian rhythms)**
- **Wild or domestic stock**
- **Inbreeding**

Abiotic Factors Affecting Aquatic Toxicity Results

- **Exposure regimen/conditions**
- **Light intensity and periodicity**
- **Randomization, selection and arrangement of test chambers**
- **Randomization of animals in treatments**
- **Replication thru time and space**
- **Water quality parameters**

Comparison of Sheepshead Minnow Acute Toxicity for Aldicarb

Condition	**LC_{50} (ppb)**	**95% Confidence Interval (ppb)**
Static	168	102 - 320
Flow-through	41	55 - 72

Trifluralin Toxicity to Various Aquatic Receptors

Organism	**Temp (C)**	**Acute EC_{50}/LC_{50} (ppb- µg/L)**
Water flea (*D. pulex*)	21	560
Amphipod	21	2200
Bluegill	22	58
Rainbow trout	12	41
Fathead minnow	18	105
Channel catfish	22	2200

Water Quality Parameters Affecting Toxicity

- **Temperature**
- **pH and Alkalinity (buffering capacity)**
- **DO (Dissolved Oxygen)**
- **Hardness**

Temperature

- Effects on aquatic life

  Many rate constants in cold blooded animals double with 10°C ↑ T (increase metabolism and activity)

- Effects on chemical behavior:

  No single pattern, change T in a given direction may ↑, ↓ , or not change toxicity, but...

  As T ↑, biodegradation usually ↑

  As T ↑, solubility of organics ↑

  T ↑ generally ↑ toxicity if there is an effect

Temperature Examples

- Endrin is several hundred times more toxic at 27C than 7C for carp

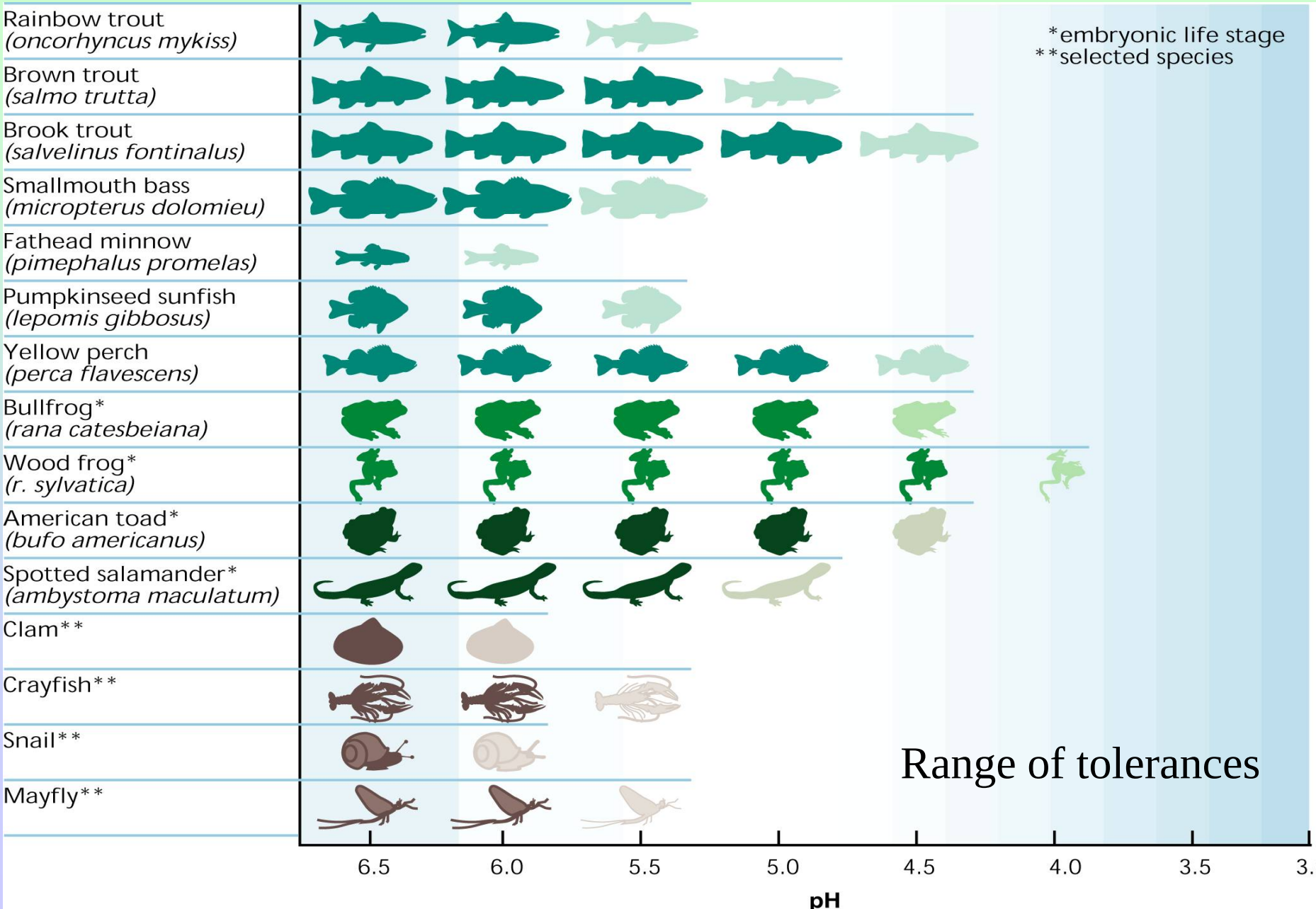
- Heptachlor is 5 times more toxic at 24C than 7C for sunfish

- As temp. decreases so does the toxicity of DDT and methoxychlor

Compensation:

- Changes in toxicity can be ameliorated with acclimation
- Effects generally not as pronounced for chronic toxicity

pH Effects on chemical behavior: pH causes greater changes in 96hr LC_{50}s than any other water chemistry parameter.



Hardness [Ca^{2+} and Mg^{2+}]

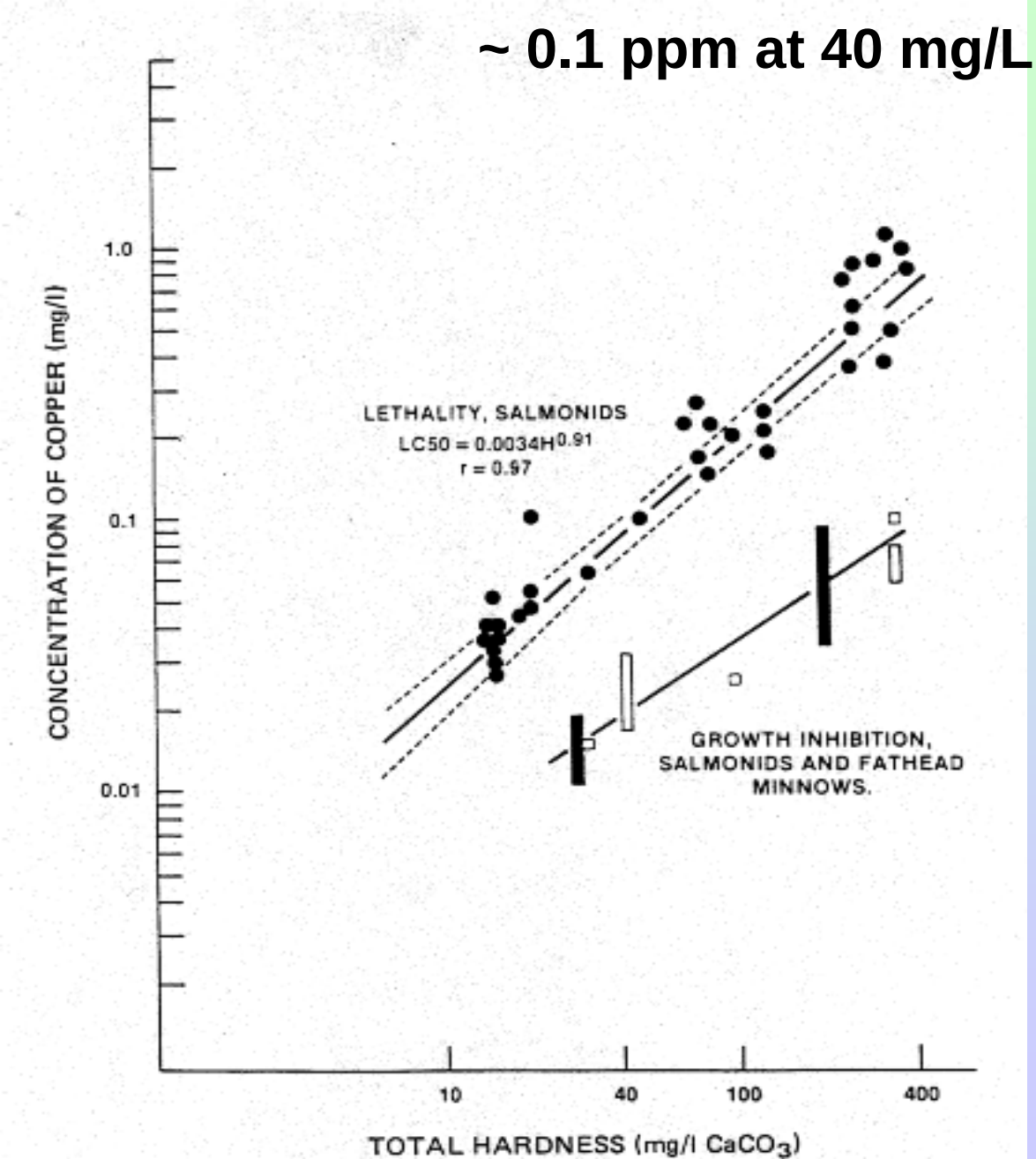
Hardness scale:

<75 mg/l (as $CaCO_3$)	Soft
75 to 150 mg/1	Moderately hard
150 to 300 mg/1	Hard
> 300 mg/1	Very Hard

Important relationship to toxicity of metals
- increased toxicity at lower hardness

Salmonid LC_{50} for Cu: **~1.0 ppm at 400 mg/L $CaCO_3$**

~ 0.1 ppm at 40 mg/L $CaCO_3$



Toxicity of Pesticides are in Fact Water Quality Dependent

- **TFM** (lampricide): higher toxicity in acidic, soft water than hard, alkaline water
- **Bayluscide** (molluscicide): higher toxicity in soft than hard water
- **Antimycin** (piscicide): higher toxicity in hard, neutral pH waters than in soft, high pH water.

Dissolved Oxygen (DO)

- Affects toxicity by adding stress
- Can alter toxicity by a factor of <2X for tests conducted at low DO

LC50s of Zn to Bluegills at Reduced Oxygen Levels (Sprauge 1985)

Oxygen (% saturation)	LC50 (mg/l)
67	11.3
38	10.6
21	7.3

Dissolved Oxygen (cont.)

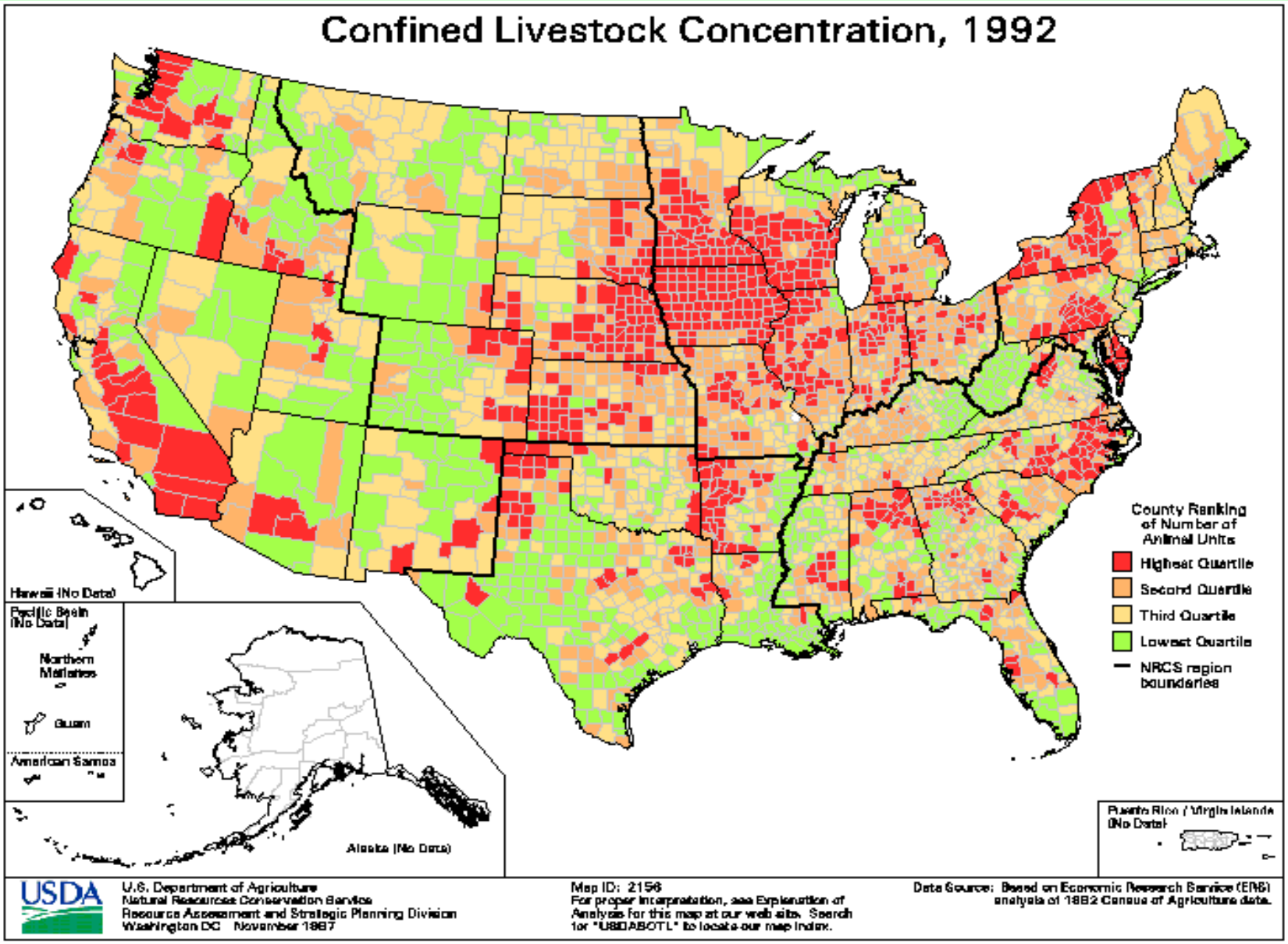
- Lethal levels are quite variable by species

- Sublethal effects include - feeding, growth, behavior, productivity

- Low DO and fish kills
  - perhaps the most common natural cause of kills
  - demand exceeds inputs
  - worst in calm, cloudy, hot weather

Fish Kill

Confined Animal Feeding Operations



Another Important Consideration: Sublethal Effects

- Decreased Fertility
- Altered Growth
- Deformities
- Slowed or Less Effective Movement
- Altered Behavior
- Effects on Organ, Tissue, Cell Hormone, or Enzyme Functions
- Genetic Defects

Sublethal Effects of Pesticides

Receptor	Pesticide	Sublethal Effect
Algae	Kepone	Reduced growth
Oyster	Chlordane	Reduced Shell growth
Fathead Minnow	Dursban	Altered schooling behavior
Fathead Minnow	Disulfoton	Altered schooling behavior
Fathead Minnow	Permethrin	Altered schooling behavior
Yellow Perch	Methoxychlor	Altered Respiration
Pinfish	Naled	Inhibited cholinesterase
Sheepshead Minnow (SHM)	Kepone	Scoliosis
SHM	Dursban	Avoidance
SHM	Malathion, DDT, Endrin, 2,4-D	Avoidance

Recent Literature

AUTHORS	JOURNAL	RESULT
Belden & Lydy	Environmental Toxicology & Chem. 2000	Atrazine ↑ toxicity of other pesticides in midge
Davidson, C.	Ecological Applications - 2004	↑ pesticide use (OPs/carbs) ↓ amphibian populations
Relyea, R.	Environmental Toxicology & Chem. 2004	Malathion ↑ toxicity to tadpoles with predators
Haendel et al.	Toxicological Sciences - 2004	Sodium metam developmental toxicity in zebrafish
Relyea, R.	Environmental Toxicology & Chem. 2004	Pest. mixtures ↓ survival and growth of tadpoles
Sandahl et al.	Environmental Toxicology & Chem. 2005	Chlorpyrifos ↓ swimming and feeding in salmonids

Endrocrine System

The endocrine system -- also referred to as the hormone system -- is made up of glands located throughout the body of organisms, hormones which are synthesized and secreted by the glands into the bloodstream, and receptors in the various target organs and tissues which recognize and respond to the hormones.

The function of the system is to regulate a wide range of biological processes, growth and function of reproductive systems (sexual characteristics), regulation of metabolism, brain and nervous system development, and development of an organism from conception through their entire life cycle.

Endrocrine Disruptors

Chemicals can disrupt the endocrine system by a range of mechanisms, but the impact of this disruption is often influenced by its timing, with development the most crucial time, and not as much as the dose.

A variety of chemicals are known to disrupt the endocrine systems of animals in laboratory studies, and compelling evidence has accumulated that endocrine systems of certain fish and wildlife have been affected by chemical contaminants, resulting in developmental abnormalities and reproductive impairment.

Endocrine Disrupters in the Boardroom

Pesticides with Known or Suspected Endrocrine Disruptor Properties

2,4,5-T	DBCP	h-epoxide	nitrofen
2,4-D	DDT	kelthane	oxychlordane
alachlor	DDD, DDE	kepone	permethrin
aldicarb	dicofol	malathion	synthetic pyrethroids
amitrole	dieldrin	mancozeb	toxaphene
atrazine	endosulfan	maneb	transnonachlor
benomyl	esfenvalerate	methoxychlor	tributyltin oxide
beta-HCH	ethylparathion	metiram	trifluralin
carbaryl	fenvalerate	metribuzin	vinclozolin
chlordane	lindane	methomyl	zineb
cypermethrin	heptachlor	mirex	ziram

Atrazine's effects on aquatic receptors

Summary of Atrazine's Current Uses

Most commonly used herbicide in U.S.

(79 million lbs –1994)

- **<u>Agricultural:</u>**
  - **Corn (field and sweet), guavas, macadamia, sorghum, sugarcane, wheat**
- **<u>Non-Agricultural:</u>**
  - **Golf courses, forestry, sod, residential (FL and Southeastern US)**
- **<u>Other:</u>**
  - **Range grasses for establishing grass cover in OK, NE, TX, OR**

Aquatic Ecological Threshold Effect Levels Identified by EPA

- **EPA review of data suggests detrimental effects to aquatic systems at 10-20 ug/L atrazine**

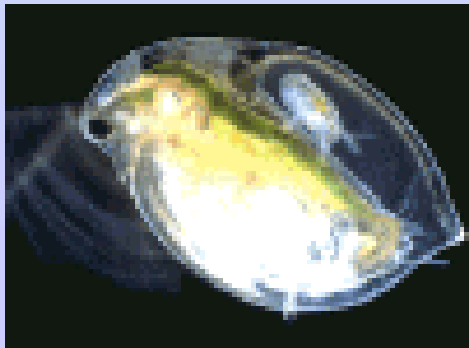
- **EPA's review was completed in Oct. 2003**
  - **The SAP concluded that there was conflicting science and that the manufacturer will conduct a monitoring program but that no new mitigation measures were needed at this time.**

- **Listed as endocrine disruptor-**
  - **Induction of aromatase which catalyzes conversion of testosterone to estrogen**

Summary of Potential Endocrine Modulating Effects of Atrazine to Invertebrates

- **Increased production of male *Daphnia pulicaria* at atrazine concentrations as low as 0.5 µg/L (?)**

- **A study conducted under conditions of continuous food supply demonstrated no effects on male induction in *D. pulicaria* at concentrations up to 87 µg/L**



Summary of Potential Endocrine Modulating Effects of Atrazine to Reptiles

- **Red-eared turtles**
  - **Reduction in proportion of males produced at 1, 10 and 100 µg/egg dissolved in solvent**
  - **No effects on sex ratios when eggs treated with 350 µg/L atrazine in water**

*Summary of Potential Endocrine Modulating Effects of Atrazine to Amphibians (*Xenopus laevis)

- **4% increased incidence of testicular abnormalities at 25 µg/L**
- **16-20% increased incidence in testicular abnormalities at 0.1 µg/L**

- **Altered male metamorph with laryngeal muscle size at greater than 1 µg/L**
- **No effect on male metamorph laryngeal muscle size at much higher concentrations**

Effects of Atrazine to Amphibians cont.

- **Change in rate of metamorphosis and corticosterone concentrations in *Ambystoma tigrinum* (salamander) at 75 µg/L but not 250 µg/L**

Questions?


... AND THEN, AFTER SPENDING SEVERAL YEARS ABSORBING GOODNESS KNOWS WHAT TOXINS, IN SOME OF THE MOST HEAVILY POLLUTED WATERS ON THE PLANET, I DRAGGED MY MERCURY-LADEN CARCASS BACK UP THIS STREAM TO DIE... BON APPÉTIT.
PTTTOOOO