WASHINGTON STATE UNIVERSITY

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Fall 2005

ES/RP 531
Fundamentals of Environmental Toxicology

Lecture 10

Developmental & Reproductive Toxicity
Part I--Developmental Hazards

Historical Aspects

- Teraton (Greek root for "monster"
- Teratogenesis--older term given to developmental problems, especially malformations
  - Supplanted today by more common usage of the phrase "developmental toxicity"
- Rubella was first "teratogen"
  - Mothers exposed to virus had higher probability of congenital cataracts
  - Two important observations
    - Time of exposure during pregnancy was important to incidence
    - Virus had to cross the placenta
- Radiation was second noted teratogen

Historical Aspects

- Post 1950: the tragedy of thalidomide
  - Given to women in Europe, Australia, and Japan as anti nausea drug and sedative
  - Over 8000 babies born with severe limb and extremities malformations
  - Not approved in U.S. because no developmental studies
    - In these studies pregnant rats are dosed
  - Lesson learned
    - Compounds innocuous to the mother could be teratogenic
    - A lot of species variation in sensitivity is possible
      - For example, the lowest dose causing malformations in humans is <1 mg/kg/day but in rats it is 50 mg/kg/day

Basic Principles

- Developmental toxicity definition
  - Any structural or functional alteration, reversible or irreversible, caused by an environmental insult, which interferes with
    - Homeostasis
    - Normal growth
    - Differentiation
    - Development (in the context of functionality)
    - Behavior

Basic Principles

- Targets for Effects:
  - Fertilized egg or zygote
  - Embryo during organogenesis
  - Fetus in postembryonic period of histogenesis and the neonatal or early postnatal period

Basic Principles

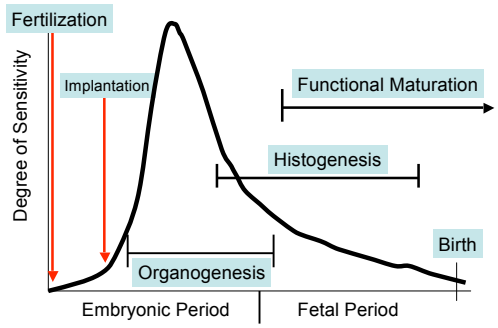
- Expressions of Developmental Toxicity
  - Death
  - Structural malformations
  - Functional deficits
  - Developmental delays

Why Is the Embryo So Vulnerable?

- Embryo composed of small mostly undifferentiated cells with limited detoxification or repair mechanisms
- Need for proper spatial and temporal sequencing of specific cell numbers and types and specific cell products for normal differentiation
  - includes need for programmed cell death
- Sensitivities of certain cell types to insults unique to specific periods of cell movement, induction, or differentiation
- Immunosuppressive system that recognizeds "self" and detects/repiars toxicant or lesions my be absent or undeveloped

Karnofsky's Law

- Any toxicant at proper dosage can cause disturbances in embryonic development
  - Threshold effect
  - Variation in sensitivity with stage of development



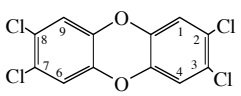
Developmental Stages
& Their Relative Sensitivity to Teratogens

Mechanisms Obscure

- General mechanism
  - Selective cell death
  - Altered biosynthesis leading to structural or functional malformations
  - Energy inhibition acting to slow grown
- Specific mechanisms hypothesized
  - TCDD
    - Interaction with the epidermal growth factor receptors
  - Retinoic acid
    - Interaction with retinoic receptors

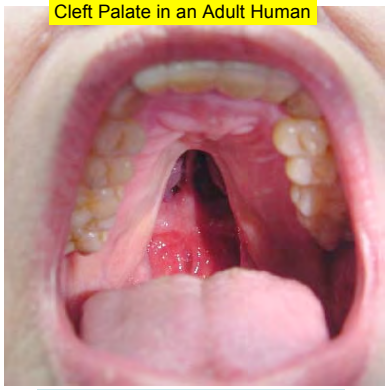
TCDD

- One of 78 congeners of polychlorinated dibenzodioxins
- Causes cleft palate in neonatal rats exposed during gestation



2,3,7,8-tetrachlorodibenzo-*p*-dioxin

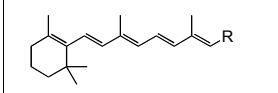
Cleft Palate in an Adult Human



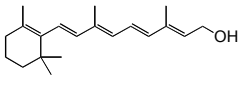
URL: www.ghorayeb.com/ CleftPalate.html

Retinoic Acid

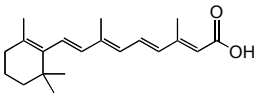
- Vitamin A metabolites
  - Precursors of vitamin A are metabolized to its nutrient form, retinol
    - Retinol is a type of retinoid, one type of biologically active terpenoid compound (remember juvenile hormone and methyl farnesoate from previous lectures)
  - Derivatives of retinol, including retinoic acid, bind to retinoid receptors and control cell differentiation in many embryonic epithelial tissues and prevent metaplasias (transformation of one differentiated cell type into another type)
- Isotrentinon (13-cis-retinoic acid) causes structural craniofacial abnormalities and head and limb defects in newborn infants
  - Sold by prescription as Accutane for treatment of severe acne



General Structure for Parent Retinoids

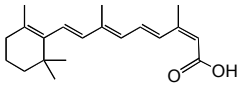


retinol (dietary Vitamin A)

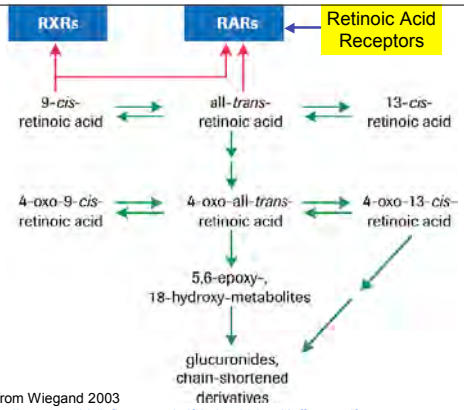


retinoic acid

(all trans-tetinoic acid)



13 cis-retinoic acid (isotretinon)



Copied from Wiegand 2003
URL: http://www.touchbriefings.com/pdf/27/ept031_t_Hoffman.pdf

ENVIRONMENT

Bad Days on the Lily Pad

Deformed frogs are turning up in dozens of states, and scientists finally have suspects

BY SHARON BEGLEY

VERY TIME HERPETOLOGIST Heppe goes for a nature wal

Global Amphibian Decline

- Possibility of global pattern of decline reported at the First World Congress of Herpetology in 1989
- By 1993, more than 500 populations of frogs and salamanders on five continents were listed as declining or of conservation concern
- Many of the declines first noted in comparatively pristine habitats at higher elevations

Possible Causes of Declines

- Ultraviolet radiation
  - Ozone hole-related??
- Predation
  - Introduction of predatory fish
- Interspecific competition with introduced species
- Habitat modification
  - Removal of trees
  - Drainage of wetlands
  - Changes in vegetation structure

Possible Causes of Declines

- Water Quality
  - Changes in pH
  - Contaminants, including pesticides
- Increased parasitism (disease)
- Global climate change
- Interactions among environmental factors
  - "Synergism"

Adults with Tails

Deformed Tails

Extra Feet

Fused Limbs

Missing Legs

Missing Eyes

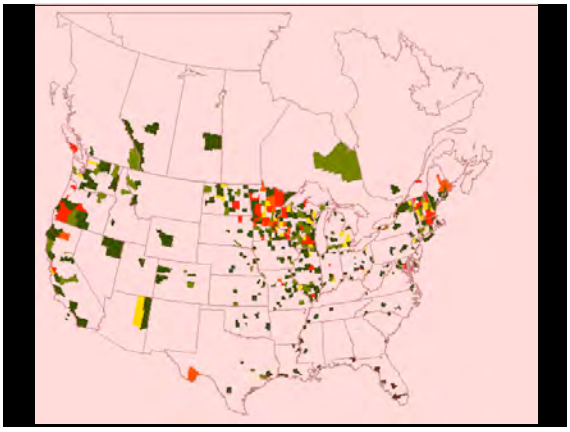
A Plague on Frogs?????

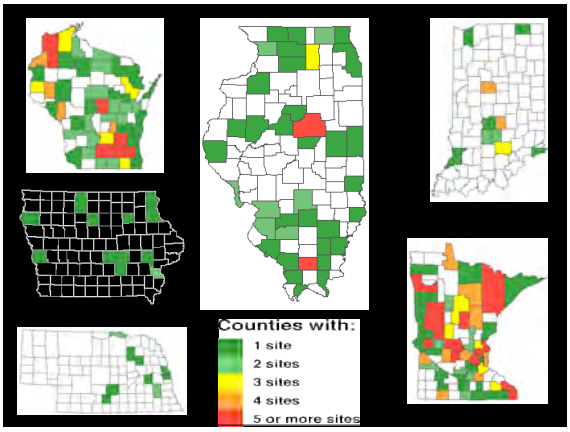
High Yield Farming for Gourmet Restaurants??

NORTH AMERICAN AMPHIBIAN MONITORING PROGRAM

NAAMP

Northern Prairie Wildlife Research Center
Biological Resources Division
U.S. Geological Survey
Jamestown, North Dakota
http://www.npwrc.usgs.gov/narcam

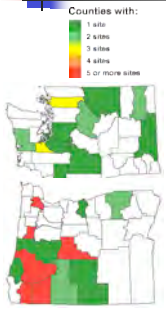




Malformed Misperception?

State	Number Frogs Examined	Percent Malformed
Illinois	1,496	0.6
Indiana	3,566	0
Iowa	504	1.8
Minnesota	15,002	6.4
Wisconsin	2,104	1.9

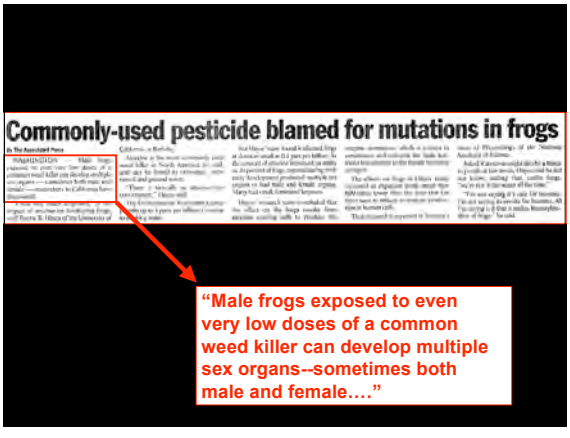
Problems in Paradise??



State	# Frogs	% Malformed
Washington	471	10.8
Oregon	455	18.5

When It Comes to Frogs, Semantics Matter

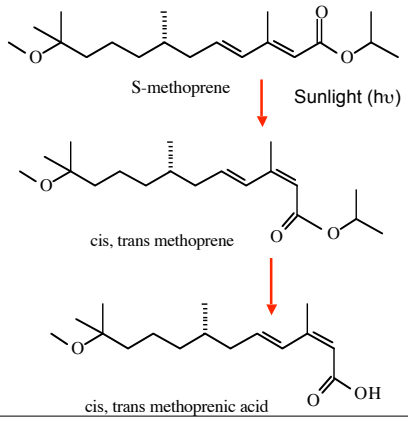
- Sower et al. report in the abstract of their published paper [Environ. Health Perspectives 108 (11):1085] that 81% of the sites sampled in New Hampshire had frogs with malformations (n=13 of 16 sites studied)
- However, when the article is read, we find out that 1,436 frogs were examined and the total malformation rate was only 3.9%
  - 4.3% among bullfrogs (n=42 of 983 frogs)
  - 2.4% among green frogs (n=11 of 453 frogs)

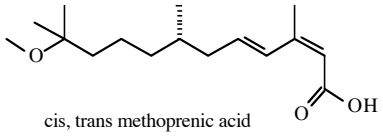


Retinoic Acid Like Products and Frogs

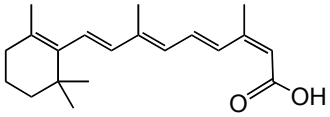
- Methoprene, an insect growth regulator, breaks down in sunlight, giving retinoic acid type structures
  - Methoprene is a juvenile hormone analog used as an insecticide to control the larval stages of mosquitoes and fleas
- Frogs exposed in an assay called FETAX (Frog Embryo Teratogenesis Assay) developed abnormalities in the craniofacial region

La Clair et al. (1998) Environ. Sci. Technol. 32:1453





Note the structural similarities between these retinoids



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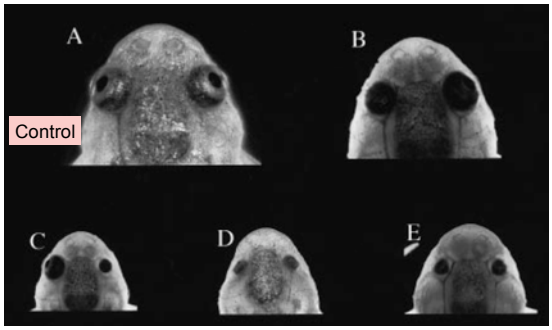
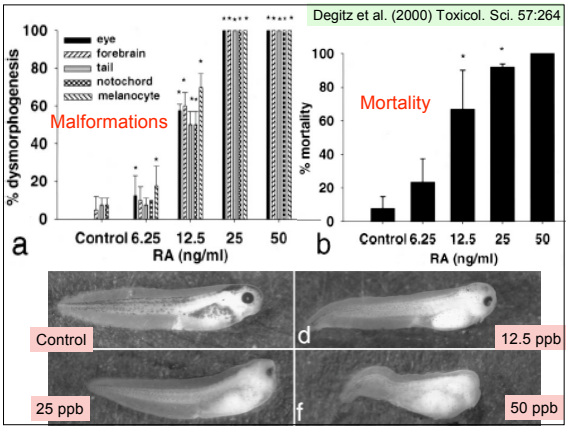


La Clair et al. (1998) Environ. Sci. Technol. 32:1453

Can Retinoids Be Causative Agents in Skeletal Malformations of Frogs?

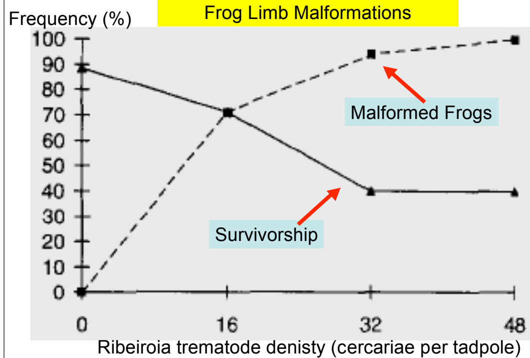
- Degitz et al. (2000, 2003) studied this question and concluded that frogs would die before they manifested an skeletal abnormalities
  - Abnormalities at low concentrations were located in the cranofacial region
  - However, the concentrations required to produce abnormalities were likely to also be acutely toxic (lethal)



Other Hypothesis for Limb Malformations

Parasitic worms blamed for deformities in frogs

Effect of Trematode Parasites on Frog Limb Malformations



Johnson et al. (1999) Science 284:802

Teratogenicity of Metals in FETAX Assay

Metal	EC50 (µM)	LC50 (µM)	Teratogenesis Index	LOAEL for Growth Inhibition (FETAX)
As	285	1400	4.9	466
Cd	3.7	32	8.6	18
Cu	2.5	22	8.8	10
Hg	<0.2	0.3	-	-
Zn	40	850	21	300

Sunderman (2000) Chap. 54, p. 1203, Gen'l. Appl. Toxicol.