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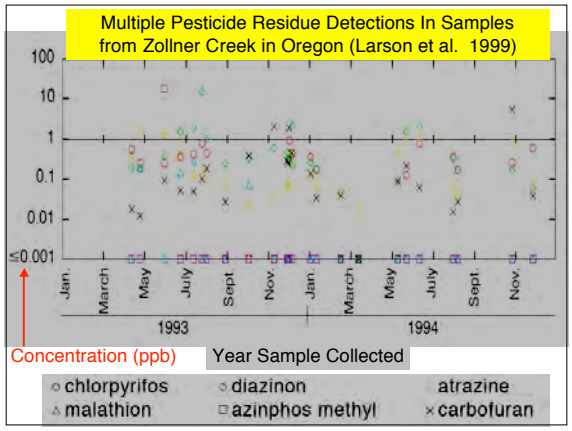
# ES/RP 531
# Fundamentals of Environmental Toxicology

Lecture 19
Chemical Interactions & Mixtures
(Dose/Response Assessment 2)

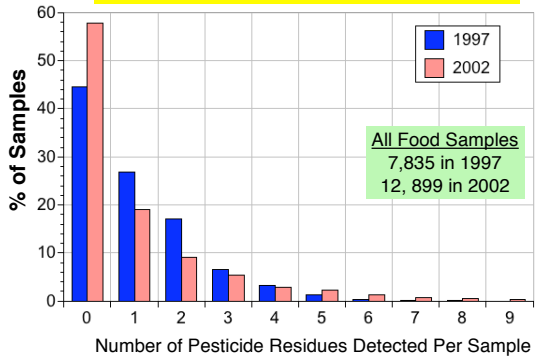
## *Chemical Mixtures & Interactions-- Why Care?*

- PNW waters with multiple pesticide residues
- Multiple pesticide residues on certain commodities
- Routine use of multiple pesticides & surfactants in tank mixes of pesticides
- Ubiquitous residues of multiple congeners of PAHs, PCBs, dioxins (PCDDs)
- Household consumer products are formulations are mixtures
- Gasoline is a mixture of solvents
- Heavy metals

Multiple Pesticide Residue Detections In Samples from Zollner Creek in Oregon (Larson et al. 1999)




**USDA Pesticide Data Program '97 & '02**
**Percentage of Samples with Multiple Residues**




## *Chemical Interactions & Biological Responses*

- It's All Natural
- Chemical Interaction Basics
- Chemical Mixtures & Health
- Chemical Mixtures & Ecological Effects
- Chemical Mixtures & Agroecosystems

## *You Are What You Eat*

**Selected Flavor Components of Camembert Cheese**

- **Acetic acid**
- **Propionic acid**
- **Butanoic acid**
- **Oleic acid**
- ***Methanol***
- ***Ethanol***
- **Octanol**
- ***Acetone***
- ***Ethyl acetate***
- **Diethyl phthalate**
- **Hydrogen sulfide**
- **Methyl mercaptan**
- **Phenol**
- **Cresol**

*Sable & Cottenceau 1999 JAFC 47:4825*

Isabella Macander displays a large brick of Swiss cheese Friday at the Polonez Gourmet Delicatessen in Silver Spring, Md. It seems the holes in traditional Swiss are so large the cheese gets torn up in new high-speed slicing machines used in the food service industry. Cheese makers have asked the Agriculture Department to change its standards to allow for smaller holes in Grade A Swiss.

Government ponders size of cheese holes

By The Associated Press

WASHINGTON — Anyway you slice it, the Swiss on your ham and cheese isn't going to be the same.

It seems the holes in traditional Swiss are so large that the cheese gets torn up in the new high-speed slicing machines used by the food service industry. So, cheese makers have asked the Agriculture Department to change its standards to allow for smaller holes — or "eyes" — in Grade A Swiss.

e-mail to USDA. "Busywork is one thing (and usually indicates a pressing need to downsize), but this is patently ridiculous."

Babagalleh Barry, who manages a deli in Bethesda, Md., doesn't have any problem with the size of the holes in his Swiss. But, he said, "If it's a little smaller that might be easier to slice."

Taxpayers won't be out a nickel, USDA officials say. The entire cost of changing the standard, including mail

## *Possible Chemical Interactions*

Neutrality--no measurable effect

Additivity

1X dose A + 1X dose B = 2X effect

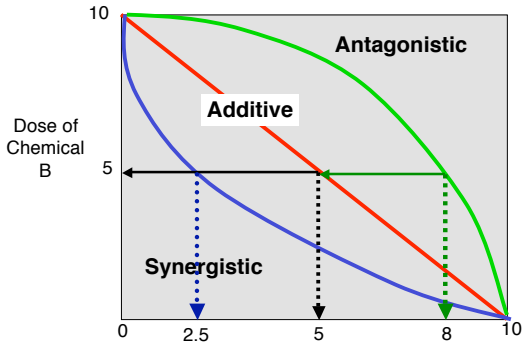
Synergism & Potentiation

1X dose A + 1X dose B = 10X effect

Antagonism

1X dose A + 1X dose B = 0.5X effect

Isoboles (Lines of Equal Effect) for Chemical Interactions




## *Toxic Units Approach*

$$TU_{mix} = \frac{LC_{50}\ A_{(mix)}}{LC_{50}\ A_{(alone)}} + \frac{LC_{50}\ B_{(mix)}}{LC_{50}\ B_{(alone)}}$$

- If TU = 1, then interaction is additive
- If TU < 1, then synergistic
- If TU > 1, then antagonistic
- Note: have to allow for variability in responses; thus, values as low as 0.8 would still be additive

Interaction of OP Insecticides Found in Western Waters

***Interactions Are Additive***

| Test ID | $LC_{50}$ ppb in water | | Toxic Units |
|---|---|---|---|
| | Diazinon | Chlorpyrifos | |
| Single Component | 0.43 | 0.068 | |
| Mixture | 0.25 | 0.032 | 1.1 |

(Bailey et al. 1997 ETAC 16:2304)

***Synergism Between an Insecticide & Herbicide Effects on Oat Growth***

| Phorate Dosage (ppm) | Diuron Dosage (ppm) | | | | |
|---|---|---|---|---|---|
| | 0 | 0.25 | 0.50 | 0.75 | 1.00 |
| 0 | 1.98 | 1.95 | 2.28 | 1.86 | 1.83 |
| 3 | 1.90 | 1.90 | 2.00 | 1.89 | 1.20 |
| 6 | 1.71 | 1.76 | 2.11 | 1.75 | 0.90 |
| 9 | 1.79 | 1.95 | 1.84 | 1.43 | 0.68 |
| 12 | 1.86 | 1.92 | 1.91 | 1.32 | 0.62 |

Nash 1981 Weed Sci. 29:147

## Need Data?

| Number of Chemicals | Number of Tests | Cost of Entire Test Series ($Millions) | | | |
|---|---|---|---|---|---|
| | | Cost of Individual Test | | | |
| | | 1,000 | 10,000 | 100,000 | 1,000,000 |
| 1 | 1 | 0.001 | 0.01 | 0.1 | 1 |
| 2 | 2 | 0.002 | 0.02 | 0.2 | 2 |
| 5 | 120 | 0.12 | 1.2 | 12 | 120 |
| 10 | 3,628,800 | 3,629 | 36,288 | 362,880 | 3,628,800 |

## Cannot Practically Test Every Combination

- Rely on basic biochemical mechanisms of toxic action for individual compounds
  - Determine whether mode of action is similar or dissimilar between chemicals
- Rely on metabolic pathway and clearance (excretion) rates
  - Toxicokinetics
  - Determine whether one chemical could interfere with metabolism of other

## Mode of Action

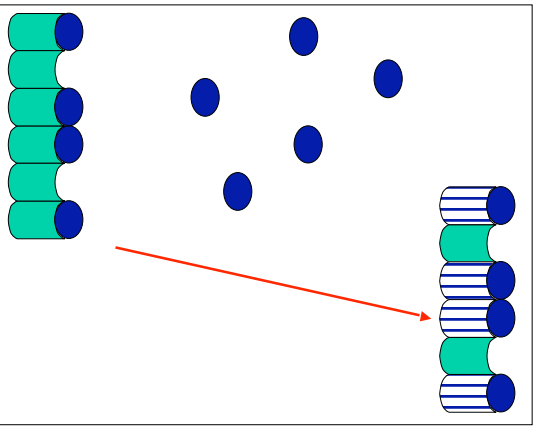
- Acetylcholinesterase inhibition
  - OP & carbamate insecticides
- Nerve membrane interactions (Na gate)
  - DDT, pyrethroid insecticides
- Amino acid synthesis inhibition
  - SU, imidazolinone herbicides
  - Glyphosate
- Auxin agonists
  - Phenoxy and pyridinyl carboxylate herbicides

## Joint Toxicity & Same MOA

- Invertebrate toxicity & OP insecticides
  - Additive interactions shown
  - Concentrations a significant percentage of $LC_{50}$
    - ✓ The 99th percentile levels of chlorpyrifos and diazinon in some basins (San Joaquin-Tulare) are within the range needed to produce measurable additive interactions
  - No studies on interactions at known environmental levels (ppt)

## Joint Toxicity & Same MOA

- Must consider concentration of chemicals in the mix
  - If concentrations are significantly below the NOEL, they are likely not going to interact sufficiently with the biochemical target site to cause a physiological effect
  - Must consider affinity (Km) and binding constants (Ki)
    - ✓ Kinetics are concentration dependent



## Joint Toxicity & Dissimilar MOA

- Invertebrate toxicity & OP/Herbicide mixtures
  - Atrazine concluded to synergize several OPs (Pape-Lindstrom & Lydy, 1997, ETAC 16:2415-2420)
    - ✓ OPs were significant percentage of $LC_{50}$
  - However, atrazine levels ranged between 5,000 and 20,000 ppb
    - ✓ Maximum level of atrazine in Willamette Basin was **4.5 ppb**
  - **Atrazine & methoxychlor were anatagonistic!!**

## All Chemicals Considered….

- Four basic metabolic pathways
  - Oxidations
    - ✓ Microsomal oxidases (cytochrome P-450 isozyme complex)
  - Hydrolyses
    - ✓ Esterase enzymes
  - Reductions
  - Conjugations
    - ✓ After oxidation or hydrolysis, linkage to a peptide or sugar and excretion
    - ✓ Involves glutathione transferases

## OP Insecticide Metabolism

- Most current OP insecticides need to be metabolically activated in body
  - P=S ----------> P=O transformation
    - ✓ **Mediated by P-450 oxidation**
- P-450 could also detoxify OPs
- Esterases detoxify OPs
- Glutathione transferases important in detoxifying certain insecticides and herbicides

## Predicting OP Toxicity When in Mixtures

- Interactions Mediated Through P-450
  - If P450 is inhibited, then toxicity will change (+ or -)
  - If P450 is induced, then toxicity will change (+ or -)
- Interactions Mediated Through Esterases
  - If esterases are inhibited, then toxicity will increase

## Well Known Synergists

- Methylene dioxyphenyls
  - Piperonyl butoxide (PBO)
  - Secondary plant compounds
    - ✓ myristicin (parsnips & carrots)
    - ✓ piperonal & piperine (black pepper)
  - Both inhibit cyt. P-450 & induce it
    - ✓ Can synergize compounds like phorate
    - ✓ Definitely known to synergize pyrethrum

## A Case of OP Synergism

- Malathion is detoxified by an esterase known as carboxyesterase
- Carboxyesterases can by inhibited by certain OP insecticides
  - EPN
  - isomalathion
    - ✓ A toxic rearrangement product (or by-product of manufacture) that can be produced under certain conditions in malathion formulations

## *Dealing with Toxicity of Mixtures & "Aggregating" Measures of Environmental Concentration & Potential Exposure*

- Toxic Equivalents (TEQ)
- Toxic Equivalency Factor (TEF)

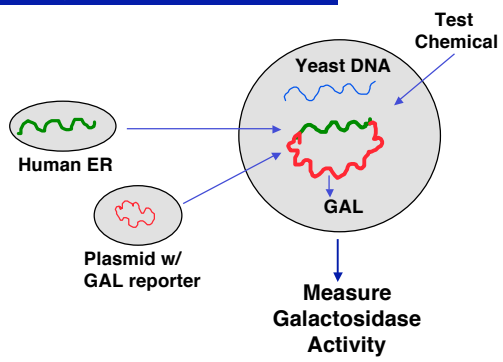
$$\mathbf{TEQ}_{2,3,7\text{-}8TCDD} = \text{sum}[PCDD_i \times TEF_i] + \text{sum}[PCDF_i \times TEF_i]$$

| Congener | EPA TEFs | Recent WHO recommendation |
|---|---|---|
| 2,3,78-TetraCDD (TCDD) | 1 | 1 |
| 1,2,3,7,8-PentaCDD | 0.5 | 1 |
| 1,2,3,4,7,8-HexaCDD | 0.1 | 0.1 |
| 1,2,3,6,7,8-HexaCDD | 0.1 | 0.1 |
| 1,2,3,7,8,9-HexaCDD | 0. 1 | 0.1 |
| 1,2,3,4,6,7,8-HeptaCDD | 0.01 | 0.01 |
| OctaCDD | 0.001 | 0.0001 |
| | | |
| 2,3,78-TetraCDF | 0.1 | 0.1 |
| 1,2,3,7,8-PentaCDF | 0.05 | 0.05 |
| 2,3,4,7,8-PentaCDF | 0.5 | 0.5 |
| 1,2,3,4,7,8-HexaCDF | 0.1 | 0.1 |
| 1,2,3,6,7,8-HexaCDF | 0.1 | 0.1 |
| 1,2,3,7,8,9-HexaCDF | 0.1 | 0.1 |
| 2,3,4,6,7,8-HexaCDF | 0.1 | 0.1 |
| 1,2,3,4,6,7,8-HeptaCDF | 0.01 | 0.01 |
| 1,2,3,4,7,8,9-HeptaCDF | 0.01 | 0.01 |
| OctaCDF | 0.001 | 0.0001 |

## *Recent Scares*

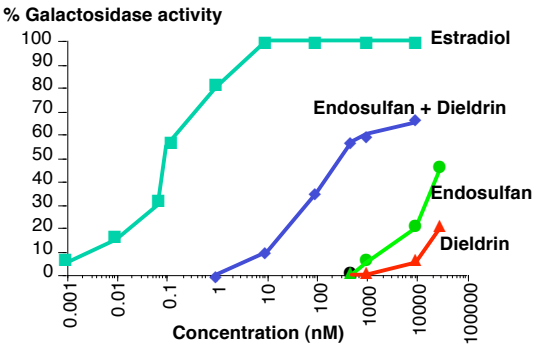
- Synergism among pesticides that are endocrine disrupters
- Bad brains & kids

## Yeast Estrogen System

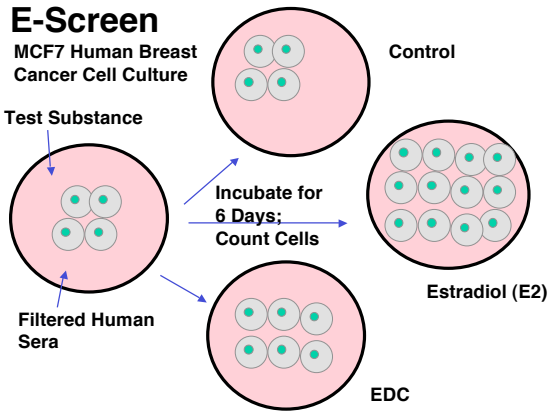


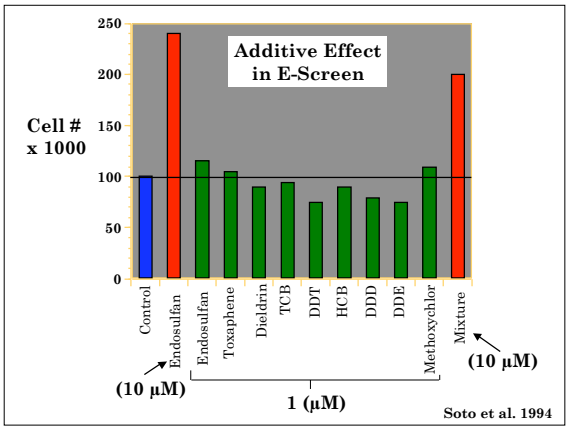

## *Endocrine Disrupter Synergism?*

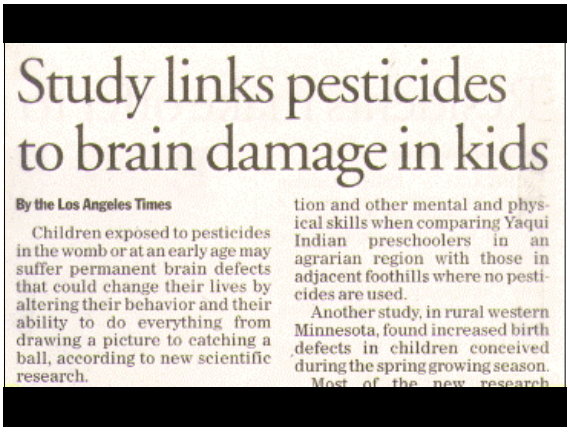
Arnold et al. 1996




## E-Screen





Study links pesticides to brain damage in kids

By the Los Angeles Times

Children exposed to pesticides in the womb or at an early age may suffer permanent brain defects that could change their lives by altering their behavior and their ability to do everything from drawing a picture to catching a ball, according to new scientific research.

tion and other mental and physical skills when comparing Yaqui Indian preschoolers in an agrarian region with those in adjacent foothills where no pesticides are used.

Another study, in rural western Minnesota, found increased birth defects in children conceived during the spring growing season.

Most of the new research

## *The Porter "Bad Brains" Study*

- Gave young rats drinking water with mixtures of nitrate, aldicarb, and atrazine
  - Concentrations were ~ 3X the MCLs (Maximum Contaminant Level)
  - Dosing duration on order of weeks
- Parameters measured
  - Spleen weight
  - Thyroid hormone levels
  - Immunocompetency (plaque forming ability)
  - Behavior (aggression index)

## *The Porter "Bad Brains" Study*

- Exposure tests repeated up to 8 times over a two year period
- Conclusions
  - Mixtures of chemicals caused adverse biochemical and behavioral effects not observed with single chemical exposures

**Ratio of Number of Experiments with Calculated Statistically Significant Outcomes Relative to the Total Number of Experiments (Porter et al. 1999)**

| Exposure Mix | Spleen Weight | Thyroid Hormone | Aggression Score | Plaque Assay |
|---|---|---|---|---|
| aldicarb (ald) | 1/7 | 1/9 | 0/6 | 1/8 |
| atrazine (atz) | 0/7 | 0/9 | 0/6 | 1/8 |
| nitrate (N) | 0/7 | 0/9 | 1/6 | 0/8 |
| ald-atz | 1/7 | 0/9 | 0/6 | 2/8 |
| ald-N | 1/7 | 0/9 | 0/6 | 2/8 |
| atz-N | 0/7 | 1/9 | 1/6 | 3/8 |
| ald-atz-N | 0/7 | 1/9 | 1/6 | 0/8 |

## *Conclusions*

- Impossible to study all mixture combinations
- Can predict effects by studying basic biochemical mechanism and two compound interactions
- Interactive effects occur at doses that represent substantial percentages of the $LD_{50}$
  - Usually additive
- Synergisms actually rare
- Interactions at environmentally relevant concentrations unlikely, but not well studied