November 5, 2003

Lecture 17: Chemical Mixtures & Interactions (Dose-Response Assessment 2)

I. Living in a Chemical Soup

A. Although the vast majority of risk assessments are conducted on chemicals one at a time, exposure is to a plethora of chemicals, naturally occurring and synthetic.

B. No one seems to flinch at the prospects of simultaneous or sequential multiple chemical exposure when we eat food.

1. However, food is full of bioactive secondary plant metabolites that have as great a probability of testing positive in rodent assays for carcinogenicity or for interactions with the endocrine system that could be interpreted as adverse if viewed from the same perspective that we take for synthetic chemicals.
2. For example, here is a list of selected compounds that have been found in Camembert cheese and are associated with its complex flavor (Sable & Cottenceau 1999, J. Agric. Food Chem. 47:4825.
   a. Acetic acid; propionic acid; butanoic acid; oleic acid; methanol; ethanol; octanol; acetone; ethyl acetate; diethyl phthalate; hydrogen sulfide; methyl mercaptan; phenol; cresol.

C. An examination of pesticide residues in food by the USDA Pesticide Data Program, shows that two or more different types of pesticide residues occur together in about 25% of analyzed foods. (Figure 1)

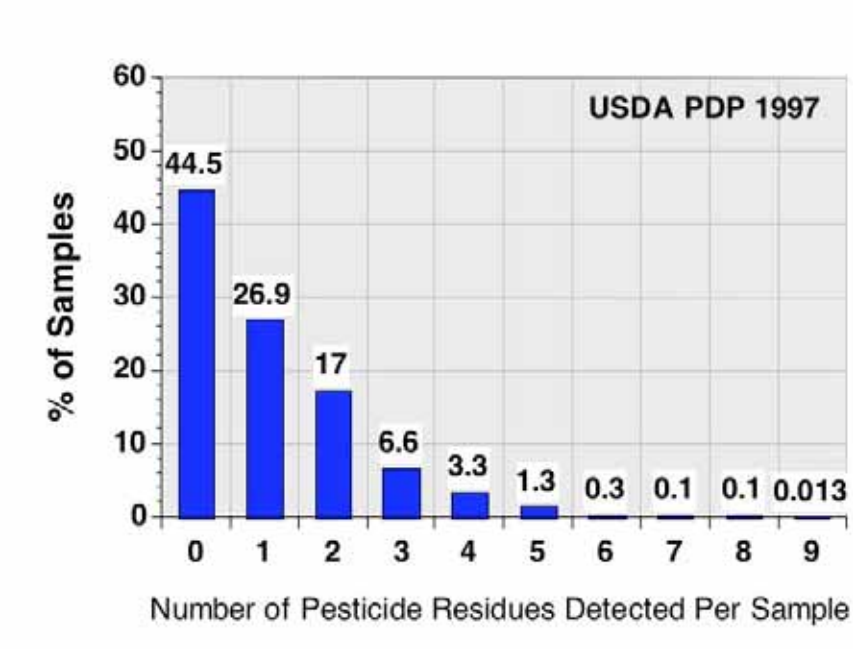

Figure 1. Proportion of food samples analyzed by the USDA in its Pesticide Data Program (PDP) that have one or more pesticide residues detected.

D. The USGS NAWQA program shows multiple detections of some pesticides in the same sample.

1. The most frequent simultaneous occurrence is atrazine and some other herbicide. More infrequent are insecticide detections, but in Oregon, along the Willamette Basin tributaries, a number of insecticides were found simultaneously in water samples. (Figure 2)

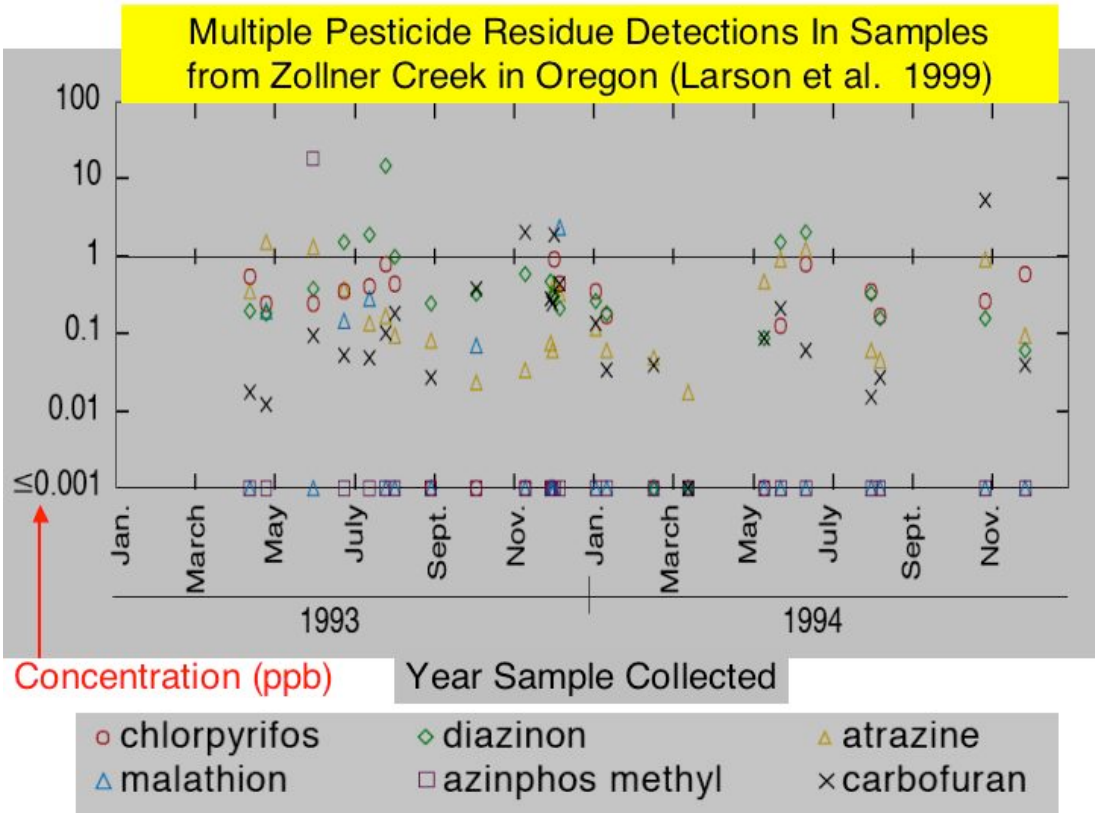

Figure 2. Detection of multiple pesticide residues in water samples collected from Zollner Creek in the Willamette Basin watershed of Oregon (Larson et al. 1999)

E. Under the Food Quality Protection Act, which is a 1996 amendment to the overarching federal pesticide regulatory law, FIFRA (Federal Insecticide Fungicide and Rodenticide Act), EPA was mandated by Congress to cumulate exposure for risk assessment when multiple residues of compounds with identical mechanisms of toxicity (i.e., identical pharmacodynamics) were present in food and/or water.
   1. Thus regulatory science policy, at least for pesticide regulations, must take into account multiple occurrence of residues, although they must have the same mechanism of causing toxicity.
      a. The OP insecticides were the first subjected to this "mixture" exposure analysis because they all have the same basic mechanism of toxicity through inhibition of brain acetylcholinesterase.
   2. Historically, the FDA as far back as 1957 mandated that companies examine possible synergistic effects of OP insecticides for purposes of tolerance establishment. (Discussed in Hayes 1991)

a. It had been noted in studies of this time that some OP insecticides, when simultaneously administered to rodents, could substantially increase the expected toxicity.

II. Classification of Potential Interactions Between Chemicals

A. Independent (or neutral) Effects
   1. Substances exert their own toxicity independently of one another
   2. The toxicity of one substance does not affect the toxicity of a second substance.

B. Additive Effects (Two types) (Koneman and Pieters 1996)
   1. Dose (Concentration) Addition: Compounds having similar mechanisms of toxicity cause a response that is simply the sum of the effects produced by the individual compounds alone.
      a. Example: 1X Dose Compd. A + 1X Dose Compd. B = 2X Effect
   2. Response Addition (also called Independent Action): Chemicals can act on completely different physiological systems, or on the same physiological system but they are functionally independent.
      a. Response additivity will occur only when the individual compounds exceed their own thresholds of tolerance.
      b. If the individual compounds do not exert an effect on their own, response additivity is unlikely to occur.

C. Antagonistic Effects
   1. One compound interferes with the expression of toxicity of another compund resulting in a combined effect that is lower than expected from one compound alone.
   2. Example: 1X Dose Compd. A + 1X Dose Compd. B = 0.5X Effect

D. Potentiation (or Synergism)
   1. Two compounds given simultaneously or close in time cause an effect that is greater than the sum of either alone.
   2. One of the compounds may not cause a reaction at all, but in combination with another biologically active compound toxic effects are greatly magnified.
   3. Example: 1X Dose Compd. A + 1X Dose Compd. B = 10X Effect

E. Dose Response Relationship (Figure 3)
   1. The relationship between the dose and response for any two chemicals can be visualized by examining the isobole graph in Figure 3.

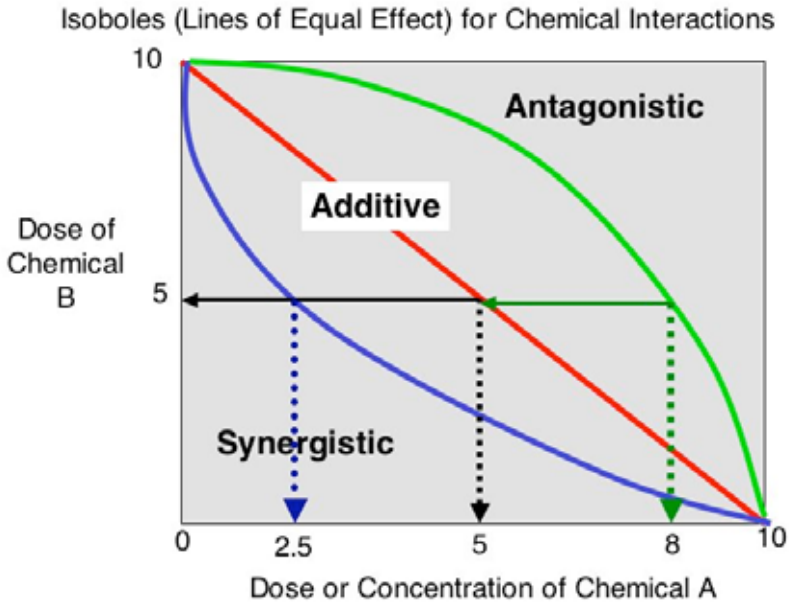

Figure 3. Lines of equal effect (isoboles) for different doses of chemicals in mixtures.

2. For example, in a synergistic interaction, a 2.5X dose of chemical A mixed with a 5X dose of chemical B causes a toxic effect of equal magnitude to a 5X A plus 5X B dose.

III. Testing for Combined Interactions of Chemical Mixtures

A. Risk assessment as practiced today normally analyzes compounds in isolation.
   1. The reason is not due to lack of desire probably as much as practicality and the myriad of possible interactions. (Table 1)

Table 1. Cost of testing multiple chemicals, assuming the single test of one compound is $1000.

		Cost of Entire Series ($ Millions)			
		Cost of Individual Test			
Number of Chemicals	**Number of Tests Needed**	**$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

B. Despite the cost, more studies are being published today that look at interactions between chemicals, especially those having similar modes of toxicity.
C. However, by knowing the mechanism of toxicity and the toxicokinetics, predictions can be made as to whether there might be an interaction or a neutral effect.

1. The primary assumption of additivity applies to chemicals having the same toxicodynamics. (See above definition of response additivity wherein the response is additive if the two chemicals are above their threshold dose for an effect).
2. If one compound is known to inhibit a detoxification enzyme, then the interaction could be potentiating or synergistic.
   a. In this case, the compound inhibiting the detoxification enzyme may or may not possess bioactivity at the administered dose.
   b. The inhibition of a detoxification enzyme is well known from earlier research with some OP insecticides.
      1. Early studies of potentiation among OP insecticides focused on malathion, which is probably the least toxic of this group. The acute oral toxicity of malathion, as measured by the dose lethal to 50% of test animals (LD_{50}), increased substantially when rats were also injected or fed EPN, an OP which is no longer registered (Frawley et al. 1957). For example, the LD_{50} to rats of malathion or EPN alone was estimated to be 1400 milligrams per kilogram of body weight (mg/kg) or 65 mg/kg, respectively. When mixed together and simultaneously administered to rats, the LD50 for malathion and EPN fell to 167 and 6.6 mg/kg, respectively. In other words, the doses required to kill 50% of the animals had dropped by nearly a factor of 10. If malathion and EPN were only additive in their interaction, then the doses corresponding to the LD50 should have dropped by at most a factor of two.
      2. Subsequent studies showed that potentiation between OP insecticides occurred only when one compound contained a certain chemical structure that made it susceptible to break down (detoxification) by a group of enzymes known as carboxyesterases. Malathion, once broken down by carboxyesterase, loses its toxicity. Malathion's toxicity could be potentiated by a second OP that was capable of inhibiting the activity of carboxyesterase (Seume and O'Brien 1960). Thus, more malathion would remain in the body longer, causing greater toxicity.
3. One compound could induce the synthesis detoxification enzymes, causing more rapid metabolism of a second compound and thus result in antagonism.
   a. On the other hand, one compound could induce an enzyme that metabolizes another compound into a toxicologically active form.
4. One compound could react with a target receptor of a second compound and either inhibit binding (which would likely cause an antagonistic relationship) or it could alter the receptor structure and make it more "receptive" to the second toxicant (causing either an additive effect, depending on the potency or activity of the first compound, or a synergistic effect).

D. Determining whether two or more chemicals are additive or synergistic or antagonistic

1. A typical experiment for two compounds interacting would start by estimating the LC50 (or other toxicological endpoint) for the chemicals alone and then test them in combination at different doses and estimate a new LC50.
   a. For example, in an experiment with chlorpyrifos and diazinon, the highest concentration tested of each compound was about twice the LC50 for *Ceriodaphnia*, and then 50% dilutions of this concentration were prepared. (Bailey et al. 1997)
      1. Thus, each tested concentration in combination represented a fraction of the LC50 for each of the compounds.
      2. The determination of whether interactions are synergistic or additive is determined using the toxic units approach shown below.
      3. Note, that this study did find additive interactions.
         a. Pertinently, when concentrations were made up in actual water from a slough receiving runoff, the results leading to a conclusion of additivity were the same as the results using laboratory water.

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

IV. Case Studies

A. In class, I will go over several "case studies" that portend to show synergism and/or additivity.
   1. The case of additivity of estrogen agonists by pesticides was discussed in Lecture 7.
      a. References:
         1. Arnold et al. 1996
         2. McLachlan et al. 1997
         3. Arcaro et al. 1998
   2. The case of synergistic interactions between a herbicide and an OP insecticide. (Nash 1981)
   3. The case of atrazine and OP interactions (Pape-Lindstrom et al. 1997)
   4. The case of atrazine, aldicarb, and nitrate interactions and mouse endocrine system effects. (Porter et al. 1999)

References

Arcaro, K. F., D. D. Vakharia, Y. Yang, and J. F. Gierthy. 1998. Lack of synergy by mixtures of weakly estrogenic hydroxylated polychlorinated biphenyls and pesticides. Environmental Health Perspectives 106 (Suppl. 4):1041-1046.

Arnold, S. F., D. M. Klotz, B. M. Collins, P. M. Vonier, L. J. Guillette, Jr., J. A. McLachlan. 1996. Synergistic activation of estrogen receptor with combinations of environmental chemicals. Science 272:1489-1492.

Bailey, H. C. and et. al. 1997. Joint acute toxicity of diazinon and chlorpyrifos to *ceriodaphinia dubia*. Environmental Toxicology and Chemistry 16(11):2304-2308.

Hayes, W. J., Fr., and E. R. Laws, Jr., ed. 1991. *Handbook of Pesticide Toxicology*, vol. 2. Academic Press, NY. pp. 954-957.

Larson, S. J., R. J. Gilliom, and P. D. Capel. 1999. Pesticides in streams of the United States--initial results from the National Water Quality Assessment Program. U.S. Geological Survey Water-Resources Investigation Report 98-4222, Sacramento, CA :99 pp (can be downloaded as PDF from http://water.wr.usgs.gov/pnsp/).

Frawley, J. P., H. N. Fuyat, E. C. Hagan, J. R. Blake, and O. G. Fitzhugh. 1957. Marked potentiation in mammalian toxicity from simultaneous administration of two anticholinesterase compounds. J. Pharmacol. & Exptl. Therapeutics 212:96-106.

Konemann, W. H. and M. N. Pieters. 1996. Confusion of concepts in mixture toxicology. Food and Chemical Toxicology 34:1025-1031.

McLachlan, J. A. 1997. Synergistic effects of environmental estrogens: report withdrawn. Science 277:462-463.

Nash, R. G. 1981. Phytotoxic interaction studies--techniques for evaluation and presentation of results. Weed Sci. 29:147-155.

Pape-Lindstrom, P. A. and M. J. Lydy. 1997. Synergistic Toxicity of atrazine and organophosphate insecticides contravenes the response addition mixture model . Environmental Toxicology and Chemistry 16(11):2415-2420.

Porter, W. P. and et al. 1999. Endocrine, immune, and behavioral effects of aldicarb (carbamate), atrazine (triazine) and nitrate (fertilizer) mixtures at groundwater concentrations. Toxicology and Industrial Health 15:133-150.

Seume, F. W., and R. D. O'Brien 1960. Potentiation of the toxicity to insects and mice of phosphorothionates containing carboxyester and carboxyamide groups. Toxicol. Appl. Pharmacol. 2:495-503.

USDA Pesticide Data Program. 1997. Annual Summary Calendar Year 1996. USDA Agricultural Marketing Service. http://www.ams.usda.gov/science/pdp/download.htm