October 31, 2005

Lecture 19: 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 exposures when we eat food.

1. However, food is full of bioactive secondary plant metabolites that have as great a probability as synthetic chemicals of testing positive in rodent assays for carcinogenicity or for interactions with the endocrine system that could be interpreted as adverse.
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).

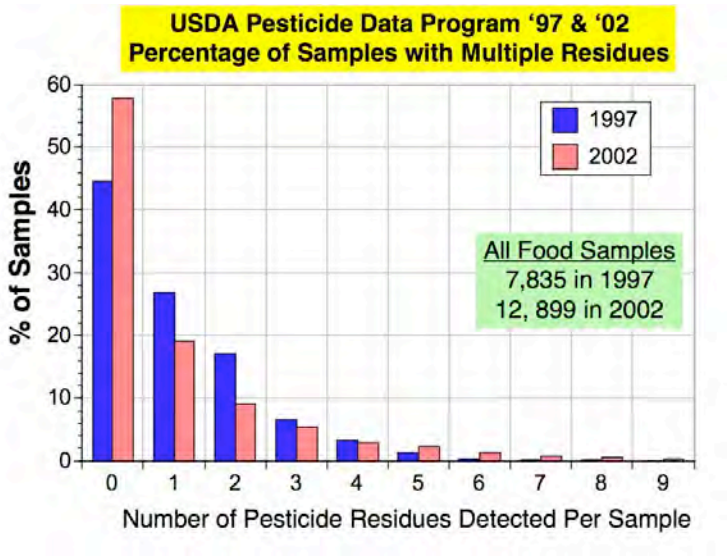

Figure 1. Proportion of food samples analyzed by the USDA in its Pesticide Data Program (PDP) that have one or more pesticide residues detected. Note that the trend is for less detection of any pesticide residues and lower frequency of detection of multiple residues. Reports can be downloaded from URL: http://www.ams.usda.gov/science/pdp/

D. The USGS NAWQA (National Water Quality Assessment) program reports multiple detections of some pesticides in the same water 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)
2. All available reports for pesticides and other water quality parameters measured in the NAWQA program can be viewed and downloaded at URL: http://www.ams.usda.gov/science/pdp/

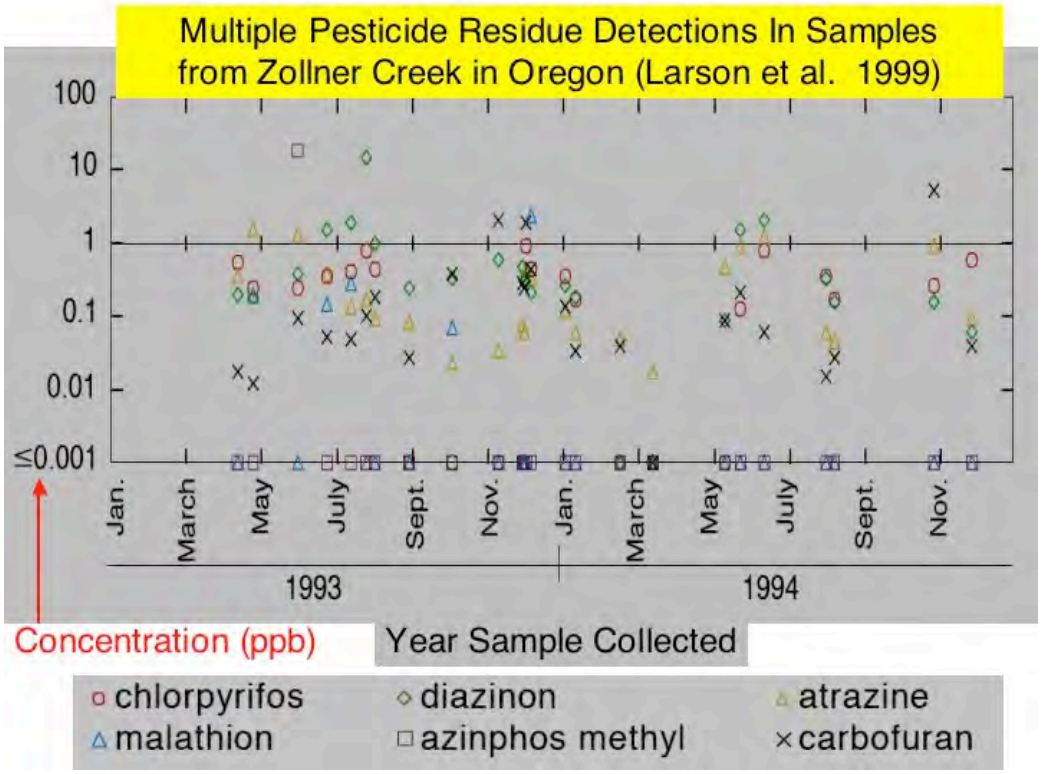

Figure 2. Detection of multiple pesticide residues in water samples collected from Zollner Creek in the Willamette Basin watershed of Oregon (Larson et al. 1999)

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 occurrences of residues, although they must have the same mechanism of causing toxicity.
      a. The OP insecticides were the first group of insecticides 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 compound 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.

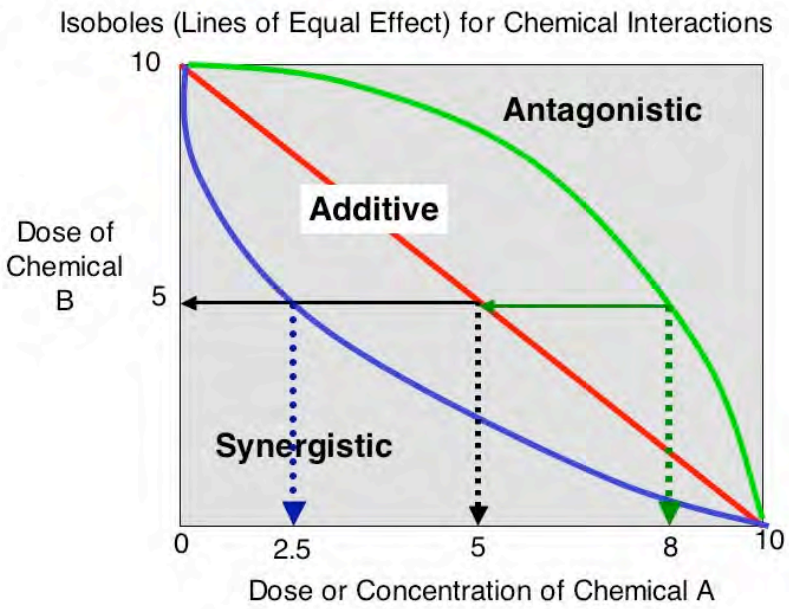

Figure 3. Lines of equal effect (isoboles) for different doses of chemicals in mixtures.

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; the lack of testing is due as much to 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 that 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.
            a. 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, but the other chemical could inhibit activity of the detoxification enzyme.
            a. 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 of 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 the Bailey et al. (1997) 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

$$TU_{mix} = \frac{LC50\ A_{(mix)}}{LC50\ A_{(alone)}} + \frac{LC50\ B_{(mix)}}{LC50\ B_{(alone)}}$$

- 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. Expressing Concentrations for Mixtures of Chemicals Having the Same Pharmacodynamic Mechanisms

A. Polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) occur in many environments as mixtures.
   1. All of these contaminants interact with the Ah receptor and induced transcription of the P450-dependent enzyme aryl hydrocarbon hydrolase (AHH).
   2. Although the mechanism of toxicity is not well understood beyond the interaction with the Ah receptor, the various congeners of the PAHs, PCBs, PCDDs, and PCDFs have different potencies in causing toxic response.
      a. TCDD (one of the PCDDs) is the most potent of these compounds; polychlorinated dioxins without chlorine substitution on at least the 2,3,7,8 positions of the dioxin ring are of very low to nil toxicity. The one

exception is the 8 chlorine dioxin, octachlorodibenzodioxin that is also of very low toxicity.

b. Because TCDD is the most potent compound that interacts with the Ah receptor, and all of the contaminants above interact with the same receptor, the concentrations of complex mixtures can be expressed as toxic equivalents (TEQs) of TCDD.
c. To transform concentrations of individual PCDDs or PCBs, etc. to TEQs of TCDD, the mass/mass concentration (i.e., pg/kg or ng/kg or μg/kg) are multiplied by a TEF (toxic equivalency factor) to yield a TEQ dioxin equivalent concentration.
    1. Based on several comparative animal toxicity assay that use immunotoxic response as one potential endpoint, TEFs have been universally agreed on.
    2. Once any single PCDD or PCB is transformed to TEQs, then for the mixture of all components the overall concentration of each TEQ is summed as shown in the equation below.
    3. Potential exposure in different matrices or body burden can then be estimated by comparing the TEQs

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

Table 2. TEF values used by the EPA (for mammals; based on in vivo data) (Van den Berg et al. 1998, Environ. Health Perspectives 106:775-792) and by the World Health Organization (WHO)

Congener	**EPA**	**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

B. OP insecticide residues have been subjected to analogous transformations using toxic equivalency factors (known in this case as relative potency factors or RPFs) to change all residues into a toxic equivalency.
   1. The OP insecticide chosen as the benchmark for summation of residues as TEQs is methamidophos.
   2. The potency for inhibition of female rat brain acetylcholinesterase is used as the toxicological endpoint.

V. 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 8.
      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)

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