Gregory Howard

Boston University School of Public Health, Boston, Massachusetts

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Assessing Synergy: Mapping the Response Surface

Overview

The dramatic growth in the number of chemicals in widespread use has led to increasing concern about the effects of interactions between low-level exposures. Mixtures can sometimes produce dramatically stronger effects ("synergy") than would be predicted by a simple model. Many general approaches and statistical tests have been proposed to address combination exposures, but there is not universal agreement on their appropriateness. A number of otherwise promising statistical tests are parametric in nature and thus make strong assumptions, not always correct, about the shapes of the individual dose-response curves.

This project has several goals: 1) We are using algebraic and numerical models of receptor-based systems to simulate interactions, particularly in complex model systems which include multiple agents and partial agonists. 2) We are developing experimental methodology for testing synergy through the use of standard laboratory techniques. 3) We are developing a new statistical test for interaction, borrowing techniques from spatial mapping to analyze the entire response surface for interactive effects without making assumptions about the shape of dose-response curves. 4) Finally, we plan to extend some of these concepts to epidemiology, in which the shape of the dose-response curve is not generally considered when evaluating interaction.

The Environmental Issue

Why are new techniques for assessing interaction important?

First, a proper characterization of interaction is critical for risk assessment. As low-level environmental exposures become ubiquitous and more numerous, the potential for interaction increases dramatically. Effects on the developing organism, which can occur (for example) by modulating hormone signaling, may be the most sensitive target. Hormonally active chemicals are now found all over the world, and have been implicated in studies ranging from the sexual development of frogs to the play behavior of Dutch schoolchildren.

Furthermore, despite concerns about synergisms, "additive" (non-synergistic) effects can be as important. A recent paper (Silva *et al.* 2001) shows that a naïve prediction of a combination effect ("ES") greatly underestimates the actual effect ("MIX") of a mixture of estrogenic xenobiotics—a mixture in which each chemical was below its No Observed Effect Concentration. This is not a synergistic interaction, but an additive one, which the method of concentration addition ("CA"; see at right) correctly predicts. This highlights the importance of using an appropriate model when predicting interaction.

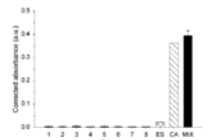


FIGURE 4. Effects of individual mixture components 1–8 at the concentrations present in 1.43 μM of the mixture. ES: effect summation, i.e., expected mixture effect obtained by calculating the arithmetic sum of individual effects of agents 1–8. CA: concentration addition prediction. MIX: observed mixture effect. Error bars are upper 95% confidence limits of the best estimate of mean responses. Concentrations of test agents in 1.43 μM of the mixture are depicted in Table 1. (Silva *et al.* 2001)

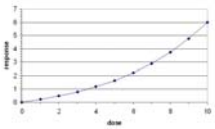
This example also suggests that threshold-type risk assessments may not apply to chemical mixtures at all, nor to individual chemicals which might interact with endogenous hormones to create a heightened effect.

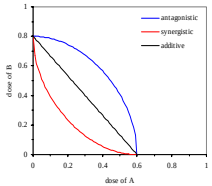
Finally, properly assessing synergistic (or antagonistic) interactions can also provide valuable information about the mechanism of toxicity of the individual exposures.

What is Synergy?

EFFECT SUMMATION

The simplest definition of synergy is also the most commonly used: "A synergistic effect occurs when the combined effects of two chemicals are much greater than the sum of the effects of each agent given alone (example: 2+2=20)" (Klaassen 1996). But this method holds only when both dose-response curves are linear. To see why, consider a sham "combination" of two doses of the same chemical with the dose-response curve shown.





CONCENTRATION ADDITION

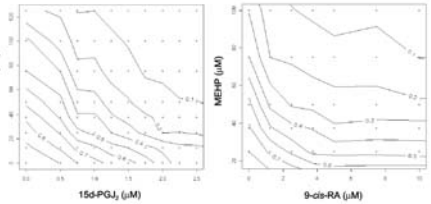
Instead of adding effects, we may add doses in concentrations proportional to their effect. In the dose-dose space, this is equivalent to replacing doses of A with isoeffective doses of B, thereby moving along the straight line (the "isobole") which connects two single doses of equal effect (for example, ED_{50A} and ED_{50B}):

$$1 = \frac{C_A}{ED_{50A}} + \frac{C_B}{ED_{50B}}$$

A popular but non-quantitative method of testing synergy uses this definition. If the isoboles are linear, the mixture must follow concentration addition, and is therefore not synergistic. Isoboles which curve towards the origin indicate synergistic mixtures; those which curve away, antagonism. Isoboles can also be thought of as contours of the response surface.

Recent Work

The "isobole method" is based on concentration addition, assuming that isoeffective concentrations of one chemical (on the x-axis) can replace the second chemical (y-axis).



Isobole analysis of MEHP interactions with 15d-PGJ2 (left) and 9-cis-RA (right). Suspension cultures of BU-11 cells were treated with ethanol (vehicle, 0.5%), MEHP (25–100 μM), 15d-PGJ2 (0.5–2.5 μM), and/or 9-cis-RA (1–10 μM) for 48 h. [3H]thymidine incorporation was determined. Levels shown are fractional responses of the Vh-Vh cell. Circles indicate locations of data points from three experiments; contours are linear interpolations. From Schlezinger *et al.* (2004).

We have used this method to compare interactions between mono(2-ethylhexyl)phthalate, 9-*cis*-retinoic acid, and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2. The roughly linear isoboles (i.e., lines of isoeffective combination dose) of the MEHP/PGJ2 combination (left) show it to be much less synergistic than the steeply curved isoboles of MEHP with 9RA (right).

We are now examining these interactions in more depth, applying other existing statistical tests, and using them as models for experimental design. Testing an interaction term in a regression model, for example, shows the MEHP/PGJ2 interaction (left) to be strongly synergistic.

Finally, analytical models of receptor-based systems like this one have helped us to describe interactions between agonists, antagonists, and partial agonists.

Acknowledgements

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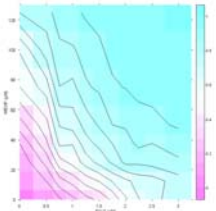
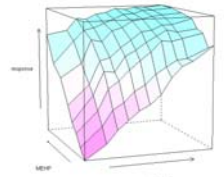
Contact

Greg Howard / gh@bu.edu / 617 414 1428
Department of Environmental Health
715 Albany St T2E, Boston, MA 02118

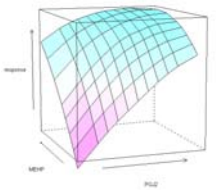
A major Aim of this project is to develop a nonparametric statistical test for synergy. We are currently exploring a method based on the construction of an "expected" response surface (i.e., the response [z] of a combination of two chemicals [x, y] under an assumption of an additive interaction obeying concentration addition).



Levelplot and contours (isoboles) of MEHP/PGJ2 interaction (see at left for experimental details).

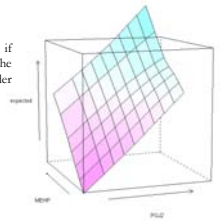


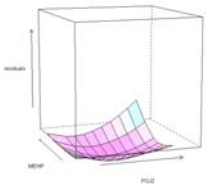
Response surface for the MEHP/PGJ2 combination.



The response surface may also be smoothed (here using LOESS).

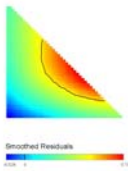
We can calculate an "expected" (non-synergistic) response surface if we know the dose-response curves of the individual chemicals in the mix. Careful experimental design is required along the axes in order to predict the whole surface.





Subtracting the expected surface from the response surface yields a surface of residuals. Hills and valleys in this surface indicate the presence of synergy or antagonism in the data.

We can now use this map of the residual surface to find interactions, with peaks and valleys corresponding to synergy and antagonism. To do so, we borrow techniques from mapping (e.g., generalized additive models), which have been used, for example, to find spatial variation in risk ratios in epidemiologic data.



Advantages of this method include:

- Unlike many other methods, it is nonparametric, making no assumptions about the shape of the dose-response curves;
- Using mapping techniques, we can test for both global and local synergy;
- These techniques are familiar from use in other fields (e.g., spatial disease risks);
- Appropriate experimental design can make the process efficient in the laboratory.

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