Methods, Models, and Experimental Designs for Evaluation of Dose-Additivity of Chemical Mixtures

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Contributing Organizations: ORD: NHEERL, NCEA; Virginia Commonwealth University; Solveritas

LTG 2 Poster 02

Science Question

➢ ***What models can be used to assess EPA's default assumption of dose-additivity for chemical mixtures?***

Research Goals

In vivo mixtures research has traditionally been limited by methods requiring large numbers of experimental animals, high dosages, or selected levels of biological effects. For example, response surface methodologies, which evaluate a range of combinations of chemicals, doses, and effect levels, may be prohibitive due to the requirement of a large number of dose groups. A more restrictive model based on isobole methodology can be used to examine different ratios of the chemicals in a mixture (mixing ratio) that are predicted to produce a single constant level of effect (*e.g.* ED_{50}). In application, this model is usually restricted to a limited number of levels of biological effects.

Our goal was to facilitate the development of more efficient experimental designs to detect deviations from dose-additivity for chemicals with a common mode of action.

Through the use of a collaborative agreement (CR-828-11401) with the Virginia Commonwealth University, and contracts with Solveritas, we furthered development of ray methodology for detecting deviations from dose-additivity (summation of the individual chemical's effects after scaling for differences in potency). The ray experimental design examines a constant mixing ratio of chemicals in various doses. The ratio can be chosen based on human exposures or other relevant parameters. This approach is useful for examining low biological effect levels, as a response threshold can be incorporated into the model.

Findings and Conclusions

➢ Methods have been developed to test deviations from dose-additivity for chemicals with a common mode of action based on a ray (dose-response) experimental design.

➢ The methods have been expanded to allow single chemical dose-response curves to have different slopes and maximal effect levels.

➢ Methods have also been developed to assess deviations from additivity when single chemical dose-response data are not available.

➢ Methods have been developed to estimate the interaction threshold – the mixture dose above (or below) which non-additive effects are observed.

➢ Means to optimize statistical power to detect overall deviations from dose-additivity (D-optimal design) or changes in specific parameters (D_s-optimal) have been developed.

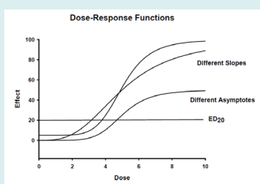
Impact and Outcomes

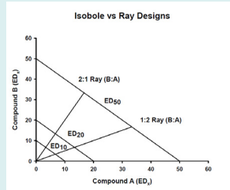
➢ This research provided OPP and the scientific community with statistical methodology to assess deviations from dose-additivity. These methods have been applied to research questions for organophosphate, *N*-methyl carbamate, and pyrethroid pesticides.

Methods/Approach

Flexible Single Chemical Required Model (SCR)

Used to predict the dose-response relationship for a mixture of chemicals along fixed-ratio rays (in terms of total dose), under the hypothesis of dose-additivity. Individual chemical dose-response data are used to create a predicted response curve for the mixture using the general equation below. The observed mixture data are used to derive the actual mixture response curve for comparison with the predicted response curve.





$$\mu_k = \begin{cases} \alpha & B_i x < \delta_i \\ \alpha + \gamma_i\left[1 - \exp\left(-\{B_i x - \delta_i\}\right)\right] & B_i x \geq \delta_i \end{cases}$$

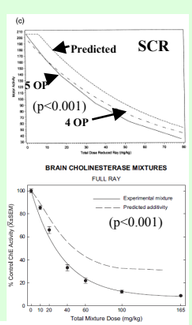
Conclusions:
1. The test of additivity is a statistical comparison of parameters between the curves.
2. The methods are useful for evaluating defined mixtures, using single-chemical data.
3. No limit on the number of chemicals in the mixture.
4. Parallel slopes and similar maximal response levels for single chemical dose-response curves are not required.

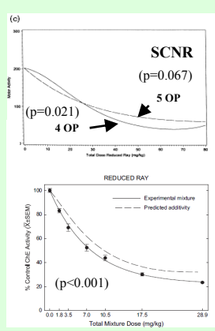
where: μ_i is mean response for single chemical or mixture ray
x_i is the dose of the chemical or mixture
α is a minimum effect parameter
γ_i is a parameter associated with the response range for each chemical
$\alpha + \gamma$ is the magnitude of the response
δ_i is the parameter associated with the threshold for a single chemical or the mixture ray
β_i is the slope parameter for a single chemical or the mixture ray

Single Chemical Not Required Model (SCNR) and SCR Models for Altered Mixture Composition

Only data along the mixture ray are necessary for detecting departure from additivity – assumption is linear response.
Significance of higher order terms in the polynomial model indicates departure from additivity.
This approach is useful for evaluation mixtures of unknown composition or unknown proportions.

$$g(\mu_{mix}) = B_0 + B_1 t + \sum_{i=1}^{c} B_i t^i$$





Conclusions:
1. Both the SCNR and SCR method resulted in similar conclusions. However, the SCR method is more powerful for detecting deviations from additivity.
2. SCR analysis of the reduced ray detected the impact of malathion on the dose-response to the other four pesticides.
3. The collection of single-chemical data is recommended for maximal power.

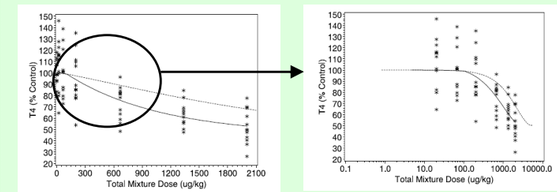
Doses (mg/kg) of Each Pesticide Comprising the OP Mixtures

Full ray	Reduced ray	Chlorpyrifos	Acephate	Diazinon	Dimethoate	Malathion[a]
10	1.75	0.31	0.4	0.02	1.02	8.25
20	3.5	0.62	0.8	0.04	2.04	16.5
40	7.0	1.24	1.6	0.08	4.08	33.0
60	10.5	1.86	2.4	0.12	6.12	49.5
100	17.5	3.1	4.0	0.2	10.2	82.5
165	28.9	5.12	6.6	0.33	16.83	136.13

[a]Malathion was not included in the reduced ray.

Determination of Interaction Thresholds

When an overall deviation from additivity is indicated, this procedure may be used to determine the threshold for non-additive responses.



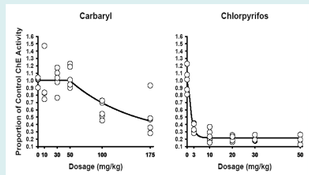
Interaction Threshold: 105.3 µg/kg
95% Confidence Interval: 0-540 µg/kg

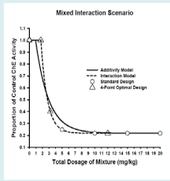
Conclusions:
1. Allows statistical criteria for determining doses above which non-additive effects may be of concern.
2. Not limited to interaction thresholds in low dose region.
3. May impact uncertainty factors for exposures above the interaction threshold.

$$\mu_k = \begin{cases} \left\{ \mu_{0(add)} \ni t_{add}\left(\mu_{0(add)}\right) = \left[\sum_{i=1}^{c} \alpha_i / \left[\left[\log\left(\mu - \alpha_{i(add)}\right) / \gamma_{i(add)}\right) / \beta_{i(add)}\right] + \delta_{i(add)}\right]\right]^{-1} \right\} & t < \Delta_i \\ \alpha_i + \left(\mu_{\Delta i} - \alpha_i\right)\exp\left(\beta_i\left(t - \Delta_i\right)\right) & t \geq \Delta_i \end{cases}$$

where: μ_i is mean response for the ith mixture ray
$\mu_{0(add)}$ is a specified value of the mean response for the additive mixture ray
t_{add} $(\mu_{0(add)})$ is the total dose of the mixture that produces $\mu_{0(add)}$
α_i is a maximum effect parameter for the ith mixture ray
$\alpha_{i(add)}$ is a maximum effect parameter for the ith additive mixture ray
$\gamma_{i(add)}$ is a parameter associated with the response range for the ith additive mixture ray
$\beta_{i(add)}$ is the slope parameter for the ith additive mixture ray
$\delta_{i(add)}$ is the parameter associated with the threshold for the ith additive mixture ray
t is the dose of the mixture
Δi is the interaction threshold for the ith mixture ray
$\mu_{\Delta i}$ is the mean response at the interaction threshold

Optimal Experimental Designs





Design Table for the Mixed Interaction Scenario

Design	Design Points	Number of Observations	Power	Generalized Variance[1]
1	0, 5, 10, 15, 20	10, 10, 10, 10, 10	0.10	773.7
2	0, 4, 8, 12, 16	10, 10, 10, 10, 10	0.14	5.02
3	0, 3, 6, 9, 12	10, 10, 10, 10, 10	0.08	0.061
4	0, 1.75, 3.2, 11.6, 13.4	9, 11, 15, 7, 8	0.78	0.00068
5	0, 1.75, 3.2, 12.0	9, 15, 19, 7	0.84	0.00056

[1]Variability proportional to the area of the confidence region of the slope and threshold parameters.

Conclusions:
1. Low power in D-Optimal designs can result from similar mixture and additivity curves, because the additivity hypothesis tested by comparing the similarity of the two curves.
2. Reduction in model variance is accomplished by placing treatment groups at points that efficiently estimate the model parameters. These are regions where the mixture model is different (*e.g.* change in ED_{20}, change in slope) from the additivity model. Unequal allocation of observations may also be used to optimize the parameter estimation.
3. The D-Optimal depend on: 1) the assumed values of the model parameters, 2) specification of the predicted type of interaction (greater than additive, less than additive, combination of greater and less than additive), 3) the shape of the mixture dose-response curve, and 4) the magnitude of the change (effect size) to detect.
4. If only selected model parameters are of interest (*e.g.*, threshold), D_s-Optimal designs can be used to concentrate power on estimating the reduced number of parameters of interest.

Future Directions

➢ This portion of the ORD/NHEERL mixtures research is completed. Future work will involve complex mixtures with multiple modes of action (See Simmons *et. al.*, Poster LTG 2-3).

Researchers Involved

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Solveritas

Cumulative Risk

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