Detecting Departure From Additivity Along a Fixed-Ratio Mixture Ray With a Piecewise Model for Dose and Interaction Thresholds

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For mixtures of many chemicals, a ray design based on a relevant, fixed mixing ratio is useful for detecting departure from additivity. Methods for detecting departure involve modeling the response as a function of total dose along the ray. For mixtures with many components, the interaction may be dose dependent. Therefore, we have developed the use of a three-segment model containing both a dose threshold and an interaction threshold. Prior to the dose threshold, the response is that of background; between the dose threshold and the interaction threshold, an additive relationship exists; the model allows for departure from additivity beyond the interaction threshold. With such a model, we can conduct a hypothesis test of additivity, as well as a test for a region of additivity. The methods are illustrated with cytotoxicity data that arise when Chinese hamster ovary cells are exposed to a mixture of nine haloacetic acids.

Key Words: Dose threshold; Dose-dependent interaction; Synergy.

1. INTRODUCTION

As a result of complicated biotransformation, elimination and/or repair processes, many biological systems exhibit some level of tolerance to a toxic insult (Cox 1987). In such instances, in order for a chemical to cause an observable change in response, the body must

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be exposed to quantities sufficient to overwhelm these processes. It is often reasonable, therefore, to assume that a chemical must be administered above an unknown dose threshold if it is to produce an effect that is different from background. Cox (1987) and Ulm (1991) described single chemical dose–response relationships using models in which the dose threshold is a parameter to be estimated. Schwartz, Gennings, and Chinchilli (1995) extended the methodology to the estimation of the response surface associated with a mixture of chemicals. Gennings et al. (1997) applied the dose threshold to the estimation of an additivity surface using single chemical data only. More recently, Casey et al. (2004) developed methodology for including the dose threshold in the analysis of a chemical mixture along a fixed-ratio ray.

Gennings et al. (2002) found that interaction in the mixture of arsenic, cadmium, chromium and lead was synergistic for some dose combinations and antagonistic for others, while no interaction was detected for still other dose combinations. Such a finding is not unheard of. A literature review by Carpy, Kobel, and Doe (2000) demonstrated that interactions are rare at low exposure levels. This assumption of low-dose additivity, acknowledged by the U.S. EPA (2000), can be incorporated into the statistical analysis of a chemical mixture using an interaction threshold. Hamm (2004) developed a model for describing the dose–response relationship of a chemical mixture along a fixed-ratio ray, in which the interaction threshold is a parameter to be estimated. Hamm's interaction threshold model describes additivity for mixture doses smaller than the interaction threshold and allows for departure from additivity for larger doses. Gennings et al. (2007) recently extended the methodology to allow for differences in the shapes of the individual components' dose response curves.

In 2002, the Society of Toxicology commissioned an expert panel to suggest directions for future research in the risk assessment of chemical mixtures. A number of the alternative hypotheses presented by the panel dealt with interaction thresholds. Specifically, the panel stressed the importance of demonstrating additivity among the chemicals in a mixture at low exposure levels. The panel also emphasized the importance of establishing that the interaction threshold is higher than the dose threshold. This suggests the need for statistical methodology capable of simultaneously estimating both a dose threshold and an interaction threshold.

To our knowledge, however, these concepts have not been combined in a single model to describe the dose–response relationship for a chemical mixture along a fixed-ratio ray. The situation in which both thresholds exist is considered as follows. For mixture doses smaller than the dose threshold, the response is equivalent to background. For mixture doses between the dose threshold and the interaction threshold, the chemicals behave as expected under additivity (as predicted with single chemical data). For mixture doses larger than the interaction threshold, the model allows for interaction among the chemicals in the mixture. The development of such a model is the primary objective of this paper.

In Section 2, we present the motivating example for the proposed methodology. The cytotoxicity of a mixture of nine haloacetic acids (HAAs) along a fixed-ratio chlorination ray is analyzed using the threshold additivity model. We demonstrate that the threshold additivity model reflects the dose–response relationship reasonably well for low doses but

Table 1. Haloacetic acid by-products of the chlorination process.

Chemical	Abbreviation	Ratio in the Mixture
Chloroacetic acid	CA	0.03
Dichloroacetic acid	DCA	0.30
Trichloroacetic acid	TCA	0.26
Bromoacetic acid	BA	0.06
Dibromoacetic acid	DBA	0.03
Tribromoacetic acid	TBA	0.02
Bromodichloroacetic acid	BDCA	0.12
Dibromochloroacetic acid	DBCA	0.04
Bromochloroacetic acid	BCA	0.14

not for high doses. In Section 3, we present the dose and interaction thresholds model, and we describe the estimation process and related hypothesis tests. In Section 4, the model is applied to the chlorination ray data.

2. MOTIVATING EXAMPLE

When drinking water is disinfected, 'disinfectant by-products' (DBPs) are produced in the water. There are approximately 500 known DBPs (and many unknown ones), and a substantial proportion have yet to be studied for adverse health effects (Richardson, Simmons, and Rice 2002). Nine HAAs result from the disinfection process known as chlorination and are currently under investigation by the EPA. Table 1 contains a list of these HAAs and the relative proportion of each resulting from the chlorination process.

The primary research objective is to determine whether these nine HAAs interact when combined according to the chlorination mixing ratio. To investigate, an in vitro Chinese hamster ovary (CHO) cell assay was conducted to study the cytotoxic effects of the single chemicals. A similar study of the chlorination mixture was also conducted. The procedures and the calibration for the CHO cell chronic cytotoxicity assay were previously published (Plewa et al. 2000, 2002, 2004). The response is cell viability, defined as the percentage of the negative control surviving the administered concentration. Single chemical plots can be found in Stork et al. (2007).

As a preliminary analysis of the data, we used the threshold additivity model (Casey et al. 2004) to predict additivity along the chlorination ray using the single chemical data. Figure 1 demonstrates the fit of the additivity model to the mixture data. While a concentration threshold is not evident in the mixture data, a threshold was included in the additivity model to reflect those observed in the single chemical data.

The predicted additivity model fits the chlorination ray data fairly well for total concentrations less than approximately 0.1 mM. For larger total concentrations, the additivity model predicts lower cell viability than indicated by the observed mixture data. This region of disagreement between the mixture data and the predicted additivity model may be indicative of dose-dependent interaction, suggesting the existence of an interaction threshold. The inclusion of both a concentration threshold and an interaction threshold in a single

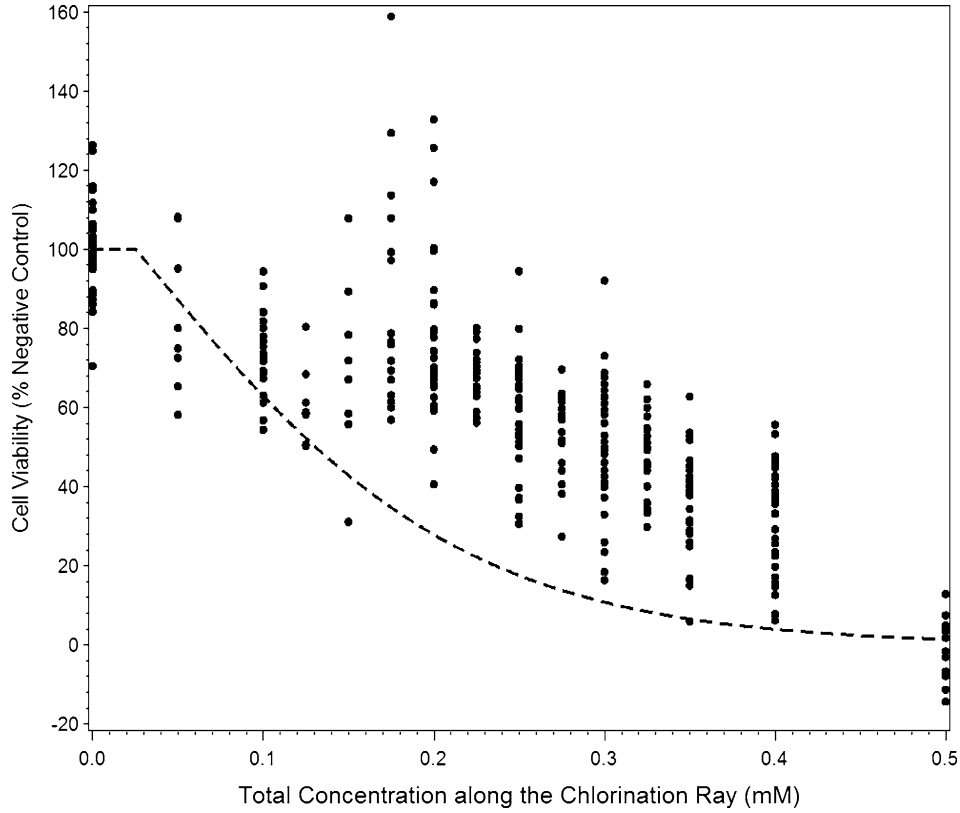

Figure 1. Cell viability vs. total concentration of the mixture along the chlorination ray. The dashed line represents the additivity model, as determined by the single chemical data alone.

model would allow us to test the null hypothesis of additivity; it would also allow us to test for a region of additivity. A significant result on the latter test would indicate that an active region of additivity exists along this ray, and the resulting confidence intervals would be used to describe the location of the region.

3. METHODOLOGY

3.1. MODEL DEFINITION AND FIT

The dose and interaction thresholds model is supported by both single chemical data and mixture data along the fixed-ratio ray. Below the interaction threshold, single chemical slopes are estimated, under the assumption of additivity, using both single chemical and mixture data. Beyond the interaction threshold, model parameters associated with departure from additivity are estimated based on mixture data alone.

Each fixed-ratio ray is defined by its mixing ratio, $\mathbf{a}_{(h)} = [a_{(h)1}\ a_{(h)2} \cdots a_{(h)c}]$, for $h = 1, \ldots, c+1$, such that the first c rays describe the single chemical data, and the $(c+1)$st ray describes the mixture data. The mixing ratio for the ith single chemical is defined such that the ith element of $\mathbf{a}_{(h)}$, $a_{(h)i}$, is 1, and the remaining elements are 0.

Let $x_{(h)ji}$ be the dose of the ith chemical at the jth dose on the hth ray, for $i = 1, \ldots, c$ and $j = 1, \ldots w_{(h)}$, where $w_{(h)}$ represents the number of dose levels for the hth ray. Then $t_{(h)j} = \sum_{i=1}^{c} x_{(h)ji}$ is the jth total dose along the hth ray, and $x_{(h)ji} = a_{(h)i} t_{(h)j}$. Let $y_{(h)jk}$ represent the kth response from the jth dose of the hth ray, for $k = 1, \ldots, n_{(h)j}$, where $n_{(h)j}$ represents the number of observations at the jth dose level on the hth ray. The total sample size for both the single chemical and the mixture ray data is $N = \sum_{h=1}^{c+1} \sum_{j=1}^{w_{(h)}} n_{(h)j}$.

The threshold additivity model for the mixture, equivalent to that described by Casey et al. (2004), is expressed for decreasing dose-response curves as

$$g(\mu_{\text{add}}, \boldsymbol{\gamma}) = \begin{cases} \beta_0, & t < \delta_{\text{add\_}t}, \\ \beta_0 + \left(\sum_{i=1}^{c} \beta_i a_i\right)(t - \delta_{\text{add\_}t}), & t \geq \delta_{\text{add\_}t}, \end{cases} \tag{3.1}$$

where μ_{add} is the mean response of the mixture under additivity, g is the appropriate link function, and $\boldsymbol{\gamma}$ represents the vector of nonlinear parameters necessary to describe the dose-response relationship. Following traditional notation, β_0 is the unknown parameter associated with the intercept and β_i is the unknown slope for chemical i. Here $\delta_{\text{add\_}t} = \delta_{\text{add}} / \sum_{i=1}^{c} \beta_i a_i$ is the threshold along the mixture ray, where δ_{add} is an unknown parameter associated with the threshold. For increasing dose-response curves, the inequalities are reversed.

For decreasing dose–response relationships with an approximate sigmoid shape, consider $\boldsymbol{\gamma} = [\alpha\ \gamma]$, where α represents the minimum response and γ represents the range of the response. Then the mean response, μ, can be represented by any nonlinear function of the form $\alpha + \gamma F(g(\mu))$, where $F(\cdot)$ is any decreasing, sigmoidal function that takes on values between zero and one. For increasing dose-response relationships, $F(\cdot)$ is any increasing, sigmoidal function that takes on values between zero and one.

The dose and interaction thresholds model is an extension of the threshold additivity model, where a third segment describes departure from additivity for large mixture doses. This model has the following form:

$$g(\mu_{(h)}, \boldsymbol{\gamma}) = \begin{cases} \beta_0, & t_{(h)} < \delta_{\text{add\_}t(h)}, \\ \beta_0 + \left(\sum_{i=1}^{c} \beta_i a_{(h)i}\right)(t_{(h)} - \delta_{\text{add\_}t(h)}), & \begin{cases} t_{(h)} \geq \delta_{\text{add\_}t(h)}, h \leq c, \\ \delta_{\text{add\_}t(h)} \leq t_{(h)} < \Delta, h = c+1, \end{cases} \\ \beta_0 + \left(\sum_{i=1}^{c} \beta_i a_{(h)i}\right)(t_{(h)} - \delta_{\text{add\_}t(h)}) \\ \qquad + \theta_{\text{mix}}(t_{(h)} - \Delta), & t_{(h)} \geq \Delta \text{ and } h = c+1. \end{cases} \tag{3.2}$$

In this parameterization, let $\sum_{i=1}^{c} \beta_i a_{(h)i} = \theta_{\text{add}(h)}$, the slope of total dose in the region of additivity along the hth ray, and θ_{mix} represents the change in the slope for total doses above the interaction threshold. Here, $\delta_{\text{add\_}t(h)} = \delta_{\text{add}} / \theta_{\text{add}(h)}$ is the dose threshold along the hth ray (Casey et al. 2004), and the interaction threshold for the mixture ray is represented by Δ. The region of additivity is supported by both single chemical and mixture data, while the interaction region is supported only by the mixture data. Higher order terms can be added to the third segment of the model as necessary.

Let $\boldsymbol{\beta} = [\beta_0\ \beta_1\ \beta_2 \cdots \beta_c\ \delta_{\text{add}}\ \theta_{\text{mix}}\ \Delta]$ be the $p \times 1$ parameter vector represented in the dose and interaction thresholds model given in Equation (3.2), such that $E\{y_{(h)jk}\} = \mu_{(h)j}(\boldsymbol{\beta})$, the mean response for the jth dose of the hth ray. Let $\text{var}\{y_{(h)jk}\} = \tau V(\mu_{(h)j}(\boldsymbol{\beta}))$ specify that the variance is proportional to some function, V, of the mean response, with proportionality constant τ. Then the quasi-likelihood method can be used to estimate model parameters following the estimation procedures described in McCullagh and Nelder (1989). Under this framework, τ is unknown and estimated in the modeling process.

3.2. INFERENCE

The quasi-likelihood ratio test can be used to compare two nested models. Let Q_{full} be the maximum quasi-likelihood achieved under the full model (the dose and interaction thresholds model), and let Q_{red} be the maximum quasi-likelihood achieved under the reduced model (the threshold additivity model). Then the likelihood ratio test statistic is $\text{LRT} = -2\{Q_{\text{red}} - Q_{\text{full}}\}/\tau$, which has an approximate χ^2 distribution with M degrees of freedom (McCullagh 1983), where M is the number of parameters constrained by the reduced model and τ is known under the full model. Replacing τ with a consistent estimate $\hat{\tau}$, the likelihood ratio test statistic

$$Q\text{LRT} = -2\{Q_{\text{red}} - Q_{\text{full}}\}/\hat{\tau}M \tag{3.3}$$

is asymptotically distributed F with M and $N - p$ degrees of freedom.

Alternatively, we can use the covariance matrix of $\hat{\boldsymbol{\beta}}$ to construct hypothesis tests and confidence intervals on the parameter vector. Let $\tau\boldsymbol{\Omega}$ be the covariance matrix of $\hat{\boldsymbol{\beta}}$. Then the Wald-type statistic for testing $H_0 : \boldsymbol{\beta} = \boldsymbol{\beta_0}$,

$$W_{QL} = (\hat{\boldsymbol{\beta}} - \boldsymbol{\beta}_0)'(\hat{\tau}\hat{\boldsymbol{\Omega}})^{-1}(\hat{\boldsymbol{\beta}} - \boldsymbol{\beta}_0)/M, \tag{3.4}$$

is asymptotically distributed F with M and $N - p$ degrees of freedom. The Wald test can be extended for nonlinear hypotheses of model parameters using the delta method as described by Seber and Wild (2003). Let $\boldsymbol{\lambda}$ denote a $q \times 1$ vector of functions of the parameter vector, $\boldsymbol{\beta}$, representing some nonlinear contrast, so that the null hypothesis can be written as $H_0 : \boldsymbol{\lambda} = \mathbf{0}$. Letting $\mathbf{D} = [\partial\boldsymbol{\lambda}/\partial\boldsymbol{\beta}']$ represent the $q \times p$ matrix of derivatives, the corresponding Wald test for nonlinear hypotheses is of the form

$$W_{QL\_\boldsymbol{\lambda}} = (\hat{\boldsymbol{\lambda}})'\left(\mathbf{D}(\hat{\tau}\hat{\boldsymbol{\Omega}})\mathbf{D}'\right)^{-1}(\hat{\boldsymbol{\lambda}})/q, \tag{3.5}$$

which is asymptotically distributed F with q and $N - p$ degrees of freedom.

3.2.1. Test of Additivity

Following the Single Chemical Required method of Casey et al. (2004) for dose threshold models and the extension of Hamm (2004) for interaction threshold models, the test of additivity is a test of coincidence. If the dose and interaction thresholds model does not coincide with the threshold additivity model, then we can conclude departure from additivity. Note that evidence of departure from additivity is captured in the parameter θ_{mix}. If the interaction threshold is equal to the dose threshold, and the parameter θ_{mix} is equal to zero,

then the dose and interaction thresholds model reduces to the threshold additivity model. The null hypothesis of additivity, then, is given by $H_0 : \boldsymbol{\lambda} = \mathbf{0}$, for

$$\boldsymbol{\lambda} = \begin{bmatrix} \Delta - \delta_{\text{add\_}t(c+1)} \\ \theta_{\text{mix}} \end{bmatrix},$$

which is a nonlinear contrast of model parameters, since $\delta_{\text{add\_}t(c+1)} = \delta_{\text{add}}/\theta_{\text{add}(c+1)}$ and $\theta_{\text{add}(c+1)} = \sum_{i=1}^{c} \beta_i a_{(c+1)i}$. This hypothesis can be tested using the properties of either the quasi-likelihood ratio test described in Equation (3.3) or the Wald test for nonlinear hypotheses described in Equation (3.5). If the test of additivity is significant, then we can conclude that there is departure from additivity somewhere along the ray.

3.2.2. Test for a Region of Additivity

If we can conclude that departure from additivity exists along the mixture ray, a secondary goal is to determine where that departure exists. If the interaction threshold is significantly larger than the dose threshold, then we can conclude that there is a region of additivity that is associated with a response other than background. Therefore, the one-sided test of $H_0 : \Delta - \delta_{\text{add\_}t(c+1)} \leq 0$ is a test for an active region of additivity along the mixture ray. This hypothesis can be tested using a Wald test as described in Equation (3.5), where $\lambda = \Delta - \delta_{\text{add\_}t(c+1)}$.

4. APPLICATION TO THE CHLORINATION RAY

Recall the presentation of the chlorination ray data in Section 2. We have data from $c = 9$ single chemicals and the chlorination mixture, for a total of $h = 10$ rays. We visually demonstrated lack of fit of the threshold additivity model for large mixture concentrations (Figure 1) and hypothesized that such lack of fit suggests the existence of an interaction threshold. In this section, we apply the dose and interaction thresholds model to the chlorination ray data and test the additivity hypothesis.

4.1. MODEL DEFINITION AND FIT

The response of interest is cell viability, which is captured as a percent of negative control; therefore, it is reasonable to assume that the range of the mean response would fall in the interval between and including 0 and 100. At a total concentration of 0 mM, we would expect survival roughly equal to that observed in the negative control group (100%); as the total concentration increases, we expect that the percentage surviving will decrease until it plateaus at approximately 0%. In fact, the dose–response data appear to support this assumption. The vector of nonlinear parameters described in Equation (3.1) was chosen to represent these constants ($\alpha = 0, \gamma = 200$), with $\beta_0 = 0$.

The logistic model chosen to describe the concentration-response relationship along the chlorination ray and demonstrated in Figure 1 is of the form given in Equation (3.2). The temporary increase in observed cytotoxicity visible in Figure 1, however, indicates that a

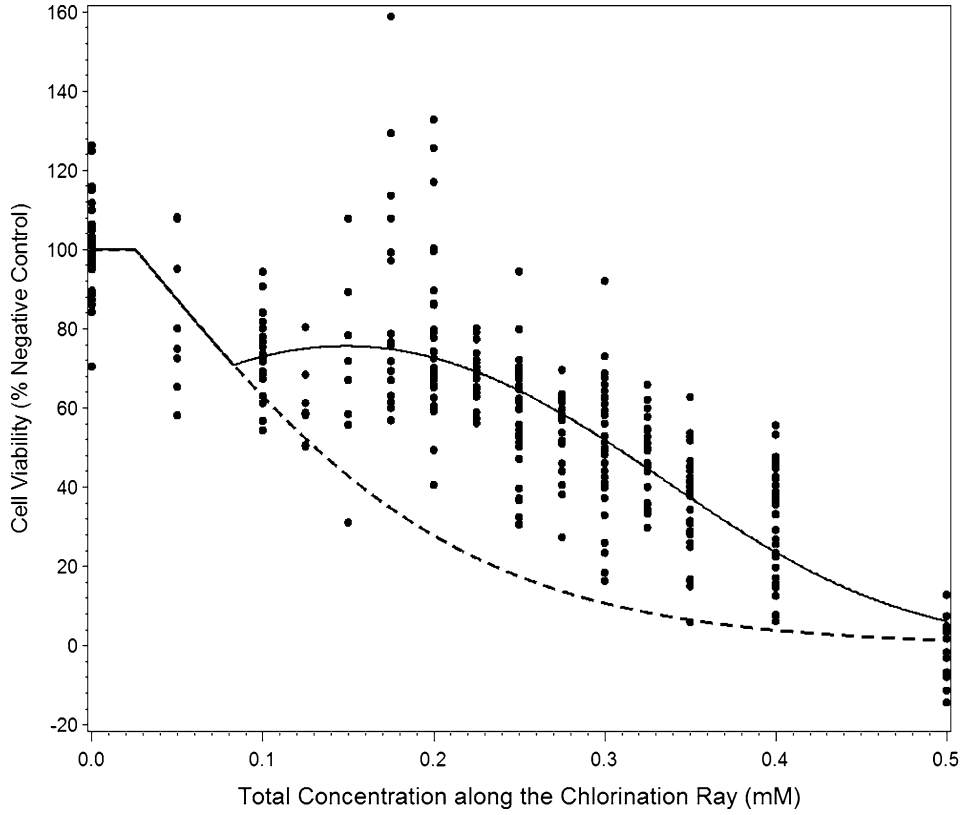

Figure 2. Assessment of model fit. The dashed line represents the additivity model, as determined by both the single chemical and the mixture data. The solid line represents the dose and interaction thresholds model.

higher order term (θ_{mix2}) is necessary to describe the departure from additivity. Therefore, we considered the dose and interaction thresholds model of the form

$$\mu_h = \begin{cases} 100, & t_{(h)} < \delta_{\text{add\_}t(h)}, \\ \dfrac{200}{1+\exp\{-((\sum_{i=1}^{9}\beta_i a_{(h)i})(t_{(h)}-\delta_{\text{add\_}t(h)}))\}}, & \begin{cases} t_{(h)} \geq \delta_{\text{add\_}t(h)}, h \leq 9, \\ \delta_{\text{add\_}t(h)} \leq t_{(h)} < \Delta, h = 10, \end{cases} \\ \dfrac{200}{1+\exp\{-((\sum_{i=1}^{9}\beta_i a_{(h)i})(t_{(h)}-\delta_{\text{add\_}t(h)})+\theta_{\text{mix}}(t_{(h)}-\Delta)+\theta_{\text{mix2}}(t_{(h)}-\Delta)^2)\}}, & t_{(h)} \geq \Delta \text{ and } h = 10 \end{cases}$$

for the analysis of the chlorination ray data.

Quasi-likelihood methods, implemented via the Gauss–Newton algorithm in Proc NLIN (SAS), were used to fit the model to the single chemical data and the mixture data simultaneously. The form of the variance function that most adequately fit the data was $\text{var}\{Y\} = \tau(\mu + \mu^2/k)$, where k was estimated to be -124. This function resulted in an R^2 value of 0.65, indicating that 65% of the variability present in the variance (i.e., from a model of the sample variance as a function of the sample mean) can be accounted for by this relationship. Figure 2 demonstrates the fit of this model, as well as the fit of the

Table 2. Parameter estimates resulting from fit of dose and interaction thresholds model to the single chemical and mixture data along the ray.

Parameter	Estimate	Standard Error	P-value
β_1(CA)	−1.83	0.077	<0.0001
β_2(DCA)	−0.22	0.008	<0.0001
β_3(TCA)	−0.51	0.022	<0.0001
β_4(BA)	−149.2	5.32	<0.0001
β_5(DBA)	−2.93	0.108	<0.0001
β_6(TBA)	−19.9	0.896	<0.0001
β_7(BDCA)	−1.99	0.074	<0.0001
β_8(DBCA)	−7.25	0.339	<0.0001
β_9(BCA)	−1.80	0.066	<0.0001
δ_{add}	−0.2692	0.0268	<0.0001
θ_{mix}	13.61	0.952	<0.0001
θ_{mix2}	−23.9	3.33	<0.0001
Δ	0.083	0.007	<0.0001
τ	12.47		
$\theta_{\text{add(10)}}$	−10.5	0.334	

threshold additivity model. As evidenced by the figure, the dose and interaction thresholds model more closely represents the data than the additivity model.

Table 2 lists the parameter estimates resulting from the fit of the dose and interaction thresholds model. All of the single chemicals are statistically significant in the dose and interaction thresholds model, and all of the corresponding single chemical slopes were negative. The estimate of $\delta_{\text{add\_}t\,(10)}$, the concentration threshold in terms of total concentration along the chlorination ray, is 0.026 mM (SE 0.002). The estimate of $\theta_{\text{add(10)}}$, the slope of total concentration under additivity, which is a function of the single chemical slopes and the proportions defining the chlorination ray, is −10.5 (SE 0.334). In the additivity region of the model, as the concentration of the mixture increases, cell viability decreases. The estimate of the interaction threshold, Δ, is 0.083 mM (SE 0.007), which is statistically significant. The estimate of θ_{mix} is statistically significant and positive; for total concentrations larger than 0.083 mM, the slope of the total concentration of the mixture is less negative than the slope in the additivity region. The estimate of θ_{mix2} is statistically significant and negative.

4.2. Testing for Interaction Along the Chlorination Ray

Recall that the primary research objective was to determine whether the nine HAAs involved in the mixture interact when combined according to the chlorination ray. The null hypothesis of additivity can be written

$$H_0 : \begin{cases} \Delta = \delta_{\text{add\_}t\,(10)}, \\ \theta_{\text{mix}} = 0, \\ \theta_{\text{mix2}} = 0 \end{cases}$$

which is tested according to the methods described in Section 3.2.

Table 3. Threshold parameter estimates.

Agent	Threshold Estimate (mM)	Standard Error	Approximate 95% Lower Confidence Bound	Approximate 95% Upper Confidence Bound
CA	0.147	0.013	0.122	0.173
DCA	1.23	0.111	1.01	1.45
TCA	0.526	0.049	0.430	0.622
BA	0.002	0.0002	0.002	0.002
DBA	0.092	0.008	0.076	0.108
TBA	0.014	0.001	0.011	0.016
BDCA	0.136	0.012	0.112	0.159
DBCA	0.037	0.003	0.030	0.044
BCA	0.149	0.013	0.124	0.175
Chlorination Ray Mixture (Concentration Threshold)	0.026	0.002	0.021	0.030
Chlorination Ray Mixture (Interaction Threshold)	0.083	0.007	0.070	0.096

The hypothesis of additivity can be tested using a quasi-likelihood ratio test as described in Equation (3.3). The threshold additivity model was fit to both the chlorination ray data and the single chemical data simultaneously. The achieved quasi-likelihood is that of the reduced model, Q_{red}, while the quasi-likelihood achieved under the dose and interaction thresholds model is that of the full model, Q_{full}. The resulting quasi-likelihood ratio test was statistically significant ($F_{3,1765} = 159.7$, pvalue < 0.0001), indicating that there is departure from additivity along the chlorination ray.

Alternatively, the hypothesis of additivity can be tested using the Wald test for nonlinear hypotheses. The constraints imposed by the null hypothesis can be represented by $H_0 : \boldsymbol{\lambda} = \mathbf{0}$, where

$$\begin{aligned}\boldsymbol{\lambda}' &= \begin{bmatrix}\Delta - \delta_{\text{add\_}t(10)} & \theta_{\text{mix}} & \theta_{\text{mix2}}\end{bmatrix} \\ &= \begin{bmatrix}\Delta - \delta_{\text{add}}/\sum_{i=1}^{c}\beta_i a_{(10)i} & \theta_{\text{mix}} & \theta_{\text{mix2}}\end{bmatrix},\end{aligned}$$

which is a vector of functions of the parameter vector $\boldsymbol{\beta}$. The Wald-type test statistic of this hypothesis is given in Equation (3.5) and has an approximate F distribution with 3 and 1765 degrees of freedom. The result is statistically significant ($F_{3,1765} = 132.1$, pvalue < 0.0001). Therefore, we conclude that there is departure from additivity along the chlorination ray.

The process for constructing confidence intervals around the threshold parameters is similar. The estimates of the threshold for each single chemical, as well as the concentration threshold and the interaction threshold for the mixture, are given in Table 3. The corresponding standard errors and confidence intervals are also provided. We are approximately 95% confident that, for the chlorination ray mixture, cell viability will be that of background for concentrations below 0.021 mM, the lower confidence bound for the concentration threshold of the mixture along the chlorination ray.

The Wald test for a region of additivity described in Section 3.2.2 was found to be statistically significant ($F_{1,1765} = 76.1$, pvalue < 0.0001). Therefore, we conclude that there is a significant active region of additivity along the ray. The location of this region can be described using the estimate of the interaction threshold, Δ, and the corresponding confidence interval.

According to the dose and interaction thresholds model, then, there is no change in cell viability prior to a total concentration of approximately 0.021 mM, the lower confidence bound for the concentration threshold along the chlorination ray. Conservatively, the agents behave as expected under additivity for total concentrations less than approximately 0.070 mM, the lower confidence bound for Δ. It may be that the agents behave as expected under additivity for total concentrations up to 0.096 mM, the upper confidence bound for Δ. In this additivity region, cell viability decreases significantly as total concentration increases. Beyond the interaction threshold, the model predicts higher cell viability than that predicted under additivity. Therefore, when the mixture is administered at a concentration larger than 0.096 mM, the model describes a less-than-additive response (i.e., antagonism according to Berenbaum's (1981) definition).

5. CONCLUSION

The dose and interaction thresholds model allows the investigator to simultaneously estimate both a dose threshold and an interaction threshold. If a significant test of additivity is followed by a significant test for a region of additivity, we can conclude that an active region of additivity (dose-dependent interaction) exists along the ray. The threshold parameter estimates can then be used to describe the location of this region, which may provide useful information in the risk assessment of chemical mixtures.

The flexibility of this model allowed us to accurately reflect the dose-response relationship of the chlorination ray data. In large part, we were able to consider a higher order term in the interaction region because of the sheer size of the experiment. The experiment included 15 dose groups, spaced throughout the entire concentration range; each group contained between 8 and 32 observations. The breadth of data in the interaction region suggested the "hump" as a true effect, since several concentrations were involved. In a smaller study of three to five dose groups, such a relationship would have been difficult to see.

The dose and interaction thresholds model is complicated in its parameterization, and care should be taken so that the experimental design is sufficient to support the fit of this model. Because this model uses single chemical data to support the region of additivity, the experimental design requires support primarily in and around the hypothesized interaction region. Design points above the interaction threshold should include doses near the hypothesized points of inflection to aid in parameter estimation. Design points below the interaction threshold can be included for verification purposes.

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REFERENCES

Berenbaum, M. C. (1981), "Criteria for Analyzing Interactions Between Biologically Active Agents," *Advances in Cancer Research*, 35, 269–335.

Carpy, S. A., Kobel, W., and Doe, J. (2000), "Health Risk of Low-Dose Pesticides Mixtures: A Review of the 1985–1998 Literature on Combination Toxicology and Health Risk Assessment," *Journal of Toxicology and Environmental Health, Part B*, 3, 1–25.

Casey, M., Gennings, C., Carter, W. H. Jr., Moser, V. C., and Simmons, J. E. (2004), "Detecting Interaction(s) and Assessing the Impact of Component Subsets in a Chemical Mixture Using Fixed-Ratio Mixture Ray Designs," *Journal of Agricultural, Biological, and Environmental Statistics*, 9, 339–361.

Cox, C. (1987), "Threshold Dose-Response Models in Toxicology," *Biometrics*, 43, 511–523.

Gennings, C., Carter, W. H. Jr., Campain, J. A., Bae, D., and Yang, R. S. H. (2002), "Statistical Analysis of Interactive Cytotoxicity in Human Epidermal Keratinocytes Following Exposure to a Mixture of Four Metals," *Journal of Agricultural, Biological and Environmental Statistics*, 7, 58–73.

Gennings, C., Carter, W. H. Jr., Carchman, R. A., DeVito, M. J., Simmons, J. E., and Crofton, K. M. (2007), "The Impact of Exposure to a Mixture of Eighteen Polyhalogenated Aromatic Hydrocarbons on Thyroid Function: Estimation of an Interaction Threshold," *Journal of Agricultural, Biological and Environmental Statistics*, 12, 96–111.

Gennings, C., Schwartz, P., Carter, W. H. Jr., and Simmons, J. E. (1997), "Detection of Departures from Additivity in Mixtures of Many Chemicals with a Threshold Model," *Journal of Agricultural, Biological, and Environmental Statistics*, 2, 198–211.

Hamm, A. K. (2004), "Analysis of an Interaction Threshold in Drug/Chemical Mixtures," unpublished Ph.D. Dissertation, Virginia Commonwealth University Department of Biostatistics, Richmond, VA.

McCullagh, P. (1983), "Quasi-Likelihood Functions," *The Annals of Statistics*, 11, 59–67.

McCullagh, P., and Nelder, J. A. (1989), *Generalized Linear Models* (2nd ed.), New York: Chapman & Hall.

Plewa, M. J., Kargalioglu, Y., Vankerk, D., Minear, R. A., and Wagner, E. D. (2000), "Development of Quantitative Comparative Cytotoxicity and Genotoxicity Assays for Environmental Hazardous Chemicals," *Water Science and Technology*, 42, 109–116.

——— (2002), "Mammalian Cell Cytotoxicity and Genotoxicity Analysis of Drinking Water Disinfection Byproducts," *Environmental and Molecular Mutagenesis*, 40, 134–142.

Plewa, M. J., Wagner, E. D., Jazwierska, P., Richardson, S. D., Chen, P. H., and McKague, A. B. (2004), "Chemical Characterization, Mammalian Cell Cytotoxicity and Genotoxicity of Halonitromethane Drinking Water Disinfection By-Products," *Environmental Science and Technology*, 38, 62–68.

Richardson, S. D., Simmons, J. E., and Rice, G. (2002), "Disinfection Byproducts: The Next Generation," *Environmental Science and Technology*, 36 (9), 198A–205A.

Schwartz, P. F., Gennings, C., and Chinchilli, V. M. (1995), "Threshold Models for Combination Data From Reproductive and Developmental Experiments," *Journal of the American Statistical Association*, 90, 862–870.

Seber, G. A. F., and Wild, C. J. (2003), *Nonlinear Regression*, New York: Wiley.

Stork, L. G., Gennings, C., Carter, W. H. Jr., Johnson, R. E., Mays, D. P., Simmons, J. E., Wagner, E. D., and Plewa, M. J. (2007), "Testing for Additivity in Chemical Mixtures Using a Fixed-Ratio Ray Design and Statistical Equivalence Testing Methods," *Journal of Agricultural, Biological, and Environmental Statistics*, 12, 514–533.

Ulm, K. (1991), "A Statistical Method for Assessing a Threshold in Epidemiological Studies," *Statistics in Medicine*, 10, 341–349.

U.S. Environmental Protection Agency (2000), "Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures". USEPA EPA/630/R-00/002. National Center for Environmental Assessment, Office of Research and Development, Washington, D.C. 51 FR 34014. Available: *http://www.epa.gov/NCEA/raf/chem_mix.htm*.