國立交通大學

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碩 士 論 文

化學物混合交互作用之再研究

A Re-visit of
Assessment of Interactions in Chemical Mixtures

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中華民國 一百 年 六 月

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化學物混合交互作用之再研究

國立交通大學統計學研究所

摘要

這篇文章目的在於提出較無爭議之化學物混合之效用的分解。我們提出此分解之母體主效用及交互作用。在常態分配之假設下我們計算這些作用並且以表格呈列，我們也進一步介紹統計估計方法，並模擬分析其估計效果。

A Re-visit of Assessment of Interactions in Chemical Mixtures

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SUMMARY

This paper propose new but unambiguous systematic decompositions of the effect of covariates on a response variable into main effect and interaction effects. The population type main effects and interaction effects under the normal distribution are formulated. These effects under some examples of distributional parameter settings are computed and presented. Then, this settings make the study of main effects and interactions available through classical statistical inferences techniques.

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Key words: ANOVA; antogonistic effect; grand mean; interaction; linear regression; main effect; synergistic effect.

1. Introduction

In biological sciences, it often needs to verify if several covariates (factors or characteristics) measured from the subjects are risk factors for a caues of disease (cancer) or death. For examples, Ponce, et al. (2005) investigated if maternal age (categorized as $< 20, 20-29, 30-34, \geq 35$ years), maternal race/ethnicity (African American, White, Hispanic, other races) and some others are risk factors for preterm birth and Kifley et al. (2007) studied if smoking (current smoking and past smoking), alcohol use (light, moderate and heavy) are some others casuses of retinal venular carliber. The observations of a covariate are generally classified into intervals (categories) that aims not only for detection if presence of this covariate forms a risk factor for the cause but also find support for cumulative lifetime exposure to risk factor such as smoking would cause more chance of disease or death.

The toxicological research has long been devoted to assess the risk with exposure to single chemicals in the environment. However, organisms are rarely environmentally exposed to single chemicals in isolation. More typically, exposures occur to multiple chemicals simultaneously. It has long understood that the behavior of one chemical in the body is affected by other chemicals. Recently most researches in the literature have been investigated on the important area of toxicology of mixed chemicals. One very important study in chemical mixtures is the detection for existence of interactions and characterization of an interaction being synergistic or antagonistic effect. It is important for this study since one may overestimate or underestimate the true risk.

There are several approaches for studying the chemical interactions. The most common technique in analysis of toxicologic interactions is by classifying the chemicals into interval levels and verifying it through the analysis of variance (ANOVA). This technique can detect the existence of interactions, however, there is no description of the interaction to be given. The isobolographic method has a long history but is recently popular as an alternative method for the study of chemical interactions. Berenbaum (1981) defined the interaction index through fixed ratio ray designs to detect if the chemical mixture is additive, synergistic or antagonistic. However, this techniques of isobole require experimental iterations to obtain the doses of the studying chemicals that will cause the same magnitude of effect which is not only labor extensive and require a large number of animal experiments but is not applicable in real data analysis. For references of various interaction detecting techniques and discussions, see Rider and LeBlane (2005), Ei-Masri, Reardon and Yang (1997), Charles et. al. (2002) and Mumtaz et al. (1998).

A systematic investigation of mixed chemicals in the environment or workplace is highly desired while the isobolographic method is not applicable for this practical investigation of interaction characterization. It is interesting to see if we can develop an ANOVA like model deserving the benefit of providing valuable insights into the detection of interactions being synergistic or antagonistic that is done by the isobolographic method.

In Section 2, we state the fundamental framework of a grouping ANOVA model for one response variable and several chemical variables that states new concepts of main effects and interactions. In Section 3, we introduce the parameter type main effects and interactions where results are computed and discussed when specifications of normal distribution are given. In Section

4, we perform a simulation study for parametric estimation of these effects. The simulated results indicate that the proposed estimating method is quite promissing. In Section 5, the proofs are provided.

2. Decomposition of Total Effect Into Covariate Contributions

Let Y be the effect of exposure to two chemicals X_1 and X_2 where random variables Y, X_1 and X_2 have a joint distribution with mean μ and covariance matrix Σ as

$$\mu = \begin{pmatrix} \mu_y \\ \mu_1 \\ \mu_2 \end{pmatrix} \text{ and } \Sigma = \begin{pmatrix} \sigma_y^2 & \sigma_{1y} & \sigma_{2y} \\ \sigma_{y1} & \sigma_1^2 & \sigma_{12} \\ \sigma_{y2} & \sigma_{21} & \sigma_2^2 \end{pmatrix}. \tag{2.1}$$

A key in statistical analysis for toxic effects of a mixture is the formulation of combined effects as linear function of main effects and interactions. Let $A_1 = (0, a_1], A_2 = (a_1, a_2], ..., A_m = (a_{m-1}, \infty)$ and $B_1 = (0, b_1], B_2 = (b_1, b_2], ..., B_\ell = (b_{\ell-1}, \infty)$ be respectively, the interval types partitions of the spaces of X_1 and X_2 where a_i's and b_j's are two known increasing sequences. The conditional mean of y on rectangular level $A_j \times B_g$ is denoted by $\mu_{jg} = E(Y| \begin{pmatrix} X_1 \\ X_2 \end{pmatrix} \in A_j \times B_g)$. By defining the group variable $Y_{jg} = Y|X_1 \in A_j, X_2 \in B_g$, the error variables as $\epsilon_{jg} = Y_{jg} - \mu_{jg}$, the effect variable Y in each rectangular level $A_j \times B_g$ may be represented in a location model as

$$\begin{array}{ccccc} & B_1 & B_2 & \dots & B_\ell \\ A_1 & Y_{11} = \mu_{11} + \epsilon_{11} & Y_{12} = \mu_{12} + \epsilon_{12} & \dots & Y_{1\ell} = \mu_{1\ell} + \epsilon_{1\ell} \\ A_2 & Y_{21} = \mu_{21} + \epsilon_{21} & Y_{22} = \mu_{22} + \epsilon_{22} & \dots & Y_{2\ell} = \mu_{2\ell} + \epsilon_{2\ell} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ A_m & Y_{m1} = \mu_{m1} + \epsilon_{m1} & Y_{m2} = \mu_{m2} + \epsilon_{m2i} & \dots & Y_{m\ell} = \mu_{m\ell} + \epsilon_{m\ell} \end{array} \tag{2.2}$$

The two way classical ANOVA technique applying on this interval grouping problem is assuming the following ANOVA model

$$Y_{jg} = \mu + \alpha_j + \beta_g + \gamma_{jg} + \epsilon_{jg}, j = 1, ..., m, g = 1, ..., \ell \tag{2.3}$$

with restrictions $\sum_{j=1}^m \alpha_j = \sum_{g=1}^\ell \beta_g = \sum_{j=1}^m \gamma_{jg} = \sum_{g=1}^\ell \gamma_{jg} = 0$. Applying this classical ANOVA method for toxicological study can only detect the existence of interactions but can not tell us if they are synergistic or antagonistic due to these restrictions when they exist.

The central to the problems in interaction study is that the statistical modeling does not involve the joint distributions of effect variable and chemical variables so that relative contributions of chemicals can not accurately defined. The population mean μ_y is a combination of mean of non-polluted Y and polluted Y. The population mean of a non-polluted subject Y may be formulated as

$$\mu_{0x} = E(Y|X \leq 0).$$

Definition 2.1. Let $A_1, ..., A_m$ be the interval levels. Suppose that there are constants μ and b such that the conditional mean $\mu_j = E[Y|X \in A_j]$ may be written as $\mu_j = \mu + bE(X - \theta_x|X \in A_j)$. We call $\mu_x = \mu - \mu_{0x}$ the X contributed grand mean and $\eta_j = bE(X - \theta_x|X \in A_j)$ the X contributed main effect. We also call μ the grand mean.

The main effect measures the contribution of covariate X_1 on the mean of effect variable Y. With the established main effect formulations, the effect variables at levels may be formulated as

$$\begin{array}{cccc} A_1 & A_2 & \cdots & A_m \\ y_1 = \mu + \eta_1 + \epsilon_1 & y_2 = \mu + \eta_2 + \epsilon_2 & \cdots & y_m = \mu + \eta_m + \epsilon_m \end{array} \tag{2.4}$$

These one way ANOVA - like models are not identical to the classical one way ANOVA models since their main effects are not restricted to have zero sums.

Now, suppose that there are two chemicals (covariates) that contribute the effect variable Y. We denote $\eta_{x_1,j}$ and $\eta_{x_2,g}$ as the main effects, respectively, for chemical variable X_1 at level A_j and for variable X_2 at level B_g. The population mean of non-polluted effect is $\mu_{0x_1x_2} = E(Y|X_1 \leq 0, X_2 \leq 0)$. The conditional mean $\mu_{jg} = E[Y|X_1 \in A_j, X_2 \in B_g]$ quantifies the effect of exposure to chemicals X_1 and X_2 at level $A_j \times B_g$. No interaction intuitively represents the fact that μ_{jg} is the sum of μ with two individual main effects.

Definition 2.2. Suppose that there is constant μ and vector b such that the the group mean $\mu_{jg} = E[Y|X_1 \in A_j, X_2 \in B_g]$ may be written as $\mu_{jg} = \mu + \mu_{com,jg}$ with $\mu_{com,jg} = b' \begin{pmatrix} E[X_1 - \theta_1|X_1 \in A_j, X_2 \in B_g] \\ E[X_2 - \theta_2|X_1 \in A_j, X_2 \in B_g] \end{pmatrix}$.
(a) We call $\mu_{x_1x_2} = \mu - \mu_{0x_1x_2}$ the (X_1, X_2) contributed grand mean and μ the grand mean in this model.
(b) We say that $\mu_{com,jg}$ the (j, g)-th combined effect and there is no interaction if the level $A_j \times B_g$ combined effect can be written as

$$\mu_{com,jg} = \eta_{x_1,j} + \eta_{x_2,g}, \text{ for all } j \text{ and } g. \tag{2.5}$$

The interaction of exposure to these two chemicals at level $A_j \times B_g$ is defined as the effect excceeding the no-interaction conditional mean.

Definition 2.3. (a) The interaction at level $A_j \times B_g$ is defined as

$$\eta_{jg} = \mu_{com,jg} - [\eta_{x_1,j} + \eta_{x_2,g}]. \tag{2.6}$$

(b) If $\eta_{jg} > 0$, we say that there is synergistic effect and, if $\eta_{jg} < 0$, we say that there is antagonistic effect at level $A_j \times B_g$.

With the above settings, the effect variable in interval level $A_j \times B_g$ of (2.2) may be formulated into an ANOVA like model in the following:

$$y_{jg} = \mu + \eta_{x_1,j} + \eta_{x_2,g} + \eta_{jg} + \epsilon_{jg}. \tag{2.7}$$

This reflects the aggregate contribution of two covariates X_1 and X_2 being $\mu_{x_1,x_2} + \eta_{x_1,j} + \eta_{x_2,g} + \eta_{jg}$. Hence the group mean μ_{jg} may not be completely contributed by X_1 and X_2 at level $A_j \times B_g$ unless $\mu_{0,x_1,x_2} = 0$. We say that a two way ANOVA model is additive if $\eta_{jg} = 0$ for all j, g's. This formulation of interactions does not make any restriction on the main effect and interaction parameters so that it is appropriate for making inferences of synergistic or antagonistic effect based on this model. This conceptual development of main effects and interactions in this section is for general study. In the next section, we restrict on the normal distribution for explicit formulation of these characteristics.

3. Main Effect and Interaction Formulation Under Normal Distribution

Consider that we have a subject that is exposed to chemical variable X and the exposed effect variable Y and X are jointly normal with distribution

$$\begin{pmatrix} Y \\ X \end{pmatrix} \sim N_2(\begin{pmatrix} \theta_y \\ \theta_x \end{pmatrix}, \begin{pmatrix} \sigma_y^2 & \sigma_{xy} \\ \sigma_{yx} & \sigma_x^2 \end{pmatrix}).$$

We first display formulations of un-polluted effect and main effect.

Theorem 3.1. With normality assumption, we have un-polluted effect as

$$\mu_{0x} = \theta_y - \frac{\rho_{yx}\sigma_y}{\sqrt{2\pi}\Phi(\frac{-\theta_x}{\sigma_x})} e^{-\frac{\theta_x^2}{2\sigma_x^2}}, \mu_x = \frac{\rho_{yx}\sigma_y}{\sqrt{2\pi}\Phi(\frac{-\theta_x}{\sigma_x})} e^{-\frac{\theta_x^2}{2\sigma_x^2}}$$

and main effects as

$$\begin{aligned} \eta_1 &= \frac{\rho_{yx}\sigma_y}{\sqrt{2\pi}[\Phi(\frac{a_1-\theta_x}{\sigma_x}) - \Phi(\frac{-\theta_x}{\sigma_x})]}[e^{-\frac{1}{2}(\frac{-\theta_x}{\sigma_x})^2} - e^{-\frac{1}{2}(\frac{a_1-\theta_x}{\sigma_x})^2}] \\ &\vdots \\ \eta_j &= \frac{\rho_{yx}\sigma_y}{\sqrt{2\pi}(\Phi(\frac{a_j-\theta_x}{\sigma_x}) - \Phi(\frac{a_{j-1}-\theta_x}{\sigma_x}))}\{e^{-\frac{1}{2}(\frac{a_{j-1}-\theta_x}{\sigma_x})^2} - e^{-\frac{1}{2}(\frac{a_j-\theta_x}{\sigma_x})^2}\} \\ &\quad , j = 2, ..., m-1 \qquad (3.1) \\ &\vdots \\ \eta_m &= +\frac{\rho_{yx}\sigma_y}{\sqrt{2\pi}(1-\Phi(\frac{a_{m-1}-\theta_x}{\sigma_x}))} e^{-\frac{1}{2}(\frac{a_{m-1}-\theta_x}{\sigma_x})^2} \end{aligned}$$

where $\rho_{yx} = \frac{\sigma_{yx}}{\sigma_y\sigma_x}$ is the correlation coefficient between Y and X and Φ is the distribution function of the standard normal distribution.

In Table 1, we set up several parameter values to display the un-polluted effect.

Table 1. Un-polluted effects μ_{0x} and μ_x

	$\sigma_{y1} = 0.2$	$\sigma_{y1} = 0.4$	$\sigma_{y1} = 0.7$	$\sigma_{y1} = 0.9$
$\theta_y = 1.5$				
$\theta_x = 1$	$\binom{1.195}{0.305}$	$\binom{0.890}{0.610}$	$\binom{0.432}{1.068}$	$\binom{0.127}{1.373}$
1.2	$\binom{1.163}{0.337}$	$\binom{0.825}{0.675}$	$\binom{0.319}{1.181}$	$\binom{-0.019}{1.519}$
$\theta_y = 2$				
$\theta_x = 1$	$\binom{1.695}{0.305}$	$\binom{1.390}{0.610}$	$\binom{0.392}{1.068}$	$\binom{0.627}{1.373}$
1.2	$\binom{1.663}{0.337}$	$\binom{1.325}{0.675}$	$\binom{0.819}{1.181}$	$\binom{0.481}{1.519}$
1.5	$\binom{1.612}{0.838}$	$\binom{1.225}{0.775}$	$\binom{0.643}{1.357}$	$\binom{0.255}{1.745}$
1.8	$\binom{1.561}{0.439}$	$\binom{1.121}{0.879}$	$\binom{0.462}{1.538}$	$\binom{0.022}{1.978}$
$\theta_y = 3$				
$\theta_x = 1$	$\binom{2.695}{0.305}$	$\binom{2.390}{0.610}$	$\binom{1.932}{1.068}$	$\binom{1.627}{1.373}$
1.2	$\binom{2.663}{0.337}$	$\binom{2.325}{0.675}$	$\binom{1.819}{1.181}$	$\binom{1.481}{1.519}$
1.5	$\binom{2.612}{0.388}$	$\binom{2.225}{0.775}$	$\binom{1.643}{1.357}$	$\binom{1.255}{1.745}$
1.8	$\binom{2.561}{0.439}$	$\binom{2.121}{0.879}$	$\binom{1.462}{1.538}$	$\binom{1.022}{1.978}$
2	$\binom{2.525}{0.475}$	$\binom{2.051}{0.949}$	$\binom{1.339}{1.661}$	$\binom{0.684}{2.136}$
2.5	$\binom{2.436}{0.565}$	$\binom{1.871}{1.129}$	$\binom{1.024}{1.976}$	$\binom{0.460}{2.540}$

We have several comments for the results in Table 1:

(a) The quantity μ_{0x} measures the effect on Y not polluted with chemical X. This effect, for fixed θ_y, decreases when θ_x increases. For fixed θ_x, this effect increases in θ_y.

(b) The quantity μ_x measures the constant effect contributed by variable X. The un-polluted effect, for fixed θ_y, increases in θ_x. For fixed θ_x, this un-polluted effect is a constant in θ_y.

We compute the main effects and display the results in Table 2.

Table 2. Main effects

	η_1	η_2	η_3
$\theta_x = 1$			
$\sigma_{yx} = 0.2$	-0.048	0.137	0.300
0.4	-0.096	0.273	0.600
0.7	-0.168	0.478	1.049
0.9	-0.216	0.614	1.349
$\theta_x = 1.5$			
$\sigma_{yx} = 0.2$	-0.078	0.137	0.300
0.4	-0.156	0.273	0.600
0.7	-0.273	0.478	1.049
0.9	-0.351	0.614	1.349
$\theta_x = 2$			
$\sigma_{yx} = 0.2$	-0.096	0.137	0.300
0.4	-0.192	0.273	0.600
0.7	-0.337	0.478	1.049
0.9	-0.433	0.614	1.349

The main effects (η_1) are negative for lower level are negative and are negative for bigger levels (η_2 and η_2). This shows that main effects positive or negative is not only relying on covariance between Y and X but also relying on low or high levels. This shows that this setting makes the main effects independently determined by the relationship between variables Y and X. This property is not allowed in the classical ANOVA model that can only detect if the main effects exist.

Assume that the subject is exposed to chemical variables X_1 and X_2 and the exposed effect variable Y and X_1 and X_2 are jointly normal with distribution

$$\begin{pmatrix} Y \\ X_1 \\ X_2 \end{pmatrix} \sim N_3(\begin{pmatrix} \theta_y \\ \theta_1 \\ \theta_2 \end{pmatrix}, \begin{pmatrix} \sigma_y^2 & \sigma_{1y} & \sigma_{2y} \\ \sigma_{y1} & \sigma_1^2 & \sigma_{12} \\ \sigma_{y2} & \sigma_{21} & \sigma_2^2 \end{pmatrix}).$$

It is known that the conditional mean of Y given $X_1 = x_1, X_2 = x_2$ is

$$\begin{aligned} \theta_{y|x_1x_2} =& \theta_y + (\sigma_{y1}, \sigma_{y2}) \begin{pmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{21} & \sigma_2^2 \end{pmatrix}^{-1} (\begin{pmatrix} x_1 \\ x_2 \end{pmatrix} - \begin{pmatrix} \theta_1 \\ \theta_2 \end{pmatrix}) \\ =& \theta_y - (\sigma_{y1}, \sigma_{y2}) \begin{pmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{21} & \sigma_2^2 \end{pmatrix}^{-1} \begin{pmatrix} \theta_1 \\ \theta_2 \end{pmatrix} \\ &+ (\sigma_{y1}, \sigma_{y2}) \begin{pmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{21} & \sigma_2^2 \end{pmatrix}^{-1} \begin{pmatrix} x_1 \\ x_2 \end{pmatrix} \end{aligned}$$

When X_1 and X_2 are uncorrelated, the conditional mean then is

$$\theta_{y|x_1x_2} = \theta_y - \frac{\sigma_{y1}}{\sigma_1^2}\theta_1 - \frac{\sigma_{y2}}{\sigma_2^2}\theta_2 + \frac{\sigma_{y1}}{\sigma_1^2}x_1 + \frac{\sigma_{y2}}{\sigma_2^2}x_2.$$

We now state the results for some types of effects defined earlier in the following theorem.

Theorem 3.2. Suppose that the normality assumption is true.
(a) The (j,g)th group mean may be decomposed as $\mu_{jg} = \mu + \mu_{com,jg}$ with

$$\mu = \theta_y - (\sigma_{y1}, \sigma_{y2}) \begin{pmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{21} & \sigma_2^2 \end{pmatrix}^{-1} \begin{pmatrix} \theta_1 \\ \theta_2 \end{pmatrix}$$

and

$$\mu_{com,jg} = (\sigma_{y1}, \sigma_{y2}) \begin{pmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{21} & \sigma_2^2 \end{pmatrix}^{-1} \begin{pmatrix} E[X_1|X_1 \in A_j, X_2 \in B_g] \\ E[X_2|X_1 \in A_j, X_2 \in B_g] \end{pmatrix}.$$

(b) The un-polluted effect for two chemicals is

$$\mu_{0x_1x_2} = \theta_y + (\sigma_{y1}, \sigma_{y2}) \begin{pmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{21} & \sigma_2^2 \end{pmatrix}^{-1} [\begin{pmatrix} E[X_1|X_1 \leq 0, X_2 \leq 0] - \theta_1 \\ E[X_2|X_1 \leq 0, X_2 \leq 0] - \theta_2 \end{pmatrix}.$$

(c) The (x_1, x_2)-contributed grand mean is

$$-(\sigma_{y1}, \sigma_{y2}) \begin{pmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{21} & \sigma_2^2 \end{pmatrix}^{-1} [\begin{pmatrix} E[X_1|X_1 \leq 0, X_2 \leq 0] \\ E[X_2|X_1 \leq 0, X_2 \leq 0] \end{pmatrix}.$$

(d) The level $A_j \times B_g$ interactions is

$$\eta_{jg} = (\sigma_{y1}, \sigma_{y2}) \begin{pmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{21} & \sigma_2^2 \end{pmatrix}^{-1} \begin{pmatrix} E[X_1|X_1 \in A_j, X_2 \in B_g] \\ E[X_2|X_1 \in A_j, X_2 \in B_g] \end{pmatrix} - [\eta_{x_1,j} + \eta_{x_2,g}]$$

where main effects $\eta_{x_1,j}$ and $\eta_{x_2,g}$ are, respectively, defined in the form of (3.1) for variables X_1 and X_2.

The mixture of chemical variables X_1 and X_2 contributes to toxicity Y through the common mechanism of the sum of individual effects and the interaction effect. However, the interaction is not contributed from any product term between conditional means of X_1 and X_2.

Let us give an example for explanation of interactions where we consider a three dimensional normal distribution for Y, X_1, X_2 with mean and covariance matrix as

$$\mu = \begin{pmatrix} \mu_y \\ 1 \\ 1 \end{pmatrix} \text{ and } \Sigma = \begin{pmatrix} 1 & \sigma_{y1} & \sigma_{y2} \\ \sigma_{1y} & 1 & \sigma_{12} \\ \sigma_{2y} & \sigma_{21} & 1 \end{pmatrix}. \tag{3.2}$$

The interval levels are determined with $a_1 = F_{x_1}^{-1}(2/3), a_2 = F_{x_1}^{-1}(5/6)$ and $b_1 = F_{x_2}^{-1}(2/3), b_2 = F_{x_2}^{-1}(5/6)$. In Tables 3 and 4, we display the true interactions for these inetrval levels.

Table 3. Unpolluted effect

	$\sigma_{y1} = 0.2$ $\sigma_{y2} = 0.7$	$\sigma_{y1} = 0.3$ $\sigma_{y2} = 0.4$	$\sigma_{y1} = 0.7$ $\sigma_{y2} = 0.9$
$\sigma_{12} = 0.3$			
$\mu_y = 1.2$	0.093	0.338	-0.777
$\mu_y = 1.5$	0.390	0.637	-0.481
$\mu_y = 1.7$	0.591	0.840	-0.276
$\mu_y = 2.0$	0.889	1.136	0.023
$\sigma_{12} = -0.3$			
$\mu_y = 1.2$	-0.624	-0.220	
$\mu_y = 1.5$	-0.323	0.079	
$\mu_y = 1.7$	-0.124	0.282	
$\mu_y = 2.0$	0.176	0.584	

Table 4. Unpolluted effect

	$\sigma_{y1} = 0.2$ $\sigma_{y2} = 0.2$	$\sigma_{y1} = 0.3$ $\sigma_{y2} = 0.3$	$\sigma_{y1} = 0.4$ $\sigma_{y2} = 0.4$
$\sigma_{12} = 0.3$			
$\mu_y = 1.2$	0.707	0.465	0.211
$\mu_y = 1.5$	1.004	0.761	0.511
$\mu_y = 1.7$	1.207	0.961	0.712
$\mu_y = 2.0$	1.506	1.258	1.014
$\sigma_{12} = -0.3$			
$\mu_y = 1.2$	0.392	-0.019	-0.426
$\mu_y = 1.5$	0.683	0.271	-0.130
$\mu_y = 1.7$	0.886	0.487	0.067
$\mu_y = 2.0$	1.191	0.783	0.380

It is seen that the un-polluted effect $\mu_{0x_1x_2}$ may be negative. This happens because the assumption of a joint continuous distribution.

In the next, we display the interaction effects.

Table 5. Interaction effects for $\sigma_{y1} = 0.2$ and $\sigma_{y2} = 0.7$

$(\mu_y, \sigma_{y1}, \sigma_{y2})$	$= (1.2, 0.2, 0.7)$	$(1.5, 0.2, 0.7)$	$(1.5, 0.3, 0.4)$
$\sigma_{12} = 0.3$			
η_{11}	0.736	0.736	0.586
η_{12}	0.741	0.741	0.454
η_{13}	0.720	0.719	0.454
η_{21}	0.575	0.572	0.475
η_{22}	0.555	0.549	0.426
η_{23}	0.554	0.558	0.354
η_{31}	0.427	0.428	0.371
η_{32}	0.384	0.383	0.321
η_{33}	0.429	0.437	0.245
$\sigma_{12} = -0.3$			
η_{11}	1.212	1.216	0.945
η_{12}	1.302	1.303	1.041
η_{13}	1.379	1.382	1.127
η_{21}	1.394	1.404	1.064
η_{22}	1.543	1.543	1.200
η_{23}	1.584	1.596	1.276
η_{31}	1.562	1.559	1.162
η_{32}	1.712	1.727	1.286
η_{33}	1.719	1.741	1.353

There are comments for the results displayed in Table 5:

(a) The interactions are antagonistic when ρ is positive values and synergistic when ρ is negative values, and it is an additive model when ρ is zero.

(b) There is monotone property for the interactions with

$$\eta_{ij} < \eta_{i+1j} \text{ and } \eta_{ij} < \eta_{ij+1}.$$

This is interesting. Unfortunately, we are not available to provide theoretical proof.

(c) The interaction effect to be positive or negative is not solely dependent on the sign of correlation coefficient between X_1 and X_2.

We here state a second type of interaction effect.

Definition 3.3. By defining $\delta^1_{jg} = E(Y|X_1 \in A_j, X_2 \in B_g)$ and $\delta^2_{jg} =$

$E(Y|X_1 \in A_j, X_2 \in B_g, \sigma_{12} = 0)$. The type II interaction is defined as

$$\eta_{jg} = \delta^1_{jg} - \delta^2_{jg}, \text{ for all } j \text{ and } g.$$

We display the computed results of this setting of interaction in Table 6.

Table 6. Type II interaction effects for with $\sigma_{y1} = \sigma_{y2} = 0.4$

	$\mu_y = 1.2$	$\mu_y = 1.5$	$\mu_y = 1.7$
$\sigma_{12} = 0.3$			
η_{11}	0.045	0.056	0.035
η_{12}	0.034	0.055	-0.014
η_{13}	0.026	0.025	-0.073
η_{21}	-0.119	-0.137	-0.038
η_{22}	-0.159	-0.145	-0.115
η_{23}	-0.146	-0.105	-0.180
η_{31}	-0.260	-0.255	-0.140
η_{32}	-0.305	-0.281	-0.194
η_{33}	-0.263	-0.240	-0.164
$\sigma_{12} = -0.3$			
η_{11}	-0.067	-0.067	-0.065
η_{12}	0.010	0.014	0.030
η_{13}	0.107	0.099	0.147
η_{21}	0.117	0.109	0.084
η_{22}	0.245	0.229	0.197
η_{23}	0.325	0.324	0.236
η_{31}	0.295	0.266	1.156
η_{32}	0.408	0.450	0.290
η_{33}	0.414	0.465	0.312

Again, this type of interaction effect may be positive or negative for any sign of correlation coefficient between X_1 and X_2.

4. Statistical Inferences for Interactions

With specifications of main effects and interactions, it is then interesting to introduce techniques of statistical inferences for them when they are practically unknown. In this section, we will perform a simulation to verify the efficiencies of one parametric estimation of the main effect and interaction. We set the levels as $a_1 = F^{-1}_{x_1}(2/3), a_2 = F^{-1}_{x_1}(5/6)$ and $b_1 = F^{-1}_{x_2}(2/3), b_2 = F^{-1}_{x_2}(5/6)$. We assume that we have observations $\begin{pmatrix} y_1 \\ x_{11} \\ x_{21} \end{pmatrix}, \ldots, \begin{pmatrix} y_n \\ x_{1n} \\ x_{2n} \end{pmatrix}$. Let the sample means and sample variances for Y, X_1, X_2 be respectively denoted as $\bar{Y}, \bar{X}_1, \bar{X}_2$ and S_y^2, S_1^2, S_2^2. Also, we denote the sample correlation coefficients for $\{Y, X_1\}$ and $\{Y, X_2\}$ be respectively denoted as r_{y1} and r_{y2}. Some statistics are defined below:

$$\hat{a}_1 = \hat{F}_1^{-1}(2/3), \hat{a}_2 = \hat{F}_1^{-1}(5/6), \hat{b}_1 = \hat{F}_2^{-1}(2/3), \hat{b}_2 = \hat{F}_2^{-1}(5/6),$$

$$\hat{A}_1 = [0, \hat{a}_1), \hat{A}_2 = [\hat{a}_1, \hat{a}_2), \hat{A}_3 = [\hat{a}_2, \infty),$$

$$\hat{B}_1 = [0, \hat{b}_1), \hat{B}_2 = [\hat{b}_1, \hat{b}_2), \hat{B}_3 = [\hat{b}_2, \infty),$$

$$\hat{\eta}_{X_1,1} = \frac{\hat{\gamma}_{y1} S_y}{\sqrt{2\pi}[\Phi(\frac{\hat{a}_1 - \bar{X}_1}{S_1}) - \Phi(\frac{-\bar{X}_1}{S_1})]}[e^{-\frac{1}{2}(-\frac{-\bar{X}_1}{S_1})^2} - e^{-\frac{1}{2}(\frac{\hat{a}_1 - \bar{X}_1}{S_1})^2}]$$

$$\hat{\eta}_{X_1,2} = \frac{\hat{\gamma}_{y1} S_y}{\sqrt{2\pi}[\Phi(\frac{\hat{a}_2 - \bar{X}_1}{S_1}) - \Phi(\frac{\hat{a}_1 - \bar{X}_1}{S_1})]}[e^{-\frac{1}{2}(\frac{\hat{a}_1 - \bar{X}_1}{S_1})^2} - e^{-\frac{1}{2}(\frac{\hat{a}_2 - \bar{X}_1}{S_1})^2}]$$

$$\hat{\eta}_{X_1,3} = \frac{\hat{\gamma}_{y1} S_y}{\sqrt{2\pi}[1 - \Phi(\frac{\hat{a}_2 - \bar{X}_1}{S_1})]} e^{-\frac{1}{2}(\frac{\hat{a}_2 - \bar{X}_1}{S_1})^2}$$

$$\hat{\eta}_{jg} = (S_{y1}, S_{y2}) \begin{pmatrix} S_1^2 & S_{12} \\ S_{21} & S_2^2 \end{pmatrix}^{-1} \begin{pmatrix} \hat{\mu}_{1jg} \\ \hat{\mu}_{2jg} \end{pmatrix} - [\hat{\eta}_{X_1,j} + \hat{\eta}_{X_2,g}]$$

with $\hat{\mu}_{1jg} = \dfrac{\sum_{i=1}^n X_{1i} I(X_{1i} \in A_j, X_{2i} \in B_g)}{\sum_{i=1}^n I(X_{1i} \in A_j, X_{2i} \in B_g)}, \hat{\mu}_{2jg} = \dfrac{\sum_{i=1}^n X_{2i} I(X_{1i} \in A_j, X_{2i} \in B_g)}{\sum_{i=1}^n I(X_{1i} \in A_j, X_{2i} \in B_g)}$

MSE's for main effect $MSE_j = \dfrac{1}{100,000} \sum_{i=1}^{100,000} (\hat{\eta}_{ji} - \eta_j)^2,$

MSE's for interactions $MSE_{jg} = \dfrac{1}{100,000} \sum_{i=1}^{100,000} (\hat{\eta}_{jgi} - \eta_{jg})^2.$

In the simulation studies in this section, the replication numbers are all 100,000's. With sample sizes $n = 30$ and 50 and designs of parameters $(\mu_y, \sigma_{y1}, \sigma_{y2})$, we display the average of simulated main effects and their MSE's corresponding variables X_1 and X_2 in Table 7.

Table 7. Performance of main effects and their corresponding MSE's

$(\mu_y, \sigma_{y1}, \sigma_{y2})$	η_1	η_2	η_3
$n = 30$			
$(1.2, 0.2, 0.7), X_1$	-0.049 (0.003)	0.133 (0.021)	0.297 (0.086)
X_2	-0.172 (-0.168)	0.469 (0.064)	1.043 (0.178)
$(1.5, 0.3, 0.4), X_1$	-0.074 (0.004)	0.201 (0.026)	0.447 (0.096)
X_2	-0.099 (0.004)	0.268 (0.032)	0.595 (0.110)
$n = 50$			
$(1.2, 0.2, 0.7), X_1$	-0.049 (0.002)	0.135 (0.012)	0.298 (0.051)
X_2	-0.170 (0.005)	0.472 (0.038)	1.044 (0.105)
$(1.5, 0.3, 0.4), X_1$	-0.073 (0.002)	0.203 (0.015)	0.449 (0.057)
X_2	-0.098 (0.002)	0.270 (0.019)	0.597 (0.065)

From the MSE's results in Table 7, it is seen that the estimation of main effect through the above parametric estimation is effcient since the MSE's are reasonably small. We also see that the MSE's for $n = 50$ are relatively smaller than those for $n = 30$. This shows that increasing the sample size may reduce the errors in estimation of main effects.

For sample sizes $n = 30$ and 50, we also perform the simulation of estimating the interactions and the simulated results are displayed in Table 8 and 9.

Table 8. Interaction effects and their MSE's ($\sigma_{12} = 0.3, n = 30$)

$(\mu_y, \sigma_{y1}, \sigma_{y2})$	$(1.2, 0.2, 0.7)$	$(1.5, 0.3, 0.4)$	$(1.0, 0.2, 0.3))$
η_{11}	0.752 (0.049)	0.586 (0.060)	0.417 (0.064)
η_{12}	0.748 (0.055)	0.538 (0.063)	0.388 (0.064)
η_{13}	0.691 (0.125)	0.467 (0.084)	0.342 (0.075)
η_{21}	0.685 (0.501	0.551 (0.194)	0.391 (0.159)
η_{22}	0.556 (0.078)	0.433 (0.065)	0.307 (0.059)
η_{23}	0.526 (0.186)	0.369 (0.104)	0.267 (0.080)
η_{31}	0.457 (0.103)	0.423 (0.084)	0.295 (0.072)
η_{32}	0.383 (0.121)	0.340 (0.092)	0.238 (0.072)
η_{33}	0.381 (0.225)	0.296 (0.131)	0.212 (0.094)

Table 9. Interaction effects and their MSE's ($\sigma_{12} = 0.3, n = 50$)

$(\mu_y, \sigma_{y1}, \sigma_{y2})$	$(1.2, 0.2, 0.7)$	$(1.5, 0.3, 0.4)$	$(1.0, 0.2, 0.3)$
η_{11}	0.740 (0.028)	0.576 (0.033)	0.411 (0.036)
η_{12}	0.724 (0.030)	0.521 (0.032)	0.376 (0.033)
η_{13}	0.672 (0.070)	0.453 (0.043)	0.331 (0.037)
η_{21}	0.605 (0.323)	0.504 (0.110)	0.357 (0.085)
η_{22}	0.534 (0.045)	0.414 (0.034)	0.296 (0.030)
η_{23}	0.514 (0.123)	0.356 (0.058)	0.259 (0.044)
η_{31}	0.435 (0.056)	0.406 (0.043)	0.284 (0.036)
η_{32}	0.366 (0.069)	0.325 (0.050)	0.229 (0.038)
η_{33}	0.387 (0.130)	0.300 (0.074)	0.215 (0.049)

We have several comments for the results in Tables 8 and 9:

(a) Most MSE's are low enough showing that the estimators are quite efficient in predicting the unknown interactions. However, there are few (for examples, 0.501 when $n = 30$ and 0.323 when $n = 50$ for η_{21} with design $(1.2, 0.2, 0.7)$) with relatively larger MSE's. This is due to small sizes of samples falling in these cells. This also indicates that level settings is important.

(b) Comparison of the MSE's for cases of $n = 30$ and 50, we see that increasing the sampel size can reduce the MSE's for estimation of unknown interactions.

5. Appendix

We denote that the conditional pdf of y given $X_1 \in A_j$ is

$$\begin{aligned} f_{y|A_j}(y) &= \int_{A_j} f_{y|x_1}(y) \frac{1}{P(X_1 \in A_j)} f_{x_1}(x_1) dx_1 \\ &= \frac{1}{P(X_1 \in A_j)} \int_{A_j} f(y, x_1) dx_1. \end{aligned}$$

and the conditional pdf of y given $A_j \times B_g$ is

$$\begin{aligned} f_{y|A_j \times B_g}(y) &= \int_{A_j \times B_g} f_{y|x_1,x_2}(y) \frac{1}{P(\begin{pmatrix} X_1 \\ X_2 \end{pmatrix} \in A_j \times B_g)} f_{x_1 x_2}(x_1, x_2) dx_1 dx_2 \\ &= \frac{1}{P(\begin{pmatrix} X_1 \\ X_2 \end{pmatrix} \in A_j \times B_g)} \int_{A_j \times B_g} f_{y,x_1,x_2}(y, x_1, x_2) dx_1 dx_2. \end{aligned}$$

since $\frac{1}{P(\begin{pmatrix} X_1 \\ X_2 \end{pmatrix} \in A_j \times B_g)} f_{x_1,x_2}(x_1, x_2)$ is the truncated pdf of X_1 and X_2 on $A_j \times B_g$.

Proof of Theorem 3.1: From the well known property $E(y|x) = \theta_y + \frac{\rho_{yx}\sigma_y}{\sigma_x}(x - \theta_x)$ where x is a given value, we have conditional mean of Y at

A_1

$$\begin{aligned}
\mu_0 &= \int_{-\infty}^{\infty} y f_{y|A_1}(y)dy \\
&= \int_{-\infty}^{\infty} y \frac{1}{P(0 \le X \le a_1)} \int_0^{a_1} f(y,x)dxdy \\
&= \frac{1}{P(0 \le X \le a_1)} \int_0^{a_1} [\int_{-\infty}^{\infty} y f(y|x)dy] f_x(x)dx \\
&= \frac{1}{P(0 \le X \le a_1)} \int_0^{a_1} [\theta_y + \frac{\rho_{yx}\sigma_y}{\sigma_x}(x-\theta_x)] f_x(x)dx \\
&= \theta_y + \frac{1}{P(0 \le X \le a_1)} \frac{\rho_{yx}\sigma_y}{\sigma_x} [\int_0^{a_1} x f_x(x)dx - \theta_x P(0 \le X \le a_1)] \qquad (5.1) \\
&= \theta_y + \frac{\rho_{yx}\sigma_y}{\sqrt{2\pi}[\Phi(\frac{a_1-\theta_x}{\sigma_x}) - \Phi(\frac{-\theta_x}{\sigma_x})]} [e^{-\frac{1}{2}(\frac{-\theta_x}{\sigma_x})^2} - e^{-\frac{1}{2}(\frac{a_1-\theta_x}{\sigma_x})^2}]
\end{aligned}$$

from the fact that $\int_{-\infty}^{a_1} x f_x(x)dx = \theta_x P(X \le a_1) - \frac{\sigma_x}{\sqrt{2\pi}} e^{-\frac{1}{2}(\frac{a_1-\theta_x}{\sigma_x})^2}$. Formulation (5.1) states the linear function of conditional mean $E(X|X \in A_1)$. The other θ_j's may be derived analogously and are skipped. □

Proof of Theorem 3.2: The conditional mean of Y given the intercal $A_j \times B_g$ can be derived as follows:

$$\begin{aligned}
\mu_{jg} &= \int_{-\infty}^{\infty} y f_{y|A_j \times B_g}(y)dy \\
&= \int_{-\infty}^{\infty} y \frac{1}{P(\begin{pmatrix} X_1 \\ X_2 \end{pmatrix} \in A_j \times B_g)} \int_{B_g}\int_{A_j} f(y,x_1,x_2)dx_1dx_2dy \\
&= \frac{1}{P(\begin{pmatrix} X_1 \\ X_2 \end{pmatrix} \in A_j \times B_g)} \int_{B_g}\int_{A_j} [\int_{-\infty}^{\infty} y f(y|x_1,x_2)dy] f_{x_1,x_2}(x_1,x_2)dx_1dx_2 \\
&= \frac{1}{P(\begin{pmatrix} X_1 \\ X_2 \end{pmatrix} \in A_j \times B_g)} \int_{B_g}\int_{A_j} [\theta_y + (\sigma_{y1},\sigma_{y2}) \begin{pmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{21} & \sigma_2^2 \end{pmatrix}^{-1} (\begin{pmatrix} x_1 \\ x_2 \end{pmatrix} - \begin{pmatrix} \theta_1 \\ \theta_2 \end{pmatrix})] \\
& f_{x_1,x_2}(x_1,x_2)dx_1dx_2 \\
&= \theta_y + (\sigma_{y1},\sigma_{y2}) \begin{pmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{21} & \sigma_2^2 \end{pmatrix}^{-1} \begin{pmatrix} E[X_1|X_1 \in A_j, X_2 \in B_g] - \theta_1 \\ E[X_2|X_1 \in A_j, X_2 \in B_g] - \theta_2 \end{pmatrix}.
\end{aligned}$$

The result of (a) is straight forward and the result of (b) is induced from (2..6) with (a) and the above conditional mean. □

Proof of Theorem 3.4: Assuming that X_1 and X_2 are uncorrelated, they are independent in this normal case and the formula is derived from the followings:

$$\mu_{jg} = \theta_y + \frac{1}{P(X_1 \in A_j)P(X_2 \in B_g)}(\sigma_{y1}, \sigma_{y2}) \begin{pmatrix} \sigma_1^2 & 0 \\ 0 & \sigma_2^2 \end{pmatrix}^{-1} \begin{bmatrix} \int_{A_j}\int_{B_g} x_1 f_1(x_1) f_2(x_2) dx_1 dx_2 \\ \int_{A_j}\int_{B_g} x_2 f_1(x_1) f_2(x_2) dx_1 dx_2 \end{bmatrix}$$

$$- (\sigma_{y1}, \sigma_{y2}) \begin{pmatrix} \sigma_1^2 & 0 \\ 0 & \sigma_2^2 \end{pmatrix}^{-1} \begin{pmatrix} \theta_1 \\ \theta_2 \end{pmatrix}$$

$$= \theta_y + \frac{1}{P(X_1 \in A_j)P(X_2 \in B_g)}(\sigma_{y1}, \sigma_{y2}) \left[\begin{matrix} \frac{1}{\sigma_1^2} P(X_2 \in B_g) \int_{A_j} x_1 f_1(x_1) dx_1 \\ \frac{1}{\sigma_2^2} P(X_1 \in A_j) \int_{B_g} x_2 f_2(x_2) dx_2 \end{matrix} \right)$$

$$- [\frac{\sigma_{y1}}{\sigma_1^2}\theta_1 + \frac{\sigma_{y2}}{\sigma_2^2}\theta_2]$$

$$= \theta_y + \frac{\sigma_{y1}}{\sigma_1^2}(\frac{\int_{A_j} x_1 f_1(x_1) dx_1}{P(X_1 \in A_j)} - \theta_1) + \frac{\sigma_{y2}}{\sigma_2^2}(\frac{\int_{B_g} x_2 f_2(x_2) dx_2}{P(X_2 \in B_g)} - \theta_2)$$

$$= \mu_{y12} + \eta_j^a + \eta_g^b. \quad \square$$

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