An Overall Risk Probability Based Method for Quantification of Synergistic and Antagonistic Effects in Health Risk Assessment for Mixtures: Theoretical Concepts

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Abstract

Purpose In the assessment of health risks of environmental pollutants, the method of dose addition and the method of independent action are used to assess mixture effects when no synergistic and/or antagonistic effects are present. Currently no method exists to quantify synergistic and/or antagonistic effects for mixtures. The purpose of this paper is to develop the theoretical concepts of an overall risk probability (ORP) based method to quantify the synergistic and antagonistic effects in health risk assessment for mixtures.

Method The ORP for health effects of environmental chemicals was determined from the cumulative probabilities of exposure and effects. This method was used to calculate the ORP for independent mixtures and for mixtures with synergistic and antagonistic effects.

Results For independent mixtures, a mixture ORP can be calculated from the product of the ORPs of individual components. For systems of interacting mixtures, a synergistic coefficient and an antagonistic coefficient were defined respectively to quantify the ORPs of each individual component in the mixture. The component ORPs with synergistic and/or antagonistic effects were then used to calculate the total ORP for the mixture.

Conclusions An ORP-based method was developed to quantify synergistic and antagonistic effects in health risk assessment for mixtures. This represents a first method to generally quantify mixture effects of interacting toxicants.

Keywords: Health risk assessment for mixtures, overall risk probability method, synergistic effects, synergistic coefficient, antagonistic effects, antagonistic coefficient.

1 Introduction

Health risk assessment of environmental chemicals is important in many aspects of environmental management. Examples of such risk assessment include the assessments of human health risks of chlorinated disinfection by-products in drinking water (Hamidin et al. 2008), organic contaminants in indoor air (Sofuoglu et al. 2011; Hamidin 2009), and endocrine disrupting chemicals in water recycling schemes (Cao et al. 2010). Health risk assessment for environmental pollutants has a number of specific difficulties that need to be considered. These include long exposure times up to life times, chronic toxicity effects from low levels of pollutants, large variations and different statistical distributions in both exposure and dose-response data, and the evaluation of the combined effects of mixtures. Environmental pollutants are usually present in the environment as mixtures. However, despite the prevalence of environmental pollutants as mixtures, the assessment and quantification of toxic effects of mixtures are not commonly studied.

Currently, the only methods that can be used for the quantification of mixture effects are the *method of dose addition* (Bliss, 1939; Sumpter et al. 2006; Thorpe et al. 2006) and the *method of independent action (or response addition)* (Loewe et al., 1926; Silva et al. 2002; Christiansen et al. 2009). The method of dose addition can be used when the mixture components are assumed to act through a similar or common mode of action (Sumpter et al. 2006). For example, the base nonspecific toxicities of similar organic compounds such as those in a homologue series are considered to have similar modes of actions. In this case, it is assumed that one chemical in a mixture can be replaced with an equivalent (or equitoxic) amount of another chemical to produce a similar effect and the toxicity of the mixture can be described by an equivalent dose of the mixture compounds (Silva et al. 2002). Each chemical does not exacerbate or diminish the effects of another chemical, and no synergistic or antagonistic effects are present. Similarly, the method of independent action assumes that the modes of actions of the individual components in a mixture are independent to each other and no synergistic or antagonistic effects are occurring in the mixture. There is no toxicity model that can be used to quantify synergistic effects and/or antagonistic effects in mixtures. The BINWOE (binary weight of evidence) method is developed for mixture effects in which a hazard index value obtained from the method of dose addition is adjusted by an uncertainty factor raised to the power of a weight factor to take into account the interaction effects (Mumtaz and Durkin, 1992; USEPA, 1999).

In health risk assessment of toxicants, many methods have been used to evaluate and quantify the adverse effects of the toxicants. These methods can be divided into conventional non-probabilistic methods and probabilistic based methods. In a conventional method, an exposure dose (or concentration), usually in the form of an average or medium value, is compared with a threshold or reference value for a given adverse effect. For example, a hazard quotient (or risk quotient) can be calculated from the ratio of the exposure value to the reference value (Cao et al. 2011, 2010). The larger the value of the hazard quotient, the higher the health risks of adverse effects being observed. A margin of safety can also be calculated to be the inverse of the hazard quotient (Solomon et al. 2000; Straub and Stewart 2007). Typically in a conventional method of risk characterization, the statistical distributions of the exposure and/or adverse effects data are not taken into account.

Probabilistic methods are relatively newer methods for the evaluation and quantification of health risks from exposure to toxicants (Bosgra et al. 2007; Bosgra et al. 2009; Carriger and Rand 2008; Johnston and Snow 2007; Jager et al. 2001; Thompson and Graham 1996). Risk assessment is based on the use of cumulative probability plots of both the exposure and adverse effect (dose response) data sets on the same graph. In a probabilistic method, the statistical distributions of both the exposure data set and the adverse effect data set could be taken into account to evaluate the quantification of adverse effects. Therefore, a more comprehensive and accurate assessment of risk characterization can be obtained. The disadvantage is that many more data points are needed. In recent years, the probabilistic risk assessment method has been used in several case studies of health risk assessment of environmental chemicals (Straub and Stewart 2007; Hamidin et al. 2008; Cao et al. 2011; Chowdhury et al. 2009).

Risk characterization and quantification by the probabilistic approach is usually achieved through a comparison between the cumulative probability curves for exposure and effects. Under typical conditions, the exposure curve is on the left hand side of the effect curve, and the closeness or overlap between the two curves provides a qualitative indication of the relative degree of the health risk. A closer overlap between the two curves indicates a higher risk. On the other hand, if the two curves are far apart from each other and do not overlap, it can be said that the risk is low or negligible. To obtain a quantitative comparison, a hazard (or risk) quotient (HQ) can be calculated. For example, $HQ_{95/5}$ is defined as the ratio of the exposure dose (or concentration) at 95% cumulative probability on the exposure curves to that at 5% cumulative probability at the effect curve (Cao et al. 2011). A reference point of $HQ_{95/5} = 1$ corresponds to a risk probability of 1/20, or 5%. Similarly, a reference $HQ_{50/50} = 1$ represents a risk probability of 50%. This situation would also indicate that the two cumulative probability curves are largely overlapped with each other over the same dose intervals. However, while the HQ method is a simple method for risk quantification and comparison, because it is only a single point method, the statistical distributions of the cumulative probability curves are not taken into account. The HQ may not represent the true risk characteristics of the system, in particular when the distributions of exposure and effects data do not follow the normal distribution or have different slopes (Cao et al., 2011).

The method of Monte Carlo simulation with the use of the hazard quotient has also been used for the quantification of the risks. In this method, a large number of HQ values are calculated by randomly selecting values from the two cumulative probability curves. From these HQ values, the probability distribution of HQ values can be calculated and plotted out (eg Cao et al. 2010; Djohan et al. 2007). Effectively, this method transforms the two cumulative probability charts of exposure and effects into a new probability distribution of HQ values. In addition, the Monte Carlo simulation usually requires the two cumulative probability distributions to be normal distributions. This restricts the application of the method.

Recently a new method of overall risk probability (ORP) was developed to quantify the health risks from the two cumulative probability plots (Cao et al. 2011). In this method, an ORP for the health effect is calculated. This method overcomes the nature of a single point evaluation used in the HQ method and provides a straight-forward probability measure for the health risks present in the system. When compared to the HQ method, the ORP method has a number of advantages. The

ORP method provides a simple probability measure that can easily compare the relative risks of individual compounds. It is also a multiple-point method such that the statistical distributions of both the exposure and effect curves are properly taken into account. Importantly, the ORP method also has the advantage that it can quantify mixture effects of either independent or interacting mixtures. On the other hand, the ORP method needs the construction and integration of an exposure exceedence curve to obtain the ORP. This requires more computational efforts when compared to single point methods such as the hazard quotient method.

The purpose of this paper is to extend the concept of ORP to mixture systems and therefore to develop an ORP-based method for the quantification of synergistic and antagonistic effects in health risk assessment for mixtures. The theoretical concepts of the ORP method for mixture effects were developed. In this approach, for independent mixtures where there are no synergistic and/or antagonistic effects, the ORP for the mixture can be calculated from the product of the ORPs of individual components. For systems of interacting mixtures, a synergistic coefficient and an antagonistic one were defined as needed to quantify the ORPs of each individual component in the mixture. The component ORPs with synergistic and/ or antagonistic effects were then used to calculate the total ORP for the mixture.

2 Theoretical Concepts

In a risk assessment with the probabilistic approach, the risk characterization and quantification are based on the cumulative probability charts for both exposure and adverse effects plotted on the same graph (US EPA 2001; Solomon et al. 2000). These are determined from various experimental measurements. A schematic example is given in Figure 1. The x-axis in Figure 1 is either dose or concentration as the measure of exposure to the toxicant. Logarithm scale is used. The y-axis represents the cumulative probability of the exposure and effects.

From the cumulative probability curves in Figure 1, for each point on the effect curve, with the percentage of sample affected identified as x%, a corresponding exposure probability value can be obtained from the exposure curve as identified by y% at the same dose or concentration. The exposure exceedence value is then calculated as 1-y%. When the exposure exceedence value 1-y% is plotted against the percentage of sample affected, an exposure exceedence curve is obtained. This is illustrated in Figure 2. The exposure exceedence curves can be used to characterize the health risk of the system as represented by the cumulative probability charts. The origin of an exposure exceedence chart represents zero risk and any deviation from the origin represents increasing risks. The higher the exceedence curve, the higher the risk. Quantitatively, the area underneath the exposure exceedence curve represents the overall risk probability of the adverse effects (Cao et al. 2011). This area can be easily obtained through numerical integration of the exceedence curve.

The extension of the ORP method for the quantification of mixture effects is derived for the cases of independent effects, antagonistic effects and synergistic effects as follows. When the individual components in the mixture do not interact with each other, it can be assumed that the ORP of each component remain the same as if the components are present in single component systems. The product of the ORPs for the mixture can therefore be calculated from Equation (1).

$$P_m = 1 - \prod_{i=1}^n (1 - P_i) \quad \text{Equation (1)}$$

where P_m is the ORP for the mixture, P_i is the ORP for component i and n is the number of components in the mixture. Here the value of ORP for the mixture represents the overall percentage of samples that are adversely affected by exposure to the given mixture. If different endpoints are used for different individual compounds, the mixture ORP would represent the overall percentage of samples adversely affected as indicated by any one or more of the endpoints.

For cases where individual components interact antagonistically and reduce risk effects of other components, an antagonistic coefficient is introduced to quantify these effects. Here the antagonistic coefficient a_{ij} is defined to represent the probability of component j reducing the adverse effects of component i . It is further defined that the antagonistic coefficient values range from zero to -1. No antagonistic effects are present if a_{ij} is zero and maximum antagonistic effects are present if a_{ij} is -1. With the use of the antagonistic coefficients, the ORP of a component in the mixture can be calculated from the ORP values of the individual components in single component systems. The equation for this calculation is given below in Equation (2).

$$P_i = P_i^0 \prod_{j=1}^n (1 + a_{ij} P_j^0) \quad \text{Equation (2a)}$$

where P_i is the ORP of component i in the mixture and P_i^0 is the ORP of component i as if it is in a single-component system with the same cumulative probability distributions. In Equation (2a), the antagonistic coefficient of a component to itself is defined to be zero, as there can be no self-antagonistic effects. Therefore,

$$a_{ii} = 0 \quad \text{Equation (2b)}$$

The values of a_{ij} can be obtained through multi-variable regression analysis of experimental data with Equation (1) and (2a).

Some of the limiting points for antagonistic effects in mixtures can be obtained from Equation (2) and they are given in Equation (3).

$$a_{ij} = 0 \quad (1 + a_{ij} P_j^0) = 1 \quad P_i = P_i^0 \quad \text{Equation (3a)}$$

$$P_j^0 = 0 \quad (1 + a_{ij} P_j^0) = 1 \quad P_i = P_i^0 \quad \text{Equation (3b)}$$

$$a_{ij} = -1 \quad (1 + a_{ij} P_j^0) = 1 - P_j^0 \quad P_i = P_i^0 \prod_{j=1}^n (1 - P_j^0) \quad \text{Equation (3c)}$$

$$P_j^0 = 1 \quad (1 + a_{ij} P_j^0) = 1 + a_{ij} \quad P_i = P_i^0 \prod_{j=1}^n (1 + a_{ij}) \quad \text{Equation (3d)}$$

$$a_{ij} = -1, P_j^0 = 1 \quad (1 + a_{ij} P_j^0) = 0 \quad P_i = 0 \quad \text{Equation (3e)}$$

Similarly, for mixtures in which individual components interact synergistically to increase the risk effects of other components, the synergistic effects can be quantified through the introduction of a synergistic coefficient. It is defined that the synergistic coefficient s_{ij} represents the probability of component j increasing the adverse effects of component i . It is further defined here that the synergistic coefficient values range from zero to 1. If s_{ij} is zero, no synergistic effects are present. The maximum synergistic effects are present in the mixture when the s_{ij} value is 1. With the use of the synergistic coefficients, the ORP of a component in the mixture can be calculated from the ORP values of the individual components in single component systems. This is given below in Equation (4).

$$(1 - P_i) = (1 - P_i^0) \prod_{j=1}^n (1 - s_{ij} P_j^0) \quad \text{Equation (4a)}$$

The above equation can be re-written as

$$P_i = 1 - (1 - P_i^0) \prod_{j=1}^n (1 - s_{ij} P_j^0) \quad \text{Equation (4b)}$$

In Equation (4a) and (4b), the synergistic coefficient of a component to itself is also defined to be zero, as there can be no self-synergistic effects. Therefore,

$$s_{ii} = 0 \quad \text{Equation (4c)}$$

The values of s_{ij} can be obtained through multi-variable regression analysis of experimental data with Equation (1) and (4b).

Also similarly, some of the limiting points for synergistic effects in mixtures can be obtained from Equation (4) and they are given in Equation (5).

$$s_{ij} = 0 \quad (1 - s_{ij} P_j^0) = 1 \quad P_i = P_i^0 \quad \text{Equation (5a)}$$

$$P_j^0 = 0 \quad (1 - s_{ij} P_j^0) = 1 \quad P_i = P_i^0 \quad \text{Equation (5b)}$$

$$s_{ij} = 1 \quad (1 - s_{ij} P_j^0) = 1 - P_j^0 \quad P_i = 1 - (1 - P_i^0) \prod_{j=1}^n (1 - P_j^0) \quad \text{Equation (5c)}$$

$$P_j^0 = 1 \quad (1 - s_{ij} P_j^0) = 1 - s_{ij} \quad P_i = 1 - (1 - P_i^0) \prod_{j=1}^n (1 - s_{ij}) \quad \text{Equation (5d)}$$

$$s_{ij} = 1, P_j^0 = 1 \quad (1 + s_{ij} P_j^0) = 0 \quad P_i = 1 \quad \text{Equation (5e)}$$

Finally, after the synergistic or the antagonistic effects are taken into account as above (Equations 2 and 4) and the ORPs for each individual component in the mixture are calculated, the ORP for the mixture can be calculated with Equation (1) by the product of the component ORPs.

3 Some Numerical Examples to Illustrate the Use of the Technique

In order to illustrate the use of the above equations, a set of numerical examples for a simple two-component system is provided here. For this system, it is assumed that the ORP of each component from the two single component systems are determined to be

$$P_1^0 = 0.50 \quad \text{Equation (6a)}$$

$$P_2^0 = 0.50 \quad \text{Equation (6b)}$$

If the two components do not interact with each other, then

$$P_1 = P_1^0 = 0.50 \quad \text{Equation (7b)}$$

$$P_2 = P_2^0 = 0.50 \quad \text{Equation (7b)}$$

From Equation (1),

$$P_m = 1 - (1 - 0.50)(1 - 0.50) = 0.75 \quad \text{Equation (8)}$$

Or, the ORP for the mixture is 75%.

For the case that the two components interact antagonistically and the antagonistic coefficients are determined to be

$$a_{11} = 0 \quad \text{Equation (9a)}$$

$$a_{12} = -0.25 \quad \text{Equation (9b)}$$

$$a_{21} = -0.25 \quad \text{Equation (9c)}$$

$$a_{22} = 0 \quad \text{Equation (9d)}$$

From Equation (2a),

$$P_1 = 0.50 (1 - 0.25 \times 0.50) = 0.4375 \quad \text{Equation (10a)}$$

$$P_2 = 0.50 (1 - 0.25 \times 0.50) = 0.4375 \quad \text{Equation (10b)}$$

From Equation (1),

$$P_m = 1 - (1 - 0.4375)(1 - 0.4375) = 0.6836 \quad \text{Equation (11)}$$

Or, the ORP for the mixture with antagonistic effects is about 68%. The antagonistic effects have reduced the risk probability from 75% to 68%.

For the case that the two components interact synergistically and the synergistic coefficients are determined to be

$$s_{11} = 0 \quad \text{Equation (12a)}$$

$$s_{12} = 0.25 \quad \text{Equation (12b)}$$

$$s_{21} = 0.25 \quad \text{Equation (12c)}$$

$$s_{22} = 0 \quad \text{Equation (12d)}$$

From Equation (4a),

$$1 - P_1 = (1 - 0.50) (1 - 0.25 \times 0.50) = 0.4375 \quad \text{Equation (13a)}$$

$$1 - P_2 = (1 - 0.50) (1 - 0.25 \times 0.50) = 0.4375 \quad \text{Equation (13b)}$$

Or,

$$P_1 = 1 - 0.4375 = 0.5625 \quad \text{Equation (14a)}$$

$$P_2 = 1 - 0.4375 = 0.5625 \quad \text{Equation (14b)}$$

From Equation (1),

$$P_m = 1 - (1 - 0.5625)(1 - 0.5625) = 0.8086 \quad \text{Equation (15)}$$

Or, the ORP for the mixture with antagonistic effects is about 81%. In this case, the synergistic effects have increased the risk probability from 75% to 81%.

As an additional example, the ORP of two endocrine disrupting chemicals (EDCs) are calculated below. The ORP method has been used in the assessment of the adverse effect of significant level of vitellogenin induction in fish exposed to 17 β -estradiol (E2) and 17 α -ethinylestradiol (EE2) in fresh water receiving treated wastewater effluents (Cao, 2010). The ORPs obtained for E2 and EE2 in the study are as follows:

$$P_{E2}^0 = 0.081 \quad \text{Equation (16a)}$$

$$P_{EE2}^0 = 0.27 \quad \text{Equation (16b)}$$

There are evidences that estrogenic compounds act either additively (Brian et al., 2005) or mildly synergistically (Christiansen et al., 2009). If we assume the two EDCs do not interact with each other, the ORP for exposure to the mixture of two is

$$P_m = 1 - (1 - 0.081) (1 - 0.27) = 0.33 \quad \text{Equation (17)}$$

On the other hand, if we use a synergistic coefficient of 0.10 to represent the interacting effect of the two, the ORP for exposure to the mixture is

$$1 - P_{E2} = (1 - 0.081) (1 - 0.10 \times 0.27) = 0.894 \quad \text{Equation (18a)}$$

$$1 - P_{EE2} = (1 - 0.27) (1 - 0.10 \times 0.081) = 0.724 \quad \text{Equation (18b)}$$

Or,

$$P_{E2} = 1 - 0.894 = 0.106 \quad \text{Equation (19a)}$$

$$P_{EE2} = 1 - 0.724 = 0.276$$

Equation (19b)

$$P_m = 1 - (1 - 0.106)(1 - 0.276) = 0.35$$

Equation (20)

4 Conclusions

The evaluation and quantification of mixture effects are important aspects in the assessment of overall health risks from environmental chemicals. This is especially true when the individual components interact to produce synergistic and/or antagonistic effects, for which currently there is no quantification method available. Based on an extension of the concept of overall risk probability in health risk assessment, a new method was developed for the quantification of synergistic and antagonistic effects in mixture systems. The theoretical concept of an overall risk probability for mixtures was developed, and quantification of synergistic and antagonistic effects was accomplished with a set of synergistic and antagonistic coefficients based on the interacting components. This approach represents a first method to generally quantify mixture effects of interacting toxicants.

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Figure captions

Figure 1 Cumulative probability charts used for calculation of exceedence curves. $x\%$ represents the percentage of sample affected at a given dose or concentration, $y\%$ represents the cumulative probability of exposure, and $1-y\%$ represents the exposure exceedence.

Figure 2 Exceedence curves used for calculation of overall risk probabilities. A, B, and C are three separate exceedence curves with increasing risk probabilities.

Figure 1

Figure 2