Toxicology 335 (2015) 20–26

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Explanation of non-additive effects in mixtures of similar mode of action chemicals

Masashi Kamo[a,*], Hiroyuki Yokomizo[b]

[a] National Institute for Advanced Industrial Science and Technology, Research Institute of Science for Safety and Sustainability, 16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan
[b] Center for Environmental Risk Research, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

ARTICLE INFO

Article history:
Received 7 May 2015
Received in revised form 23 June 2015
Accepted 24 June 2015
Available online 29 June 2015

Keywords:
Risk assessment
Chemical mixture
Synergism
Antagonism
Mode of action

ABSTRACT

Many models have been developed to predict the combined effect of drugs and chemicals. Most models are classified into two additive models: independent action (IA) and concentration addition (CA). It is generally considered if the modes of action of chemicals are similar then the combined effect obeys CA; however, many empirical studies report nonlinear effects deviating from the predictions by CA. Such deviations are termed synergism and antagonism. Synergism, which leads to a stronger toxicity, requires more careful management, and hence it is important to understand how and which combinations of chemicals lead to synergism. In this paper, three types of chemical reactions are mathematically modeled and the cause of the nonlinear effects among chemicals with similar modes of action was investigated. Our results show that combined effects obey CA only when the modes of action are exactly the same. Contrary to existing knowledge, combined effects are generally nonlinear even if the modes of action of the chemicals are similar. Our results further show that the nonlinear effects vanish out when the chemical concentrations are low, suggesting that the current management procedure of assuming CA is rarely inappropriate because environmental concentrations of chemicals are generally low.

1. Introduction

Current frameworks of risk assessments deal in most cases with a single chemical, but at the same time, as there are multiple chemicals used together, there are concerns regarding the cumulative or combined effects of chemical mixtures (Berenbaum, 1977; Feron et al., 1995; Teuschler and Hertzberg, 1995; Groten et al., 2001; Feron and Groten, 2002; McCarty and Borgert, 2006; Boobis et al., 2011; Meek et al., 2011; Cedergreen, 2014). In particular, for pesticides or biocides, risk assessments for combined effects have been conducted, and guidelines or documents for combined effects have also been published by governmental and international organizations (USEPA, 2002, 2003; Kortenkamp et al., 2009; OECD, 2011; European Commission, 2012).

Studies of the combined effects of chemical substances have a long history. Two different models predicting combined effects were developed by Loewe and Muischnek in 1926 (Loewe and Muischnek, 1926) and by Bliss in 1939 (Bliss, 1939). Bliss assumed that toxic effects are stochastic events and constructed a model for combined effects by calculating a joint probability. The combined effect (P_{mix}) of toxicants A and B is predicted by

$$P_{mix} = 1 - (1 - P_A)(1 - P_B) = P_A + P_B - P_A P_B \quad (1)$$

where P_A and P_B are the rates of toxic effect by toxicant A and B, respectively. Bliss's model is now known as independent action (IA) or sometimes as response addition (RA) because the total response is the sum of the response of each toxicant, especially when both P_A and P_B are very low. In contrast, Loewe and Muischnek (1926) assumed that combined effects were determined by the sum of the drug concentrations scaled by each efficacy. This model is often called concentration addition (CA). When the concentrations of toxicant A and B leading to x% effect (such as death) are denoted by EC_x^A and EC_x^B, respectively, the combined effect of chemicals A (C_A) and B (C_B) is assumed to stay at x% for given concentration of toxicants [C_A] and [C_B] when

$$1 = \frac{[C_A]}{EC_x^A} + \frac{[C_B]}{EC_x^B} \quad (2)$$

holds. The toxic potency, often termed the toxic equivalency factor (TEF), is defined as EC_x^A/EC_x^B (or EC_x^B/EC_x^A). The most successful example of risk assessment using CA is that for dioxin-like

* Corresponding author.
E-mail address: masashi-kamo@aist.go.jp (M. Kamo).

http://dx.doi.org/10.1016/j.tox.2015.06.008
0300-483X/

compounds (Safe, 1998; den Berg et al., 1998; Silva et al., 2002; Kortenkamp, 2007).

It is often considered that IA is a model for toxicants with different modes of action and that CA is a model for toxicants with the same or similar mode of action (Plackett and Hewlett, 1952). From a regulatory point of view at the screening level, the use of CA rather than IA is recommended (Kortenkamp et al., 2009). This is because CA generally has a higher predictability than IA (Kortenkamp et al., 2009; European Commission, 2012). In particular, in mixtures at low concentrations, combined effects roughly obey the prediction by CA (Kodell et al., 1991; Warne and Hawker, 1995). The mechanistic reason why CA gives better prediction at low doses is an interesting topic to pursue but has not yet been well examined.

The "interaction" among chemicals is a key concept in nonadditive toxicities. A commonly accepted concept is that if the observed effects of a chemical mixture deviate from CA, then there are interactions among the chemicals; or in reverse, if there are interactions, then the combined effect of the mixture should deviate from CA. These two concepts form a tautology. Very few efforts have been made to understand what the interactions actually are, and the definition of interaction is still ambiguous (Konemann and Pieters, 1996). In some cases, the ambiguous definition of an interaction causes confusion for interpretations of observed data from toxicity tests of chemical mixtures. A biotic ligand model (BLM) (Meyer et al., 1999; Paquin et al., 2002; Niyogi and Wood, 2004) is now widely used to predict toxicities of metals, and the model has been extended to predict toxicities of metal mixtures (Farley et al., 2015). The BLM assumes a site (or sites) of action of the metal on a gill of aquatic species, termed the biotic ligand (BL), and the BL is considered to be a major metal uptake pathway. When multiple metal species are considered, competition among metals for the BL occurs since metal uptake occurs through the BL. Some researchers consider this competition to be a type of interaction because it is intuitively expected that the uptake of a metal is inhibited by other metals through the competition for the same site of action. These researchers manage metals in a more tolerant way because weaker toxicity of the metal mixture is expected. However, some researchers may not consider the competition to be an interaction. Such researchers thus try to manage metals in a more rigorous way. It is important to understand what types of interaction are required for nonlinear toxic effects to avoid such a conflict.

A definition of the deviation from the model prediction is also important, and some models testing the significance of the deviation from additive models have been developed (Jonker et al., 2005; Kim et al., 2014; Iwasaki et al., 2015). When significant deviation is detected, models are fixed so that they can predict nonadditive toxicities. For the CA, the easiest way is to include nonlinear terms such that

$$1 = \left(\frac{[C_A]}{EC_x^A}\right)^{\lambda} + \left(\frac{[C_B]}{EC_x^B}\right)^{\lambda} \quad (3)$$

where λ is a parameter for the nonlinearity. For $\lambda > 1$, combined effects are weaker than additive and are antagonistic (Berenbaum, 1989; Greco et al., 1995). For $\lambda < 1$, combined effects are stronger than additive and the effects are synergistic. The fixed model has better predictability for observed data; however, this is an ad hoc fix for interpolating the data gap and does not suggest why λ deviates from 1. Another approach is needed to understand why combined effects are not additive.

One such approach is to construct models of mechanism-based chemical interactions and seek conditions for which CA holds by analyzing the models mathematically. Webster (2013) modeled simple pharmacodynamics for the synthesis of agonist, and the main result of that study was that depending on the functional form of synthesis inhibition, combined effects become additive or nonadditive. Webster (2013) also considered two toxicants with distinctly different modes of action. A similar approach is taken in the present study, but toxicants with more similar modes of action are considered. It is commonly accepted that CA sufficiently predicts combined effects of chemicals with the same and similar modes of action (Plackett and Hewlett, 1952). The aims of this study were to examine whether this commonly accepted concept is always true, and to examine what kind of interactions are required when combined effects deviate from the prediction by CA.

2. Models

Simple enzyme–substrate reaction dynamics of the form

$$E + S \underset{k^-}{\overset{k^+}{\rightleftarrows}} ES \xrightarrow{k^o} E + P \quad (4)$$

are considered in this paper. Enzyme (E) responds to a substrate (S) and creates an enzyme–substrate complex (ES); the final product (P) is produced from ES. Lower case k parameters represent the speed of the reactions. Here, P is assumed to be an essential substance for biota, and the death rate rises as P decreases. The value of P at equilibrium is described by the Michaelis–Menten formula.

Binary mixtures of C_A and C_B are considered. These chemicals interfere with the enzyme reaction and reduce the value of P at equilibrium. If there is no adequate information about the interactions among the chemicals and enzyme, as is depicted in Fig. 1, then it is natural to consider that the modes of action of C_A and C_B are the same or similar because both have the same role, i.e., to reduce P.

2.1. Model I

Model I (Fig. 2) is the simplest case. Both C_A and C_B react with the enzyme and produce complexes EC_A and EC_B. These complexes reduce the amount of enzyme that the substrate can use and eventually reduce the amount of product. In this model, there is no direct interaction between C_A and C_B; however, they mutually compete for the enzyme, and therefore there is an indirect interaction.

2.2. Model II

In model II, two enzyme–substrate reactions are connected in sequence (Fig. 3). The product of the first reaction (S_2) acts as the substrate in the second reaction. The role of C_A is the same as that in Model I, but in this case, C_B reacts only with the second enzyme (E_2) and not the first enzyme (E_1). Since C_A and C_B have different sites of action, there is no direct interaction between C_A and C_B; however, C_A and C_B are not entirely independent because E_2, to

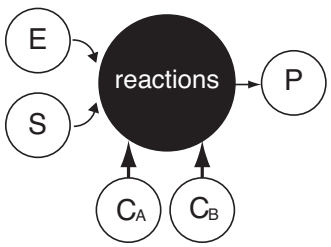

Fig. 1. The role of chemicals C_A and C_B is to reduce the amount of product (P). When the specific reactions of C_A and C_B are unknown (as in a black box), one should consider C_A and C_B to have the same or similar modes of action.