Chemosphere 150 (2016) 159–167

Contents lists available at ScienceDirect

Chemosphere

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

Prediction of mixture toxicity from the hormesis of a single chemical: A case study of combinations of antibiotics and quorum-sensing inhibitors with gram-negative bacteria

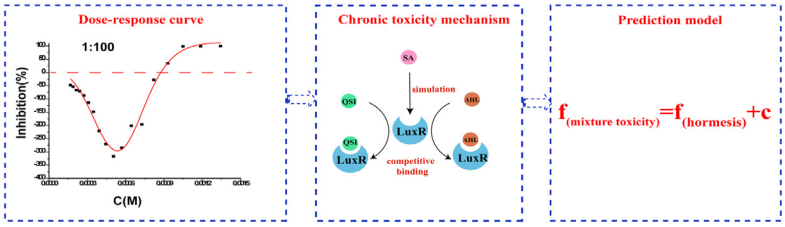
Ting Wang [a], Dali Wang [a], Zhifen Lin [a, b, c, *], Qingqing An [d], Chunsheng Yin [d], Qinghui Huang [b]

[a] State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[b] Shanghai Key Lab of Chemical Assessment and Sustainability, Shanghai, China
[c] Collaborative Innovation Center for Regional Environmental Quality, Beijing, China
[d] College of Marine Science, Shanghai Ocean University, Shanghai 201306, China

HIGHLIGHTS

- Single and mixture toxicity of SAs and QSIs were investigated.
- Toxicity mechanism for mixture toxicity was raised on gram negative bacteria.
- Toxicity mechanism for hormetic effects of SAs has been raised.
- Relationship between mixture toxicity and hormetic effect was formulated.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 October 2015
Received in revised form
4 February 2016
Accepted 4 February 2016
Available online xxx

Handling Editor: Shane Snyder

Keywords:
Mixture toxicity
Hormesis
Toxicity mechanism
Antibiotics
Quorum sensing inhibitors

ABSTRACT

The 50% effect level of a single chemical in the real environment is almost impossible to determine at the low exposure concentration, and the prediction of the concentration of a mixture at the 50% effect level from the concentration of a single chemical at the low effect level is even more difficult. The current literature does not address this problem. Thus, to determine solutions for this question, single/mixture chronic toxicities of sulfonamides (SAs) and quorum-sensing inhibitors (QSIs) were determined using Gram-negative bacteria (*Vibrio fischeri* and *E. coli.*) and Gram-positive bacteria (*B. subtilis*) as the target organisms. The results showed that the joint effects of SAs and QSIs were primarily antagonistic responses. In addition, the toxicity mechanisms of mixtures of SAs and QSIs were investigated further, and the results revealed that the chronic joint effects were primarily an antagonistic response due to the QSI competing against acyl-homoserine lactones (AHL) for luxR in *V. fischeri* and SdiA in *E. coli* generated by the SAs, leading to negative effects exerted by the QSI-luxR or QSI-SdiA complexes on luxI in *V. fischeri* or FtsZ in *E. coli*. This phenomenon eventually weakened the stimulatory effect caused by the SAs. Based on the mixture toxicity mechanism, the relationship between the mixture toxicity and the simulation effect was formulated.

1. Introduction

Complex joint effects, such as synergism and antagonism caused

* Corresponding author. State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China.
E-mail address: lzhifen@tongji.edu.cn (Z. Lin).

http://dx.doi.org/10.1016/j.chemosphere.2016.02.018
0045-6535/

by mixtures in the environment, pose a potential threat to ecological security and ecological risk assessment (Li et al., 2015; Uribe and Fuentes-Garcia, 2015) because the current environmental quality standards and ecological risk assessment are based on the toxic effects of single contaminants (Eliopoulos, 1989; Chou, 2006). Therefore, the study of the mixture toxicity of environment contaminants that always exist in the form of mixtures is urgently needed to perform objective and comprehensive ecological risk evaluation and effectively protect human health as well as ecological safety (Altenburger et al., 2003; Tian et al., 2012b, 2013; Zou et al., 2013).

In the field of mixture toxicity, many models, such as the quantitative structure-activity relationship (QSAR) (Yu et al., 2001) (Chen et al., 1996; Altenburger et al., 2003) and the concentration addition (CA) model (Hadrup et al., 2013; Kalia, 2013), have attempted to address the prediction of mixture toxicity. The calculation method of the QSAR model is shown as Figure S1(A). For model fitting in single toxicity, 50% effective concentration (EC_{50}) was the dependent variable and molecular descriptors were considered as model predictors. Then according to the contribution of single chemical to chemical mixtures in concentration, a QSAR model for chemical mixtures can be formulated with the 50% effective concentration of a single chemical and a mixture (Wang et al., 2011; Zou et al., 2012). From the above analysis, we can conclude that the same effect level that a single chemical and mixture is the restrictive condition of the QSAR model. If these restrictive conditions are not satisfied, the existing calculation methods do not work. For example, in Figure S1(B), the effect concentration is 20% for a single chemical at C1, C2 and 50% for a mixture at C3. Under these conditions, the QSAR model cannot be used to predict the mixture toxicity.

It is impossible for us to determine the EC_{50} of some chemicals because of their low solubility. If a mixture containing these chemicals, the available method cannot be used to predict the mixture toxicity. Hence, to predict the concentration of a mixture when the biological effect level is 50% from the hormetic effect of a single chemical is a beneficial complement to the classic method and has a profound significance in the risk assessment of pollutants. In addition, Tian et al. (Tian et al., 2012a, b) selected 50% as the effect level, investigated the joint effects under non-equitoxic and equitoxic conditions and formulated the relationship between the joint effects of an equitoxic mixture ($TU_{50}^{equitoxic}$) and a non-equitoxic mixture ($TU_{50}^{nonequitoxic}$). They noted that if this relationship could be formulated at a lower effect level, such as 10% or 20%, it would be meaningful for the prediction of mixture toxicity in the real environment, which no study has addressed. Hence, to predict the concentration of a mixture when the effect level is 50% from the low concentration of a single chemical would also be beneficial to extrapolate from the high concentration to the low concentration in Tian's method.

Hormesis is an adaptive response characterized by biphasic dose responses of generally similar quantitative features with respect to amplitude and range of the stimulatory response that are either directly induced or the result of compensatory biological processes following an initial disruption in homeostasis(Calabrese and Baldwin, 2002). Usually, the maximum stimulation effect (Y_{max}) is used to symbolize the amplitude of the stimulatory response and the maximal stimulatory effect (M) to symbolize the range of the stimulatory. M (in Figure S1(C)) in hormesis can be considered a specific point of low concentration. Belz et al. (Belz et al., 2008) noted that M and EC_{50} followed the same deviation patterns, which implies that a relationship may exist between M and EC_{50}. If this relationship between M and EC_{50} can be formulated, the relationship may supply a direction for prediction of the concentration of a mixture from the low concentration of a single chemical when the biological response is 50%. Hence, the formulation of a relationship between the stimulatory effect of a single chemical and mixture toxicity is the main concern of this study.

Recently, mechanism-based QSARs to the level of the receptors on the basis of a molecular modeling approach and docking analysis has been developed, for its unique advantage of higher quality than QSARs without regard to toxicity mechanism. Zou et al.(Zou et al., 2012) formulated a QSAR model to predict the acute mixture toxicity by using the CDOCKER interaction energy ($E_{binding}$) between single chemicals and their target protein. Yao et al. (Yao et al., 2013) selected different mixtures that individual chemicals bind to different binding sites on different(or same) target proteins and formulated a general QSAR model to predict the toxicity of chemical mixtures with different binding sites by using $E_{binding}$. It can be concluded from the above analysis that mechanism-based QSARs to the level of the receptors can be used to predict toxicity of mixtures. Hence, if the mechanism of simulation effect and mixture toxicity can be raised and $E_{binding}$ between single chemicals and the target proteins can be calculated, an effective method can be supplied to solve the above question.

The studies conducted by our group have found that the dose–response of SAs (sulfonamides) manifests a hormetic effect, and we speculated the nature of its mechanism: SAs are able to facilitate the expression of luxR, a quorum-sensing protein at a low dose, promoting the luminescence of *Vibrio fischeri* but inhibiting the activity of dihydrofolate synthetase(Dhps) at a high dose, and thus have a negative effect on the metabolism of folate (Deng et al., 2012). In addition, quorum-sensing inhibitors (QSIs) also affect the expression of genes by regulating natural quorum sensing, a mechanism of feedback regulation through which bacteria send messages reciprocally to each other and are sensitive to the population density (Defoirdt et al., 2013). Hence, if the mixture toxicity mechanism of SAs and QSIs, both of which exert toxicity by inferring with quorum sensing, can be raised based on the clear mechanism of the hormetic effect for SAs, the mechanism-based QSARs model may be raised between mixture toxicity and the hormetic effect.

Therefore, this article intends to choose SAs and QSIs (furanone, povidone and pyrrole) as objects. SAs are typical antibiotics widely used for livestock feeding and commonly existing in the environment. Meanwhile, SAs are demonstrated to interrupt the quorum sensing by facilitating the expression of luxR. Furanones are similar to AHLs and inhibit quorum sensing mediated activities in bacteria by competing with cognate AHL signals for their receptor site. Povidone and pyrrole have the similar structure to furanones, the relative study has demonstrated that povidone is the potent antibacterial agents. Hence, furanone, povidone and pyrrole can potentially be adapted as QSIs in the future. Consequently, SAs, furanones, povidone and pyrrole are selected as objects in this study.

In addition, in order to formulate a common quantitation prediction model between mixture toxicity and the hormetic effect, typical gram-negative bacteria (*V. fischeri*, *E. coli*) and the typical gram-positive bacteria - Bacillus subtilis (*B.subtilis*) – were selected as model organisms. *V.fischeri* is the original organism which quorum sensing was found and is the typical organism in antibiotic research. *E. coli* shares the same quorum sensing system as *V. fischeri* and also a typical gram-negative bacteria. *B.subtilis* is the typical gram-positive bacteria and its quorum sensing system has been studied systematically since 1990 s. Thus, toxicity bioassays were performed with QSIs and SAs for the following purposes: (1) to determine the chronic single/mixture toxicity of QSIs and sulfonamides on *V. fischeri*, *E. coli* and *B.subtilis*; (2) to reveal the mixture toxicity mechanism of QSIs and SAs; and (3) to develop the mechanism-based QSARs to the level of the receptors on the basis