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Toxicological interaction of multi-component mixtures to *Vibrio qinghaiensis* sp.-Q67 induced by at least three components

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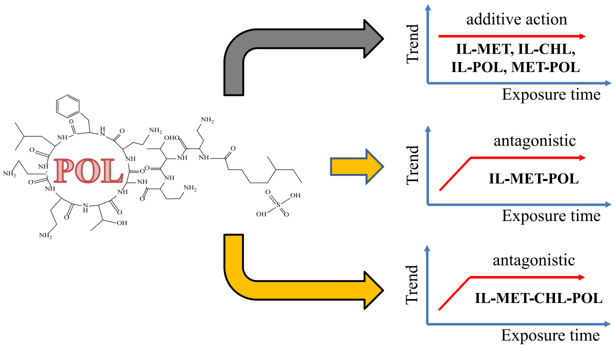
HIGHLIGHTS

- Pesticide, ionic liquids, and antibiotics have different time-dependent toxicity profiles.
- Combined toxicities of binary mixtures at different times are additive.
- Mixtures including no polymyxin B sulfate do not have toxicological interaction.
- Ternary and quaternary interactions change from weak synergism to antagonism.
- Time-dependent antagonism comes from the interaction of at least three components.

GRAPHICAL ABSTRACT



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ABSTRACT

It has been stated by researchers that the antibiotic polymyxin B sulfate (POL) is a key component inducing time-dependent antagonism in freshwater luminescent bacteria, *Vibrio qinghaiensis* sp.-Q67, exposed in the ternary mixture system of the ionic liquids, pesticide and antibiotics. However, the previous statement is limited to ternary and quaternary mixtures without considering situations such as the binary system. In order to prove the direct inducing of antagonism by POL in a more complete and systematic way, two categories of experiments (adding POL in non-antagonistic ternary system and decomposing antagonistic ternary system with POL into the binary system) have been conducted in this paper. The results showed that quaternary mixture systems (adding POL to non-antagonism ternary mixture, up verification) exhibit antagonistic action in a majority of rays, at some point in the experiment. However, by decomposing the antagonistic ternary mixtures with POL into binary mixtures (down verification), the combined toxicities of binary mixtures at all time points in the experiment are additive. Obviously, the POL has a significant contribution to antagonism only in the ternary and quaternary mixtures, but not in the binary mixtures. We can draw a new conclusion that the antagonism of the multi-component mixtures is induced by at least three components (including POL), with complex chemical interactions. Therefore, considering POL's influence of antagonism as an example, future environmental protection practitioners and academic researchers should construct more scenarios of mixtures when assessing the influences and reactions of certain chemicals causing pollutions.

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1. Introduction

Humans and other organisms living in the environment are exposed to various types of complex natural and synthetic chemicals (Zhang et al., 2016; Di Nica et al., 2017; Guo et al., 2017). Different types of chemicals usually have varying time-dependent toxicity characteristics (Dawson et al., 2016; He et al., 2017; Kim et al., 2017). Various matrices can contain hundreds, or even thousands of chemicals, the risk of which must be assessed.

Several facts show that pesticides constitute a serious environmental problem due to potential toxicity and bioaccumulation in water (Li et al., 2008; Abdo et al., 2015; Reddy et al., 2016). Ionic liquids (ILs), commonly known as green solvents due to their certain special properties (e.g. negligible vapor pressure, thermal stability, non-flammability, high ionic conductivity, and a wide electrochemical stability window) (Lei et al., 2014; Mota-Martinez et al., 2014), have a large variety of applications in all areas of the chemical industries as they require significantly less energy for regeneration (Keskin et al., 2007; Pernak et al., 2007; Shamsipur et al., 2010; Qian et al., 2017). Antibiotics are often used to treat human and animal diseases. However, resistance has eventually developed to nearly all antibiotics, shortly after they were introduced to clinical practice (Rossolini et al., 2014). On the other hand, the abuse of antibiotics and the illegal discharge of drug plant wastewater have caused serious issues in recent decades, especially in developing countries (Kummerer, 2009; Cwala et al., 2011; Fatta-Kassinos et al., 2011; Tong et al., 2014).

Chemicals, usually occurring at low concentrations and in mixtures, affect subtle physiological traits in organisms and may directly or indirectly cause long-term adverse ecological effects (Scott and Sloman, 2004; Angel et al., 2017; Church et al., 2017; Jean and Plotzke, 2017; Perez and Hoang, 2017). However, pollution prevention and control policy, based on the acute toxicity behaviour of individual contaminants, does not realistically reflect the effects of their mixture on the environment and ecosystem. The effect of a pesticide on a non-target organism depends not only on its concentration, but also on the exposure time (Jurado et al., 2012; Maazouzi et al., 2016). It was found that chronic exposure to low levels of pesticides may have a more significant effect on rainbow trout populations than acute poisoning (Ceyhun et al., 2012). Neale et al. (2017) determined the acute (0.5 h) and chronic (16 h) toxicities of five antibiotics to *Photobacterium leiognathi*, determining that the EC_{50} values of the antibiotics decreased by up to three orders of magnitude after 16 h, which means that the acute toxicities of antibiotics are less harmful than their chronic toxicities. Hormesis was clearly observed in the long-term (12 h) toxicity of 1-ethyl-3-methylimidazolium tetrafluoroborate on *Vibrio qinghaiensis sp.-Q67* (Q67), while the concentration-response curve (CRC) of its short-term (15 min) toxicity was only sigmoid (Wang et al., 2011). It was showed that, since organisms in the environment are commonly subjected to long-term exposure of pollutants, time-dependent toxicity can better reflect the environmental risks of pollutants than acute toxicity. Therefore, the identification of different classes of contaminant mixtures responsible for each toxicological interaction (additive action, synergism, or antagonism) is important for cumulative risk assessment of ecosystems (Gonzalez-Pleiter et al., 2013). It was found that the ternary mixture of insecticides, herbicides, and heavy metals, as well as all the binary mixtures of pesticides, exhibited synergism on earthworms, especially at low-concentration levels (Wang et al., 2015). Tang et al. (2016) also found that four ternary mixture systems (metal, pesticide, and ILs) display obvious synergistic interactions. These findings indicated that the toxicological interaction may not only be related to different types of chemicals, but also to the species tested.

Within the last few years, an increasing number of studies have been carried out on the toxicological interactions of mixtures in the environment (Amariei et al., 2017; Hernandez et al., 2017; Liu et al., 2017). It was observed that the quaternary mixtures exhibiting antagonism or synergism are well correlated with the mixture ratio of a key IL (Zhang et al., 2011). However, the ILs can interact in various ways, depending on the compound itself and its chemical group, the dose, and the targeted organs, eventually leading to various effects. In other words, the toxicological interaction in multi-component mixtures may be caused by two or more components, instead of a single component. It was shown that polymyxin B sulfate (POL) is a key component that induces antagonism in ternary mixtures by designing two new quaternary systems (Fan et al., 2017). However, this method alone cannot comprehensively explain how POL is a key component causing antagonism of the ternary mixture. Hence, our goal with this work was to build a complete method to analyze and identify the “key component” inducing the antagonism in the mixtures.

The five chemicals used in the experiment also have environmental significance. Metalaxyl (MET) was detected in fruit juice, vegetable samples (Farajzadeh et al., 2018), and surface water (Struger et al., 2016). Chloramphenicol (CHL) was detected in chicken meat (Rezaee and Khalilian, 2018) and lake water (Ahmed et al., 2017). Polymyxin B sulfate (POL) was detected in pharmaceutical wastewaters (Fan et al., 2017). Although there is limited study on the concentration of ILs in the environment, they can cause severe water pollution due to their high water miscibility, resulting in potential toxicity to the aquatic organism and inaccessible biodegradability (Swatloski et al., 2003; Cho et al., 2008).

In general, the main purpose of this study was to identify the number of components in which toxicological interactions could occur, using the up and down verifications, while trying to illustrate the meaning of “key component” by demonstrating that POL is a “key component” inducing antagonism in ternary mixture systems, as an example. Thus, the individual toxicity of one pesticide, two ionic liquids (ILs), two antibiotics, and mixture toxicities of five binary, four ternary, and two quaternary mixtures to Q67 was assessed using the time-dependent microplate toxicity analysis (t-MTA), while the toxicological interactions in mixtures were evaluated by the concentration addition (CA) model. Many studies have agreed on the importance of considering bioluminescent bacteria as a relevant ecotoxicological subject (Backhaus and Grimme, 1999; Bouki et al., 2013; Yu et al., 2014; Zhang et al., 2014b; Yu et al., 2015; Zhang and Liu, 2015; Qu et al., 2016), thus, we chose Q67 for it is a species of fresh water photobacterium and plays a pivotal role in the freshwater ecosystems.

2. Materials and methods

2.1. Chemicals

The pesticide, metalaxyl (CAS 57837-19-1, MET), and the antibiotic, chloramphenicol (CAS 56-75-7, CHL), were purchased from Dr. Ehrenstorfer (Germany). Polymyxin B sulfate (CAS 1405-20-5, POL) was purchased from TRC (Canada). Two ILs, 1-hexyl-3-methylimidazolium bromide (CAS 85100-78-3, [hmim]Br) and 1-hexyl-3-methyl-imidazolium chloride (CAS 171058-17-6, [hmim]Cl), were purchased from TCI (Japan). All solutions were prepared with Milli-Q water and stored in the dark. Some physical properties and stock concentrations are listed in Table S1 of ESI†. The molecular structures of the five chemicals are shown in Fig. S1 of the ESI†.

2.2. Time-dependent toxicity test

Q67 was selected as the test organism in this study. The freeze-dried Q67 was purchased from Beijing Hamamatsu Corp., Ltd. (Beijing, China). The medium formula for Q67, the culture condition, and relative light unit determination were the same as those in the literature (Yu et al., 2014). The t-MTA method (Fan et al., 2017; Li et al., 2017b) was used to determine the luminescence inhibition toxicity of various concentrations of single components and their mixture rays at 0.25, 3, 6, 9, and 12 h on Q67. Three parallels of