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Joint toxicity on hepatic detoxication enzymes in goldfish (*Carassius auratus*) exposed to binary mixtures of lead and paraquat

Xiaoming Xu[a,b], Zhaojie Cui[a,*], Shanshan Wang[b]

[a] School of Environmental Science and Engineering, Shandong University, Jinan, 250100, China
[b] School of Municipal and Environmental Engineering, Shandong Jianzhu University, Jinan, 250101, China

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ABSTRACT

Compared to single exposure, chemical mixtures might induce joint toxicity including additive, synergistic and antagonistic effects on both organisms and environment. Owing to the specific toxicity of oxidative stress and binding to proteins, lead (Pb) is generally recognized a non-essential and threatening heavy metal to animals and human. Paraquat (PQ) is a widely used herbicide in agriculture and can trigger oxidative stress as well as Pb. Little information was available about joint effects of the two chemicals on toxicological responses in organisms, especially in fish. In our present study, goldfish (*Carassius auratus*) were randomly exposed to single and combined experiments with different concentrations of Pb and PQ for 28 days. Activities of four enzyme biomarkers in liver, ethoxyresorufin-*O*-deethylase (EROD), 7-benzyloxy-4-trifluoromethyl-coumarin-*O*-debenzyloxylase (BFCOD), glutathione-*S*-transferase (GST) and UDP-glucuronosyltransferase (UGT) were evaluated in each experimental group on day 14 and 28. The results showed four enzyme levels were markedly reduced with the increase of concentrations in mixtures and prolonged exposure. The inhibitory EROD and BFCOD activities were not significantly changed in goldfish following PQ-treated groups with or without 0.5 mg/L Pb, which indicated PQ has more inhibitory toxicity on CYP450 enzymes than Pb in co-exposure groups. However, the reduced values of GST were observed only in the combinations containing high doses of Pb or PQ during experimental periods. Although the responses of UGT activity were similar to GST on 14th day, all combinations of Pb and PQ generated stronger inhibitions on UGT activities compared to individual Pb and PQ-treated group. These results suggested that combined exposure of Pb and PQ have more inhibitory toxicity on phase I enzymes than phase II enzymes.

1. Introduction

On account of diversified production and living needs, anthropogenic pollutants coming from different sources do not exist alone in environment in most cases. They can concurrently enter into or sequentially accumulate in aquatic ecosystem through direct discharge, surface runoff, infiltration and atmospheric sedimentation. These chemicals in the complex system may be changed in physical and chemical property because of the interaction with each other. As a result, mixed pollutants may lead to combined toxic effects on organisms, such as addition, synergism and antagonism, which are more complicated than those of single pollution (Uwizeyimana et al., 2017). Many environmental evaluations and toxicological effects may be overstated or underestimated only relying on the toxic mechanism of individual pollution (Kim et al., 2018). In this context, co-pollution is increasingly taken into account and the studies of joint effects also become a more reliable guidance for understanding the potential threats of environmental pollutants.

Compared to the combined pollution of inorganic or organic contaminants, the co-exposure of heavy metals and organic toxicants (chelating agents, pesticides and aromatic hydrocarbons) has been a focused issue in the field of environmental toxicology. At present, the toxic effects of heavy metals were mainly studied together with polycyclic aromatic hydrocarbons (PAHs), organochlorine and organophosphorus pesticides (Kim et al., 2014; Chen et al., 2015; Muthusamy et al., 2016; Xu et al., 2017). Many researchers found that the contents and forms of heavy metals may be altered through coordinating, precipitating and ion exchanging with organics (Liu et al., 2017). As a result, some heavy metals can decrease the toxicity because of their lower concentrations. On the other hand, increased solubility may also enhance diffusivity and transfer heavy metals which results to a possible toxic effect on organisms. Equally, organic pollutants may be changed in degradation and transformation with the presence of heavy metals.

* Corresponding author.
E-mail address: xuxiaoming@sdjzu.edu.cn (Z. Cui).

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As the representative element of heavy metals, lead (Pb) is a priority pollutant in environmental monitoring and contamination control owing to its high toxicity for human and widespread pollution in atmosphere, soil and water (Chen et al., 2016). Meanwhile, paraquat (PQ) is a cheap and effective bipyridyl herbicide still applied extensively to protect crops in more than 100 countries (Augustyniak et al., 2015). For high solubility in water and difficult degradation and decomposition (Ma et al., 2014), PQ is recognized as a high-risk toxicant which is linked to neurodegenerative diseases for human and animals (Soares et al., 2017). Both of them can lead to oxidative stress in different mechanisms of toxicity, which result in overproduction of reactive oxygen species (ROS), lipid peroxidation and DNA damage (Park et al., 2012; Soleimani et al., 2016). Numerous biomarkers such as antioxidant enzymes and malondialdehyde (MDA) have been used as the established tools to evaluate the toxic effects of Pb or PQ-induced oxidative stress (Mohanty and Samanta, 2016; Wang et al., 2016).

The responses of biomarkers can indicate the toxic effects and mechanisms of pollutants on the organisms. More sensitive and reliable biomarkers are continually developed for providing the early warning and monitoring of pollution levels. Cytochrome P450 enzymes (CYP) are pivotal heme-thiolate catalysts in phase I metabolism, which catalyze the endogenous biosynthesis and the biotransformation of various drugs and environmental pollutants (Hlavica, 2017). Ethoxyresorufin-*O*-deethylase (EROD) activity which is quantified for measuring the level of CYP1 A, has been served as one of the most sensitive biomarkers in environmental monitoring, especially the presence of PAHs in fish (Oziolor et al., 2014; Boehler et al., 2018). Another subfamily of P450, CYP3 A has also received greater concerns because of the broad catabolism on multiple xenobiotics and is evidenced by the activity of 7-benzyloxy-4-trifluoromethyl-coumarin-*O*-debenzyloxylase (BFCOD) (Koenig et al., 2012). Apart from P450 enzymes, glutathione-*S*-transferase (GST) and UDP-glucuronosyltransferase (UGT) are the important detoxifying enzymes in Phase II biotransformation. They can catalyze the conjugation reactions of exogenous substrates or metabolites to specific polar ligands (den Braver-Sewradj et al., 2016). Although GST and UGT enzymes do not directly catalyze the reactions of ROS removing, they are unanimously assigned to “indirect” antioxidants against oxidative damage. Quite a few literatures have found single Pb exposure or the mixtures of heavy metals containing Pb can significantly change the activities of these metabolic enzymes in different organisms (Rajeshkumar et al., 2013; Sayed et al., 2017; Ibor et al., 2017). As for PQ, more sights were focused on its single stress on antioxidant enzymes in animals and plants (Amirshahrokhi and Khalili, 2016; Díaz et al., 2016). Hence, it is needed to understand the joint toxic effects of Pb and PQ on cytochrome P450 enzymes and conjugated enzymes of phase II in fish.

For purpose of evaluating the joint effects of Pb and PQ exposures on activities of four enzymes biomarkers (EROD, BFCOD, GST and UGT) in liver, healthy goldfish were respectively exposed to single and combined treatments of the two chemicals with varying concentrations for 28 days. Dose and time-dependent responses of four hepatic enzymes in fish in each group were analyzed after exposure to 14 and 28 days. The changed activities of enzymes in fish following exposure to the mixtures were compared with those of individual treatments of Pb and PQ in order to acquire the joint toxicity of the two chemicals on metabolic enzymes in fish.

2. Materials and methods

2.1. Chemicals and test fish

Substrates and reactants used for analyzing the activities of enzymes were purchased from Sigma-Aldrich Shanghai Trading Co., Ltd (Shanghai, China). Protective agents and inhibitors of enzymes were purchased from Amresco (USA). The ELISA of GSH-ST was obtained from Nanjing Jiancheng Bioengineering Inst. (China). Coomassie brilliant blue G-250 (CBG) for total protein determination was purchased from BBI (Toronto, Canada). All other reagents of analytical grade in this work were purchased from Sinpharm Chemical Reagent Co., Ltd (Shanghai, China).

Goldfish (*Carassius auratus*) served in experiments were obtained together from a local commercial fishery (Jinan, China). The selected fish for experiments were of unified size with mean initial weight (5.9 ± 0.3 g) and length (6.5 ± 0.5 cm). Before the exposure experiments were conducted, all healthy fish were maintained in dechlorinated and continuous aerated water for 15 days to accommodate to experimental surroundings (temperature 20 ± 1°C, pH 7.1 ± 0.2, and DO 6.5 ± 0.3 mg/L) and fed once every two days in the stage of acclimatization. To avoid the possible influence, food was forbidden to provide on 13th day of acclimatization. Feces and food residues were taken out daily and a proper amount of water were supplemented to meet the experimental requirements.

2.2. Combined exposure assays

Before the experiments of combined exposure, we have conducted the single exposure tests of Pb and PQ with a range of 0.05–10 mg/L in fish under the same experimental conditions (Xu et al., 2018). According to the analysis results of single Pb and PQ exposure in our previous experiments, two representative concentrations of each chemical, 0.5 and 10.0 mg/L for Pb, 1.0 and 10.0 mg/L for PQ, were respectively picked out to serve for the binary exposure. Because the responses to low concentrations of the two chemicals on GST activity were different from other three enzymes (EROD, BFCOD and UGT) in fish, 0.1 mg/L Pb and 0.05 mg/L PQ were selected to respectively replace 0.5 mg/L Pb and 1.0 mg/L PQ in mixtures for the measurement of GST activity.

After 2 weeks’ acclimatization, some goldfish was equally distributed in glass tanks (40 × 23 × 25 cm^3, 10 fish per tank) where Pb and PQ were combined in pairs. The experimental concentrations of single group and the mixture were shown in Table 1. Duplicate experiments were performed for each exposure including the control group. All experimental groups were conducted at the semi-static conditions for 28 days and the aqueous solution of each treatment were replaced every other day to maintain the constant concentrations. No food was provided during the whole exposure periods and the experimental conditions (temperature, DO and pH) were in accordance with acclimatization periods described above.

2.3. Sampling and preparation of enzyme extracts

A total of 10 fish (5 fish per tank) were severally taken out from the duplicate tanks of each treatment after 14 (7 days for GST) and 28 days of exposure. The collected fish were injected with 1 mL MS-222 buffer (100 mg/L) and cleaned with deionized water. The liver was carefully separated from the dissected fish and washed by 0.15 M potassium chloride (KCl) solution (4°C). The rinsed liver samples were wiped with filter papers and weighed accurately.

Quantitative volumes of 0.1 M ice-cold phosphate buffer (pH 7.4)

Table 1
Concentration design applied to single and combined exposure.

Groups		Exposure concentrations (mg/L)			
Pb		0.5[a]	10.0	–	–
PQ		1.0[b]	10.0	–	–
Mixture	Pb	0.5[a]	0.5[a]	10.0	10.0
	PQ	1.0[b]	10.0	1.0[b]	10.0

[a] 0.5 mg/L was replaced by 0.1 mg/L for Pb in the experiment of GST activity.

[b] 1.0 mg/L was replaced by 0.05 mg/L for PQ in the experiment of GST activity.