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#### ACCEPTED MANUSCRIPT

## Effects of Glyphosate on Immune Responses and Haemocyte DNA

### Damage of Chinese Mitten Crab, Eriocheir sinensis

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**Abstract:** As a broad-spectrum organophosphorus herbicide, glyphosate is widely utilized around the world. The toxic effects of glyphosate on Chinese mitten crab, Eriocheir sinensis, were assessed using immunotoxicity and genotoxicity biomarkers in this study. The results showed that 24 h and 96 h LC<sub>50</sub> values of glyphosate for E. sinensis were estimated as 461.54 and 97.89 mg/L, respectively, and the safe concentration was 4.4 mg/L. According to the results above, glyphosate was applied at concentrations of 0, 4.4, 9.8, 44 and 98 mg/L, for 96 h in the exposure experiment. Total haemocyte count (THC) and percentage of granulocytes decreased significantly following 6 h exposure to each concentration of glyphosate and tended to gradually stabilize after 12 h except in 4.4 mg/L, which rapidly recovered to a normal level in 12 h. Phagocytic activity in all treatments decreased dramatically at 6 h and maintained stability until the 96-h mark. Comet tail has been observed early at 24 h in each treatment, and the comet ratio and percentage of DNA (% DNA) in the tail increased as the exposure experiment progressed. Immune-related enzyme activity varied during the experiment. Acid phosphatase (ACP) and alkaline phosphatase (AKP) activities in 44 and 98 mg/L treatments decreased significantly after 48 h exposure, while AKP activities in all concentrations increased markedly at the beginning of exposure. The superoxide dismutase (SOD) and peroxidase (POD) activities increased significantly after 6 h exposure to 44 and 98 mg/L of glyphosates but decreased at 24 h. In addition, the β-glucuronidase (β-GD) activities in the 9.8, 44 and 98 mg/L groups, increased after 6-h exposure and were significantly higher than those in the control at 96 h. These results indicated that glyphosate has evident toxic effect on E. sinensis by immune inhibition that is possibly due to the haemocyte DNA damage and a sharp decline in haemocyte numbers, which subsequently induced changes in activities of immune-related enzymes and haemocyte phagocytosis.

Key words: Glyphosate; THC; DHC; immune-related enzyme; DNA damage; Eriocheir sinensis

#### 1.Introduction

As a broad-spectrum organophosphorus herbicide, glyphosate-based herbicides, such as the commercial formulations Roundup and Rodeo, are utilized globally and used in both agricultural areas and aquatic waters for weed control[1]. Glyphosate is considered to have low toxicity to

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