Mixture of nitrophenols and transition metal ions: combined toxicity to wildlife and inconvenient truth for coastal wetlands

Sandy Hiotong Kam [a,b]; Guiyi Gong [a,b]; Cinderela Chy Lei [a,b]; Cheryl Hei Ting Kwong [a,b]; Gloria Hiocheng Kam [a]; Simon Ming-yuen Lee [a,b,*]

[a] State Key Laboratory of Quality Research in Chinese Medicine and Institute of Chinese Medical Sciences, University of Macau, Macao 999078, China

[b] Department of Pharmaceutical Sciences, Faculty of Health Sciences, University of Macau, Macao 999078, China

Abstract: The water and sediment pollution of coastal wetlands are mainly caused by terrigenous contaminants, both organic or inorganic, and their mixtures. As important environmental pollutants, nitrophenols and transition metal ions commonly coexist in contaminated coastal wetland, which should pose risks to ecosystem and health. Although their respective toxicity was well documented, to date, their interactive toxicity to wildlife or humans at environmentally relevant concentrations remain unclear. Take 4-nitrophenol and copper for example, our investigation had identified their combined immunotoxicity that causing excessive oxidative stress, aggravated inflammation and structural damage to zebrafish and mouse. Meanwhile, significantly excessive transcription of *nlrp3*, *il-1ß,* and *cox2b* and secretion of IL-1β were found, which suggested ROS/NLRP3/IL-1β signaling might be involved in their combined immunotoxicity. We also characterized combined cardiovascular toxicity and neurotoxicity upon co-exposure of 4-nitrophenol and manganese. Our study should provide a more comprehensive and deeper understanding of the interaction between organic and inorganic pollutants, the health effects caused by their co-exposure, as well as helpful references for safeguard of coastal wetlands against potential hazards that caused by contaminant mixtures.

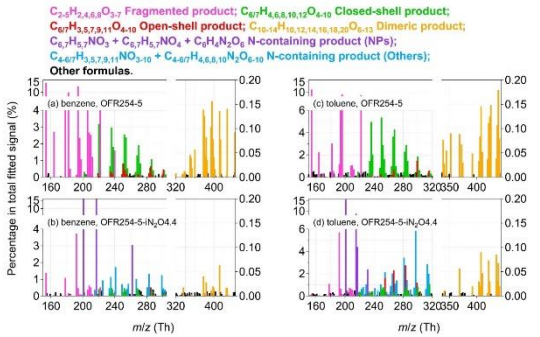

Figure 1. Oxidation products from oxidation of benzene and toluene measured with chemical ionization mass spectrometry.

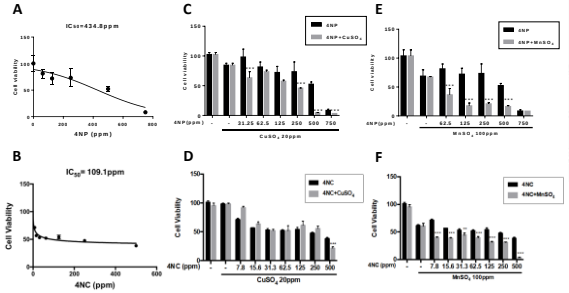

Figure 2. Cytotoxicity of classic SOA (4-nitrophenol (4-NP) or 4-nitrocatechol (4-NC)) and their joint-exposure with transition metal ion in A549 cells.

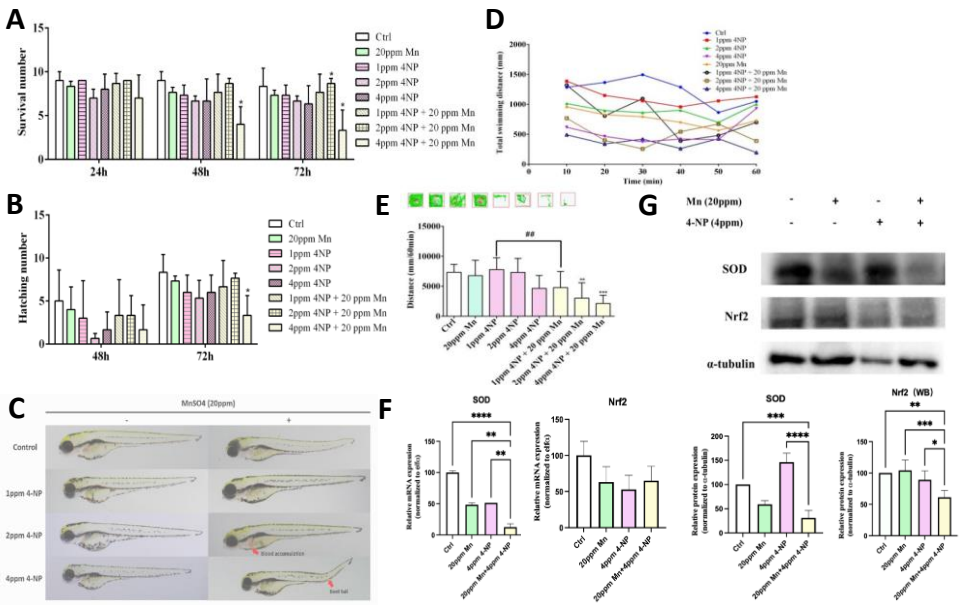

Figure 3. 4-NP and its joint-exposure with manganese ion caused toxicity, malformation, neuro-toxicity and oxidative stress in zebrafish larvae. Through zebrafish embryo toxicity test, co-exposure of 4-NP and manganese ion significantly increased lethality (A) and malformation (C) while decreased hatch-ability (B) and locomotor ability (D-E) in zebrafish. (F-G) Co-exposure of 4-NP and manganese ion significantly impair ability against oxidative stress in zebrafish through reducing expression of SOD or NRF2 related genes and proteins. Data are plotted as means ± SD. *, P < 0.05, **, P < 0.01, ***, P < 0.001 represented significant difference between groups.

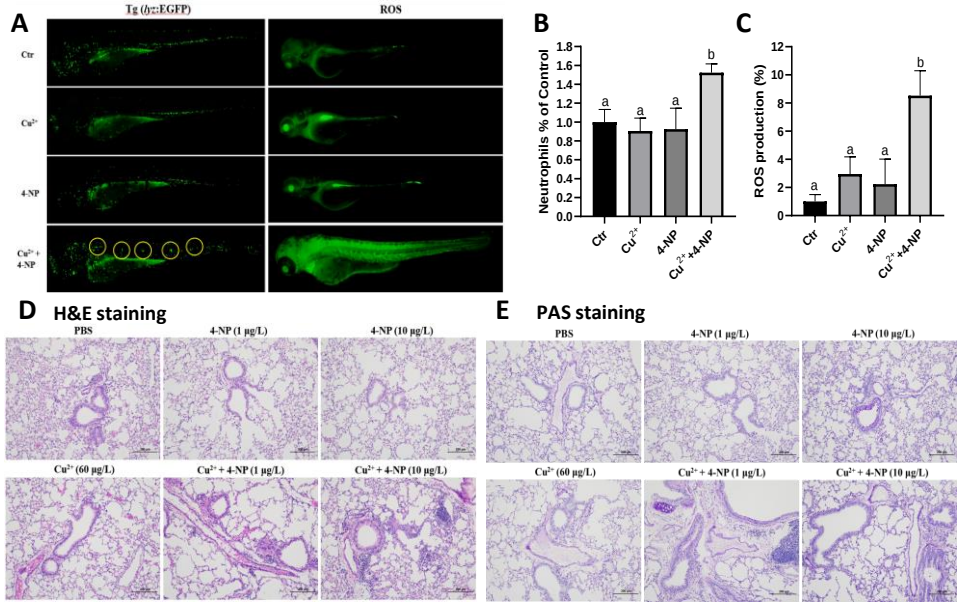

Figure 4. 4-NP and its joint-exposure with copper ion increased inflammation and ROS generation in zebrafish larvae. Co-exposure of 4-NP and copper ion significantly increased inflammation (A and B) and ROS generation (A and C) in zebrafish. Data are plotted as means ± SD. a and b represented significant difference between groups.**4-NP and its joint-exposure with copper ion increased inflammation and lysis of macrophage in lungs of mice**. Co-exposure of 4-NP and copper ion significantly increased inflammation (D) and lysis of macrophage (E) in mice lung. PAS could stain proteins produced by lysis of macrophage, which could indicate injures of alveolar cell.