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Joint toxicity of heavy metals and chlorobenzenes to *pyriformis Tetrahymena*

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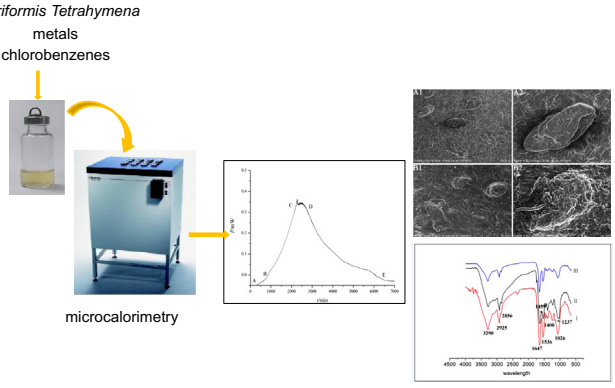
HIGHLIGHTS

- The joint toxicity of chlorobenzenes and metals to *Tetrahymena* was studied by microcalorimetry.
- The joint toxicity was evaluated by toxic unit (*TU*) and additional index (*AI*).
- The cell surface was found the micro destruction by SEM images.
- ATR-FTIR spectra were investigated the decay of functional groups.

GRAPHICAL ABSTRACT



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ABSTRACT

Chlorobenzens and heavy metals are frequently detected in the environment, but few studies have assessed the joint toxicity of organic and inorganic contaminants. The joint toxicity of heavy metals and chlorobenzenes was evaluated in the present study. Growth metabolism of the joint toxicity was studied by microcalorimetry at 28 °C, the growth constant (k) and inhibitory ratio (I) were calculated. Toxic unit (*TU*) and additional index (*AI*) were introduced to determine the outcome in combined tests, and the coexistence of Cu, Cd, Cr(III) and p-chlorobenzene was antagonism, and the effect of Cu, Cd, Cr(III) and o-chlorobenzene, Cu and 1,2,4-trichlorobenzene were synergism. In addition, micro-situation of the cell membrane surface of *pyriformis Tetrahymena* was observed by SEM. The cells suffered serious damage after sufficient acting time. ATR-FTIR spectra revealed that amide groups and PO_2^- of the phospholipid phospho-diester, both in the hydrophobic end exposed to the outer layer, were the easiest to be damaged.

1. Introduction

Human activities such as industrial and agricultural drainages have created serious risks to human and environmental health associated with exposure to heavy metals and chlorobenzenes (Kozani et al., 2007; Green et al., 2010; Jiang et al., 2010). Heavy metals and chlorobenzenes are frequently detected in the environment. Most studies that evaluate the toxicity of heavy metals and chlorobenzenes to aquatic organisms have investigated exposures to individual toxicants (Priel and Hershfinkel, 2006; Birungi et al., 2007), and study on joint toxicity have only focused on the combination of organic contaminants or inorganic contaminants (Wang et al., 2012; Schleier and Peterson, 2012; Faure et al., 2012), but few studies have assessed the joint toxicity of organic and inorganic contaminants. However, the co-existent of heavy metals and chlorobenzenes is all too common. Aquatic ecosystems are usually exposed simultaneously to a mixture of toxic

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substances. Thus, understanding the joint toxicity of these contaminants in aquatic system is needed for more accurate assessment of ecological risk (Belden and Lydy, 2006; Burton and Johnston, 2010).

In response to the need to predict effects to biota from mixtures, various models including concentration addition and independent action (Altenburger et al., 2000) have been evaluated for mixture toxicity. These models have been widely used in aquatic toxicity with reliable predictions of mixture toxicity (Swartz et al., 1995; Faust et al., 2000, 2001; Belden and Lydy, 2006). One of the most common methods of assessing concentration addition is to use toxic units (*TU*), which is a feasible method to predict adverse effects of complex chemical mixtures on the structure and functioning of aquatic ecosystems. The *TU* approach normalizes the exposure concentration for each contaminant by expressing it as a proportion of a toxicity end point and then these are summed to estimate toxicity on a proportional basis (Playle, 2004). The *TU* approach has been successfully used as a means to predict mixture toxicity and as a tool for assessing combination effects (i.e., additivity, synergism, and antagonism) (Altenburger et al., 2000; Schuler et al., 2009). And the additional index (*AI*) mode is related to *TU* mode (Marking, 1977), if $AI > 0$, synergism; $AI < 0$, antagonism; and $AI = 0$, simple addition.

Based on this background, the main goal of the present study was to determine the joint toxicity of chlorobenzenes and metals in *pyriformis Tetrahymena* by microcalorimetry which was a new method to assay biologic toxicity and damage mechanism on the *pyriformis Tetrahymena* cell membrane caused by p-dichlorobenzene, o-dichlorobenzene, m-dichlorobenzene and metals. *TU* and *AI* were used to describe the toxic effects. The results showed that binary mixture of metals and p-chlorobenzene was antagonism, and the binary mixture of metals and o-chlorobenzene, Cu and 1,2,4-trichlorobenzene were synergism. Scanning electron microscopy (SEM) images were used to observe the micro destruction on the cell surface. In addition, attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra were applied to investigate the decay of functional groups in order to sort out the details of the damage process.

2. Materials and methods

2.1. Cells and reagent

pyriformis Tetrahymena was provided by Wuhan Institute of Hydrobiology, Chinese Academy of Science, Wuhan. The culture medium contained (pH = 7.0):15 g L^{-1} tryptone, 5 g L^{-1} yeast extract, 1 g L^{-1} glucose. It was sterilized in high pressure steam at 120 °C for 30 min. The cells were grown axenically at 28 °C in the culture medium.

The compounds were 1,2-dichlorobenzene (o-dichlorobenzene), 1,3-dichlorobenzene (m-dichlorobenzene), 1,4-dichlorobenzene (p-dichlorobenzene), 1,2,4-trichlorobenzene, $CuSO_4{\cdot}5H_2O$, $CdS0_4{\cdot}8/3H_2O$ and $Cr(NO_3)_3{\cdot}9H_2O$, all of which were purchased from Sinopharm Chemical Reagent Co., Ltd. (analytical reagent grade). The chlorobenzenes were dissolved in ethylene glycol diethyl ether to make stock solutions. Concentration of stock solutions of five different chlorinated benzenes was 500 mg L^{-1}. The heavy metals were dissolved in distilled water to make stock solutions. The concentrations of Cu^{2+}, Cd^{2+} and Cr^{3+} were 100, 0.3, 86 g L^{-1}, respectively.

2.2. Microcalorimetric measurement

The microcalorimetric experiments were carried out in TAM Air 3114/3236 (Thermometric AB, Sweden), which is an eight-channel isothermal heat conduction calorimeter operating in the milliwatt range. The thermal stability of the apparatus is ±0.02 °C. The performances and the details of this instrument have been described previously (Qin et al., 2004). In the calorimetric experiment, *pyriformis Tetrahymena*, which were cultured to stationary phase in an incubator at 28 °C, were first put into the prepared 5 mL culture medium with or without the tested chemicals in 20 mL ampoule and sealed with a cap. When the temperature of measurement system reached stability, the glass ampoules were put into the microcalorimeter. The growths of *pyriformis Tetrahymena* in the absence or presence of the chemicals were monitored automatically and continuously by the microcalorimeter.

The binary toxicity of metals and chlorobenzenes to *pyriformis Tetrahymena* was determined on the basis of single toxicity under the concentration of Cu (0.25 IC_{50}), Cd (0.55 IC_{50}), and Cr (0.65 IC_{50}), respectively.

2.3. SEM observation

After the effects of heavy metals and chlorobenzenes, the samples were processed as follow before SEM observation. Glutaraldehyde was used to fix and dehydrate the protein (or lipid) in cells. The cells were then dehydrated in a series of increasing concentration of ethanol (50%, 60%, 70%, 80%, 90%, 95% and absolute ethanol). After the cells were fixed and dehydrated, they were observed by SEM (S-4800;Hitachi).

2.4. ATR-FTIR

The ATR-FTIR spectra of *pyriformis Tetrahymena* were measured by FT-IR spectroscopy (AVATAR370; Thermo Nicolet Co. of America). A: the cells in the presence of Cd^{2+} and o-dichlorobenzene with culturing for 3 d; B: the cells in the presence of Cr^{3+} and o-dichlorobenzene with culturing for 3 d; C: the cells in the presence of Cu^{2+} and o-dichlorobenzene with culturing for 3 d; D: the cells in the presence of Cd^{2+} with culturing for 3 d. E: the cells in the presence of o-dichlorobenzene with culturing for 3 d. The samples were then prepared in the following way: 0.5 mL of the liquid from the samples was evaporated on the glass vessel and dried in a vacuum chamber. Spectra were the results of 64 scans with a resolution of 4 cm^{-1} in the spectra range 600–4000 cm^{-1}.

3. Results and discussion

3.1. Thermogenic curves of pyriformis Tetrahymena growth at 28 °C

The metabolism of *pyriformis Tetrahymena* growth in culture media was studied and the thermogenic curve was recorded. From Li (Li et al., 2010) we could see that the metabolic process can be divided into four phases: the growth stagnation phase (AB), the logarithmic growth phase (BC), the stability phase (CD) and the decline phase (DE).

In the log phase of growth, the cell growth and heat output are exponential. So the kinetic equations are,

$$P_t = P_0 \exp(kt) \quad \text{or} \quad \ln P_t = \ln P_0 + kt \tag{1}$$

The growth thermogenic curves of the log phase correspond to Eq. (1). Using the data $\ln P_t$ and t taken from the curves to fit a linear equation.

It was apparent that $k = (0.002541 \pm 0.000045)\ min^{-1}$. And the relative standard deviation (RSD) was 2.35%, indicating a good reproducibility and correlation.

The power–time curves of *pyriformis Tetrahymena* growth affected by different concentrations of metals and chlorobenzenes