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两种典型持久性有机污染物对草履虫急性毒性初探

周海龙, 李国锋#, 黄仁良, 崔嵩, 刁晓平*

海南大学 海南省热带生物资源可持续利用重点实验室培育基地, 农学院, 海南 海口 570228

摘要: 滴滴涕和苯并芘这两种典型持久性有机污染物在环境中已广泛分布, 因此, 本研究利用单细胞真核模式生物-草履虫来研究其急性毒性效应, 结果发现其毒性效应存在显著的剂量效应关系。DDT 和 BaP 的半数致死浓度分别为 126.012 mg·L^{-1} 和 180.167 mg·L^{-1}, 且这两种污染物的浓度和概率间存在很好的线性关系。不同浓度的 DDT 和 BaP 对草履虫进行毒性作用时, 草履虫呈现出不同的形态; 比较而言, DDT 的毒性作用更大。由于草履虫对这两种毒性物质作用的敏感性, 因此, 草履虫可作为一种敏感指示生物来评估 POPs 的长期危害。本研究为水污染的减排和生境的保护提供了一种新途径。最后, 就这两种典型 POPs 对草履虫的毒性机理进行了讨论。

关键词: 草履虫; 滴滴涕; 苯并芘; 半数致死浓度; 毒性机理
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Initial Study on Acute Toxicity of Two Typical Persistent Organic Pollutants (POPs) against *Paramecium caudatum*

Zhou Hailong, Li Guofeng#, Huang Renliang, Cui Song, Diao Xiaoping*

Keg laboratory Incubation Base of Tropical Biological Resources Sustainable Utilization of Hainan Province, College of Agriculture, Hainan University, Haikou 570228, China

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Abstract: Dichlorodiphenyltrichloroethane (DDT) and Benzo[a]pyrene (BaP) are ubiquitous contaminants in the environment. Acute toxic effects of DDT and BaP on a model unicellular eukaryotic organism *Paramecium caudatum* were studied and a dose-dependent increase in toxicities was observed. The median lethal concentration (LC_{50}) of DDT in 1h is 126.012 mg·L^{-1} and that of BaP is 180.167 mg·L^{-1}. There exists a good linear relationship between probit and logarithm of concentrations of contaminants. Exposed to DDT and BaP at different concentrations, *Paramecia caudatum* exhibited some shapes. Comparably, DDT is more toxic to *Paramecium caudatum* than that of BaP. Sensitive to both DDT and BaP suggests that *Paramecium caudatum* can be used as a sensitive indicator for early risk assessment of long-term hazards of POPs. These results provide a novel approach for developing a strategy for the abatement of water pollution and maintenance of ecosystem viability. Finally, the toxicity mechanism of two typical POPs in *Paramecium caudatum* is discussed.

Keywords: *Paramecium caudatum*; dichlorodiphenyltrichloroethane (DDT); benzo[a]pyrene (BaP); median lethal concentration (LC_{50}); toxicity mechanism

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作者简介: Zhou hailong (1977-), E-mail: hlongzhou@gmail.com; *通讯作者 E-mail: diaoxip@hainu.edu.cn
共同第一作者: Li Guofeng (1988-), E-mail: morality9@gmail.com

1 Introduction

Persistent organic pollutants(POPs) are a type of toxic chemicals and have become ubiquitous in the environment (Iwata *et al*., 1993). The toxicity of POPs can be biomagnified through the food chain in the tissues of living organisms (Wania and Mackay, 1996; Jones and De Voogt, 1999). Many diseases may be induced by POPs in animals and human, such as cancer, damage to the nervous system, reproductive disorders, etc. POPs are easily introduced to aquatic ecosystem as well (Koumanova, 2007; Samara, 2007).

DDT and BaP are two widespread typical POPs and their application has been uncontrolled for several decades (Wang *et al*., 2007). DDT is used as insecticide worldwide. Due to its stability and bioaccumulation ability, DDT is found in animal and human bodies, releasing seriously negative effects(Turusov *et al*., 2002). DDT possibly increases the risks of cancers at various sites, to affect neurobehavioral functions and to be associated with premature births (Longnecker *et al*., 2001; Van Wendel de Joode *et al*., 2001). The presence and persistence and metabolites make DDE and DDD still a worldwide problem of great relevance to public health (Turusov *et al*., 2002). As is known, BaP is ubiquitous in the atmosphere and present in a wide variety of food items. BaP is reported as the most potent carcinogenic polycyclic aromatic hydrocarbon (PAH) carcinogen in animal experiments (Howard and Fazio, 1980), and it is also embryotoxic and teratogenic in animals (Smoke and Smoking, 2004). BaP can increase the incidence of tumors at several sites, particularly in the upper gastrointestinal tract (Kazerouni *et al*., 2001). Unfortunately, to date there is still no effective and rapid monitoring method to detect these types of POPs in aquatic environment.

Paramecium caudatum (*P. caudatum*) distribute widely as one of the typical microorganisms, which is easy accessed and cultured and sensitive to water environment. Also, *P. caudatum* can improve water quality (Shiny *et al*., 2005). Therefore, it is a perfect organism to evaluate the quality of water. Compared with chemical and physical methods, bioindicator such as *P. caudatum* can reflect the toxicity of POPs vividly and really. *P. caudatum* has been used in rapid toxicity assessment of pesticides (Komala, 1992; Juchelka and Snell, 1995; Miyoshi *et al*., 2003; Rouabhi *et al*., 2006; Garad *et al*., 2007). However, little work has been done regarding the toxic effect of DDT and BaP on *P. caudatum*. The main purpose of this work was to determine the 1-hour LC_{50} of DDT and BaP for *P. caudatum* and to observe the stress response of *P. caudatum* to these chemicals at different concentrations.

2 Materials and Methods

2.1 Materials

DDT and BaP were purchased from Sigma (St. Louis, MO). Dimethyl sulfoxide (DMSO) was obtained from Amresco Inc. (Solon, OH) which were used to dissolve these two POPs.

The test species *P. caudatum* was collected and isolated from fresh water pond within the vicinity of Hainan University, Haikou, China. *P. caudatum* was cultured in sterilized hay infusion medium (pH = 7.5—8.0) at room temperature (25±2 ℃), with a photo period of 12 h light and 12 h dark in the laboratory, and then a pure line culture was obtained. The organisms were harvested at the mid-logarithmic phase of growth and the logarithmic phase cultures were used for the present study.

2.2 Methods

Test concentrations of DDT or BaP were chosen according to the results of the initial experiments which determine the media lethal concentration (LC_{50}) for 1 h. DDT was diluted to 90, 110, 120, 135, 145, 150, 155 mg·L^{-1}. BaP was diluted to 150, 158, 167, 190, 200, 210, 220 mg·L^{-1}. *P. caudatum* was maintained in 1mL rice straw water in 12-well microplates for the toxicity test. Simultaneously, control experiments were performed without POPs and 0.01% DMSO as a vehicle control. One additional higher concentration (400 mg·L^{-1}) was also set for toxicity test for both DDT and BaP. Twenty active paramecia were put in each well and exposed to selected concentrations with four replicates. The inverted microscope (Olympus IX81) was used to observe the change in behavior and morphology of *P. caudatum*. One hour later, death number of *P. caudatums* in each concentration was counted to estimate the LC_{50} values using probit analysis (Finney, 1971). No movement showed the death of *P. caudatum*.

2.3 Statistical analyses

The LC_{50} of DDT and BaP were calculated by probit analysis (Finney, 1971), and the regression equation was obtained at the same time. This method has been recommended by the Organization for Economic Co-operation and Development (OECD) guideline as appropriate statistical method for toxicity data analysis (Lilius *et al*., 1994). Data were expressed as mean ±S.E. and analyzed statistically using SPSS (version 16.0) software. After linearization of the concentration response curve by logarithmic transformation of concentrations, 95% confidence limits and slope function were calculated to provide a consistent presentation of the toxicity data.

3 Results

Acute exposure cells showed deformities such as swelling, oval shaped deformity, and shortening of longitudinal axis in the body size at higher concentrations with blackening of cytoplasm occurred. Leaking of cytoplasmic contents was also observed.

3.1 Effect of DDT on *P. caudatum*

P. caudatums all lived well in the negative control and 0.01% DMSO (vehicle control) groups within one hour, and no death occurred at the end of the acute toxicity tests. Acute toxicity of DDT to *P. caudatum* is shown in Table 1. The LC_{50} (1 h) value of DDT is 126.012 mg·L^{-1}. A dose-dependent increase was observed for mortality rate. Probit analysis is shown in Fig. 1 and the equation of linear regression is $Y=-17.268+8.221X$ ($R^2=0.939$).

Table 1　Acute toxicity of DDT to *P. caudatum* (1h)

Concentration /(mg·L^{-1})	Concentration logarithm	Death number (Mean±SE)	Mortality rate/% (Mean±SE)	Probit(Y)
90	1.954	3.25±0.25	0.16 ±0.01	0.13
110	2.041	5.75±0.85	0.29 ±0.04	0.30
120	2.079	7.75±0.85	0.39 ±0.04	0.41
135	2.130	10.05±0.50	0.53 ±0.03	0.58
145	2.161	13.50±1.66	0.68 ±0.08	0.70
150	2.176	15.00±1.08	0.75 ±0.05	0.75
155	2.190	17.00±1.22	0.85 ±0.06	0.79

SE: Standard error of the mean

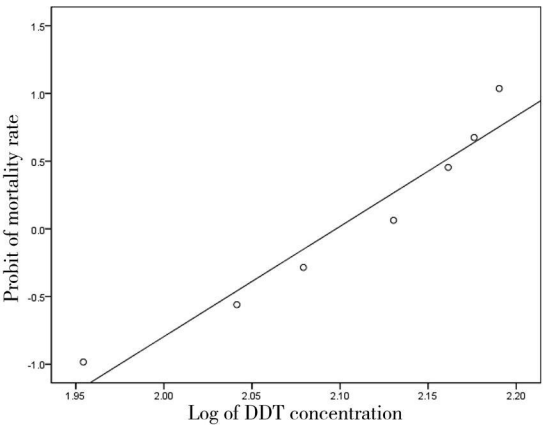

Fig. 1　Relationship between probit and logarithm of concentration of DDT in 1 hour

The aberration observation is shown in Fig. 2, where panel a is the normal body shape without DDT treatment, panel b was treated with LC_{50}, panel c was treated with 400 mg·L^{-1}.

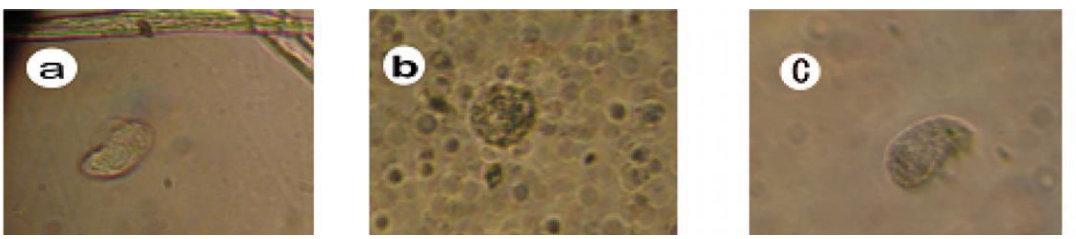

Fig. 2　*P. caudatum* was observed with microscope(100). a: normal body shape without DDT treatment; b: *P. caudatum* was treated with LC_{50} of DDT; c: *P. caudatum* was treated with 400 mg·L^{-1} of DDT

3.2 Effect of BaP on *P. caudatum*

Similarly, no dead *P. caudatum* was found in the whole exposure duration of BaP in the negative control and 0.01% DMSO (vehicle control) groups in one hour. The LC_{50} (1 h) value of BaP is 180.167 mg·L^{-1} and the mortality rate dose-dependently increases (Table 2). Fig. 3 exhibits the probit analysis and the equation of linear regression is: $Y=-32.614+14.459X$ ($R^2=0.984$).

Table 2 Acute toxicity of BaP to *P. caudatum* (1 h)

Concentration /(mg·L^{-1})	Concentration logarithm	Death number (Mean±SE)	Mortality rate/% (Mean±SE)	Probit(Y)
150	2.176	2.75±0.85	0.14 ±0.04	0.14
158	2.199	4.50±0.87	0.23 ±0.04	0.21
167	2.223	6.25±1.25	0.31 ±0.06	0.31
190	2.279	11.00±1.08	0.55 ±0.05	0.61
200	2.301	14.75±1.31	0.74 ±0.07	0.74
210	2.322	17.00±0.58	0.85 ±0.03	0.84
220	2.342	18.50±0.65	0.93 ±0.03	0.91

SE: Standard error of the mean

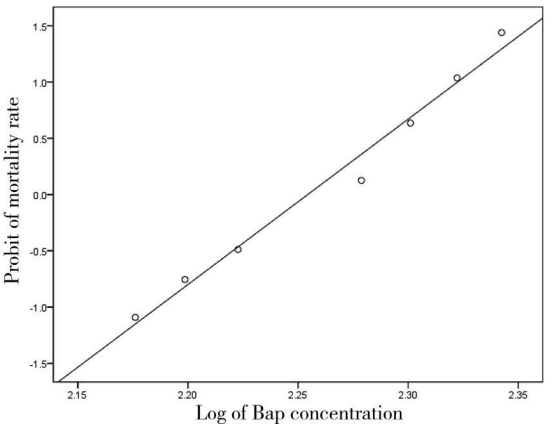

Fig. 3 Relationship between probit and BaP logarithm of concentration in 1 hour

The aberration observation is shown in Fig. 4, where panel a is normal body shape without BaP treatment, panel b is treated with LC_{50}, panel c is treated with 400 mg·L^{-1}. Round shape is found in panel b.

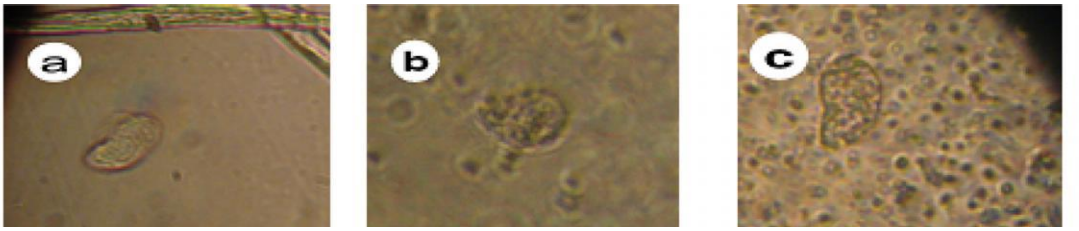

Fig. 4 *P. caudatum* was observed with microscope (100). a: normal body shape without BaP treatment; b: *P. caudatum* was treated with LC_{50} of BaP; c: *P. caudatum* was treated with 400 mg·L^{-1} of BaP

4 Discussion

Although LC_{50} values for small animals have been generally accepted as indicators of the toxicity of POPs, use of such animals in experiments seems both time-consuming and costly. The types of POPs increase rapidly and many complex experiments need to be conducted to test their toxicities. Therefore, we selected *P. caudatum* as a typical microorganism to investigate the acute toxicity of two kinds of POPs including DDT and BaP.

Rao *et al.* (2008) found that fenthion at concentration of 76 mg·L^{-1} (LC_{50} for 2h) affected cellular morphology of *P. caudatum* and inhibited its locomotion, as well as degraded the cytoskeleton leading to cell destruction. The LC_{50} values of monochrotophos and acephate at 2 h against *P. caudatum* are 40.6 mg·L^{-1} and 300 mg·L^{-1}, respectively (Rao *et al.*, 2007; Venkateswara Rao *et al.*, 2006). From Fig. 1 and Fig. 3, we can find that the death number of *P. caudatum* increases with the augment of POPs´concentrations, and there exists a linear relationship between probit and logarithm of concentration. Additionally, our results reveal that *P. caudatum* is more sensitive to DDT than to BaP.

Garad *et al.* (2007) found that mohocrotophos can reduce overall body length, length and breadth of cytopharynx, rate of feeding and cyclosis. Similarly, we also found these phenomena in our experiments. As shown in Fig. 2 and Fig. 4, it was surprisingly observed that the body shape of *P. caudatum* was round at the lethal range of DDT and BaP (20—220 mg·L^{-1} and 120—240 mg·L^{-1}) but was in normal shape at higher concentration (400 mg·L^{-1}). It was found another interesting phenomenon that when *P. caudatums* were exposed to DDT at the concentration of 190 mg·L^{-1} within 1 h, most of them did not have activity behavior. However it was found that some living *P. caudatums* in the next morning, which suggests *P. caudatum* might possess special tolerance mechanism a-

gainst DDT.

What caused the morphology changes of *P. caudatum*? We presume that there exists a protective mechanism for *P. caudatum*. When exposed to hazardous materials, such as DDT and BaP, *P. caudatums* reduced the body surface through changing their shape to round to avoid the damage of DDT and BaP. However, at higher concentration (400 mg·L^{-1}), they have not enough time to change their shapes to reduce the damage of DDT and BaP. Thus, toxicants immediately led to cell destruction. On one hand, Catallo *et al*. (1994) indicated that the protective effect of calcium involved changes in hazardous materials(quinoline) solubility and resultant decreasing in intracellular concentrations of calcium in *P. caudatum*. Moreover, the results of Iwadate and Nakaoka(2008) revealed that cell body contraction is regulated by Ca^{2+} in a dose-dependent manner in living *P. caudatum*. On the other hand, our previous study (Zhou *et al*., 2009) showed that the toxicology regulation mechanism of most POPs mainly through aryl hydrocarbon receptor (AhR) pathway in almost all vertebrates and invertebrates. What is the toxicology regulation mechanism of POPs in *P. caudatum*? It needs further research.

In conclusion, *P. caudatum* is a sensitive microorganism to DDT and BaP and can be used as a potent bioindicator for assessment of water quality and early detection of water pollution. Also, it can be used as a suitable toxicological tool for basic research.

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Biography: Diao Xiaoping (1963—), Female, Ph. D, Professor, Hainan University. Special Interests: Ecotoxicology of marine environment. Tel: 0898-66295028

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