

## Responses in the population growth and reproduction of freshwater rotifer *Brachionus calyciflorus* to four organochlorine pesticides

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**Abstract** – In China, although the production and use of organochlorine pesticides (OCPs) have been banned for decades, relatively high levels of OCP residues have still been found in some water bodies, and can result in adverse acute and chronic effects on zooplankton including rotifers, which have caused public concern for many years. Responses in the population growth and reproduction of freshwater rotifer *Brachionus calyciflorus* to four OCPs including aldrin, dieldrin,  $\beta$ -hexachlorocyclohexane ( $\beta$ -HCH) and chlordecone were studied by 3-day population growth and 4-day resting eggs (RE) production tests. In comparison with control, aldrin at  $10 \mu\text{g.L}^{-1}$ ,  $\beta$ -HCH at  $1000 \mu\text{g.L}^{-1}$  and chlordecone at  $0.05 \mu\text{g.L}^{-1}$  significantly increased the population growth rate ( $r$ ); but aldrin at  $100 \mu\text{g.L}^{-1}$ , dieldrin at  $0.001$  and  $0.1 \mu\text{g.L}^{-1}$ ,  $\beta$ -HCH at  $0.1$ – $100 \mu\text{g.L}^{-1}$  and chlordecone at  $50 \mu\text{g.L}^{-1}$  markedly decreased it. Aldrin at concentrations higher than  $1 \mu\text{g.L}^{-1}$ , dieldrin at  $0.01$  and  $1000 \mu\text{g.L}^{-1}$ ,  $\beta$ -HCH at concentrations  $0.1$  and higher than  $1 \mu\text{g.L}^{-1}$ , and chlordecone at concentrations  $0.005$  and higher than  $0.5 \mu\text{g.L}^{-1}$  significantly decreased the ratio of ovigerous females to non-ovigerous females (OF/NOF), but the reverse was true for aldrin at  $0.1 \mu\text{g.L}^{-1}$  and  $\beta$ -HCH at  $0.001 \mu\text{g.L}^{-1}$ . Dieldrin at  $0.001$ ,  $0.01$  and  $1000 \mu\text{g.L}^{-1}$  significantly decreased the ratio of mictic females to amictic females (MF/AF), but  $\beta$ -HCH at  $1$  and  $10 \mu\text{g.L}^{-1}$  highly significantly increased it. Dieldrin at  $1000 \mu\text{g.L}^{-1}$  and  $\beta$ -HCH at concentrations higher than  $10 \mu\text{g.L}^{-1}$  markedly decreased the fertilization rate (FR). Both aldrin and chlordecone have no significant effect on the MF/AF and FR of rotifers. Aldrin at concentrations higher than  $1 \mu\text{g.L}^{-1}$ , dieldrin at lower than  $0.1$  and higher than  $10 \mu\text{g.L}^{-1}$ ,  $\beta$ -HCH at  $1000 \mu\text{g.L}^{-1}$  and chlordecone at  $0.005$ ,  $0.05$  and  $50.0 \mu\text{g.L}^{-1}$  significantly decreased the mictic rate (MR) of rotifers, but the reverse was true for  $\beta$ -HCH at  $1 \mu\text{g.L}^{-1}$ . Aldrin at  $10 \mu\text{g.L}^{-1}$ , dieldrin at  $0.001$ ,  $0.1$  and  $1000 \mu\text{g.L}^{-1}$ ,  $\beta$ -HCH at concentrations higher than  $1 \mu\text{g.L}^{-1}$  and chlordecone at concentrations higher than  $0.005 \mu\text{g.L}^{-1}$  markedly decreased RE production of rotifers, but  $\beta$ -HCH at  $0.01 \mu\text{g.L}^{-1}$  significantly increased it. A clear dose–response relationship existed between the RE and the concentration of dieldrin,  $\beta$ -HCH and chlordecone, and the OF/NOF and the aldrin concentration. The RE and OF/NOF in rotifer population might be suitable endpoints for monitoring the low concentration of three OCPs (dieldrin,  $\beta$ -HCH and chlordecone) and aldrin, respectively.

**Key words:** *Brachionus calyciflorus* / ratio ovigerous females/non-ovigerous females / resting eggs production / organochlorine pesticide

### Introduction

During the past few decades, several organochlorine pesticides (OCPs) were extensively used in Asian developing countries including China for agriculture and pisciculture in order to control unwanted insects and weeds (Fernandez-Casalderrey *et al.*, 1992). OCPs can be

transported into aquatic environment through different input pathways, such as runoff from non-point sources, industrial discharge, atmospheric deposition and sediment desorption (Zhou *et al.*, 2008).

Aldrin, dieldrin, hexachlorocyclohexane (HCH) such as  $\beta$ -HCH and chlordecone all belong to OCPs. In China, although the production and use of these OCPs have been either banned for few years or decades or are non-existent, relatively high levels of these OCPs residues have still been

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found in some water bodies (Cheng, 1990; Li *et al.*, 1998, 2002; Lin *et al.*, 2000; Zhong *et al.*, 2000; Wong *et al.*, 2002; An *et al.*, 2010). Furthermore,  $\beta$ -HCH with the higher mobility transported by air, was recovered in Arctic regions (Li *et al.*, 2002). Therefore, OCPs might result in adverse acute and chronic effects on zooplankton, fishes, animals, and even human beings owing to their toxicity, persistence and bioaccumulation, which have caused public concern for many years. Hence, it is important to evaluate the effects of these OCPs on aquatic animals including zooplankton.

In freshwater ecosystems, zooplankton community is mainly composed of protozoans, rotifers, cladocerans and copepods (Pennak, 1989), and frequently used to detect anthropogenic contamination because of their sensitivity to various toxicants and their important role in the ecosystem. Among them, rotifers and cladocerans share many common characteristics that include predominantly parthenogenetic reproduction, comparable lifespan and the ability to utilize microalgae such as *Chlorella* and *Scenedesmus* as food (Lampert and Sommer, 1997; Dodson and Frey, 2000). However, compared with cladocerans, rotifers have some other important characteristics. For instance, rotifers are more diverse, have a smaller body size, a high rate of reproduction, the commercial availability of their resting eggs (RE) and sometimes more sensitive to most toxicants. Due to these characteristics, rotifers, especially *Brachionus calyciflorus* and *B. plicatilis*, are more favored test animals in aquatic toxicology (Koste, 1978; Snell and Moffat, 1992; Janssen *et al.*, 1993; Snell and Janssen, 1995).

Rotifers are a group of free-living, planktonic pseudocoelomates characterized by possessing a wheel of cilia called a corona at the anterior end (Zou, 2003). There is an alternation of parthenogenic and sexual reproduction in the life cycle of rotifers (Snell and Carmona, 1995). At the beginning of the growing season, diploid parthenogenic females hatch out from the RE of the previous season. Parthenogenic females reproduce unisexually by laying diploid eggs, which develop into females. Upon receiving appropriate environmental cues, rotifers can switch the mode of reproduction from parthenogenic reproduction to sexual reproduction, in which diploid sexual females are produced (Snell and Carmona, 1995). Diploid sexual females then produce haploid eggs through meiosis, which develop into haploid males or RE if fertilized by males (Preston *et al.*, 2000). RE are the sources of genotypic variability and permit escape from harsh environments through dormancy (Birky and Gilbert, 1971; King and Snell, 1977; Pourriot and Snell, 1983).

Life table demography and population growth studies are the two chronic toxicity tests often conducted on rotifers (Snell and Janssen, 1995). Life tables provide birth rates and death rates in an age-specific way. Therefore, different life history variables related to survivorship and reproduction can be quantified under different test conditions (Wallace *et al.*, 2006). However, the life table method does not provide information on the possible

adaptation of offspring born under the toxicant treatments chosen for the experiments. Therefore, the life table and population growth methods are complementary to each other (Sarma and Nandini, 2001; Mangas-Ramírez *et al.*, 2004).

In ecotoxicological studies using rotifers, especially *B. calyciflorus*, endpoints such as 24 or 48 h LC<sub>50</sub>, swimming speed and behavior, filtration rate and enzyme activity have been used (Snell and Janssen, 1995; Guo *et al.*, 2012a). However, only a portion of the rotifer life cycle or other endpoints are investigated in these studies, the true vulnerability of rotifer life cycle to toxicants is often underestimated (Preston and Snell, 2001). Hence, several studies assessed the effects of pesticides, heavy metals and endocrine disrupting chemicals on the entire life cycle of rotifers (Snell and Carmona, 1995; Preston *et al.*, 2000; Preston and Snell, 2001; Radix *et al.*, 2002; Xi *et al.*, 2007). These results showed that sexual reproduction and resting egg production are among the most sensitive endpoints.

Up to now, there are few studies about the effects of aldrin (Huang *et al.*, 2007), dieldrin (Huang *et al.*, 2012) and chlordecone (Zha *et al.*, 2007) to rotifer *B. calyciflorus* by means of life table techniques. However, the effects of these three OCPs on the sexual reproduction of *B. calyciflorus* are unknown. In addition, the effect of  $\beta$ -HCH on the reproduction of *B. calyciflorus* has not been reported.

The main purposes of the present study were to: (1) assess responses in the asexual and sexual reproduction of *B. calyciflorus* to four OCPs including aldrin, dieldrin,  $\beta$ -HCH and chlordecone by means of 3-day population growth and 4-day RE production tests; (2) screen out the suitable endpoints for monitoring the effects of these OCPs on the reproduction of *B. calyciflorus*.

## Materials and methods

*B. calyciflorus* were obtained by hatching RE collected from sediments of Lake Jinghu (31°33'N, 118°37'E) located in the center of Wuhu city in the east of China and then clonally culturing under controlled laboratory conditions. Stock rotifer cultures were kept under static-renewal conditions with natural illumination without artificial lighting at  $25 \pm 1$  °C in an illumination incubator for over 1 year. Rotifers were daily fed on *S. obliquus* at  $1.0\text{--}2.0 \times 10^6$  cells.mL<sup>-1</sup>. Before the experiments commenced, rotifers were cultured in the EPA medium (pH 7.4–7.8; prepared by dissolving 96 mg NaHCO<sub>3</sub>, 60 mg CaSO<sub>4</sub>, 60 mg MgSO<sub>4</sub>, and 4 mg KCl in 1 L distilled water) (USEPA, 1985) and fed on  $3.0 \times 10^6$  cells.mL<sup>-1</sup> of *S. obliquus* at  $25 \pm 1$  °C for at least two weeks. Algae were grown in a semi-continuous culture using a HB-4 medium (Li *et al.*, 1959) renewed daily at 20%. Algae in exponential growth were centrifuged and resuspended in distilled water and then stored at 4 °C. The density of the stock algal concentrate was estimated using a haemocytometer (Tiefe, 0, 100 mm, 1/400 qmm, Germany).

Aldrin, dieldrin,  $\beta$ -HCH and chlordecone (purity at least 99%) were all purchased from Sigma-Aldrich (Munich, Germany), which were first all dissolved to 1000 mg.L<sup>-1</sup> in 100% acetone, then diluted with the EPA medium to stock solutions of 10 mg.L<sup>-1</sup> which were stored at 4 °C until used, and the desired concentration containing not more than 0.1% acetone.

Considering the very low concentration (ng.L<sup>-1</sup> to  $\mu$ g.L<sup>-1</sup>) of the four OCPs existing in natural water bodies (Heberer, 2002; Xue *et al.*, 2005) and preliminary range-finding tests showed that 500  $\mu$ g.L<sup>-1</sup> of chlordecone led to 100% mortality of the rotifers during the initial 24 h exposure, but that 50  $\mu$ g.L<sup>-1</sup> of chlordecone (Zha *et al.*, 2007) and 1000  $\mu$ g.L<sup>-1</sup> of dieldrin and  $\beta$ -HCH did not cause any mortality, and 1000  $\mu$ g.L<sup>-1</sup> of aldrin nearly led to 100% mortality of the rotifers on the third day, the selected final test concentrations were: aldrin (0.001, 0.01, 0.1, 1, 10 and 100  $\mu$ g.L<sup>-1</sup>), dieldrin (0.001, 0.01, 0.1, 1, 10, 100 and 1000  $\mu$ g.L<sup>-1</sup>),  $\beta$ -HCH (0.001, 0.01, 0.1, 1, 10, 100 and 1000  $\mu$ g.L<sup>-1</sup>) and chlordecone (0.0005, 0.005, 0.05, 0.5, 5 and 50  $\mu$ g.L<sup>-1</sup>). Four replicates were carried out for each treatment. A control of the EPA medium and a solvent control containing 0.1% acetone were also tested.

All the experiments were conducted in about 8-mL custom-built glass chambers and started by introducing 10 neonates (< 3 h old, hatched from parthenogenetic eggs on the EPA medium with  $3.0 \times 10^6$  cells.mL<sup>-1</sup> of *S. obliquus*) into each chamber containing 5 mL of the test solution and  $3.0 \times 10^6$  cells.mL<sup>-1</sup> of *S. obliquus*. Thereafter, the rotifers were cultured under static conditions with natural illumination without artificial lighting at  $25 \pm 1$  °C in an illumination incubator for 3 days. During the experimental period, the original rotifers and their RE (if any) were daily transferred into freshly prepared test solution containing  $3.0 \times 10^6$  cells.mL<sup>-1</sup> *S. obliquus* and the algae deposited at the bottom of each chamber were resuspended every 12 h with a micropipette. Then, the number of each type of rotifer females was counted following the method described by Xi *et al.* (2007). Rotifers were classified as unfertilized and fertilized mictic females, amictic females by the size and morphology of their eggs, and non-ovigerous females. All the rotifers were continually cultured for another 24 h to count the number of RE (if any). Meanwhile, the population growth rate ( $r$ ,  $r = (\ln N_t - \ln N_0)/t$ , where  $N_0$  and  $N_t$  are 2nd and final rotifer population density, respectively, and  $t = 3$ ), the ratio of ovigerous females to non-ovigerous females (OF/NOF), the ratio of mictic females to amictic females (MF/AF), the mictic rate (MR), and the fertilization rate (FR) were calculated according to Radix *et al.* (2002).

One-way analysis of variance (ANOVA) by SPSS 16.0, with concentration as the independent variable, and  $r$ , OF/NOF, MF/AF, MR, FR or RE, as dependent variables, followed by Dunnett's test was conducted for pairwise comparisons of each pesticide concentration and the solvent control relative to the control (Zar, 1999). From these results, no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) were

determined and median effective concentration (EC<sub>50</sub>) was calculated using regression analysis for each compound (Stephan and Rogers, 1985).

## Results

Compared with the control, 0.1% acetone did not significantly influence all the population parameters and RE of the rotifers ( $P > 0.05$ ).

Aldrin significantly affected  $r$ , OF/NOF, MR and RE ( $P < 0.05$ ), but had no significant effect on the MF/AF and FR of the rotifers ( $P > 0.05$ ). Aldrin at 10  $\mu$ g.L<sup>-1</sup> significantly increased the  $r$ , but aldrin at 100  $\mu$ g.L<sup>-1</sup> markedly decreased it. Aldrin at 0.1  $\mu$ g.L<sup>-1</sup> highly significantly increased the OF/NOF, but aldrin at concentrations higher than 1  $\mu$ g.L<sup>-1</sup> significantly decreased the OF/NOF and MR. Furthermore, aldrin at 10  $\mu$ g.L<sup>-1</sup> markedly decreased RE of the rotifers (Fig. 1).

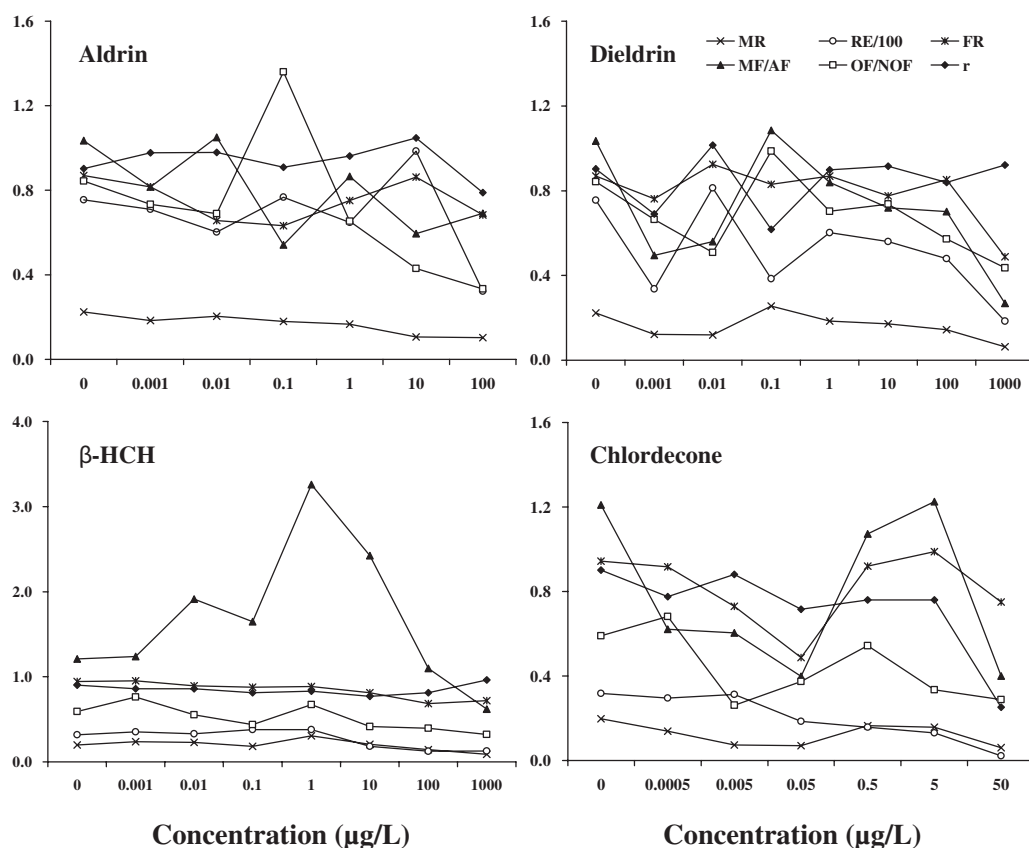
Dieldrin significantly decreased all the population parameters and RE of the rotifers ( $P < 0.05$ ). Compared with the control, dieldrin at 0.001 and 0.1, 0.01 and 1000, lower than 0.1 and 1000, lower than 0.1 and higher than 10, 1000  $\mu$ g.L<sup>-1</sup> significantly decreased  $r$ , OF/NOF, MF/AF, MR and FR, respectively. Meanwhile, dieldrin at 0.001, 0.1 and 1000  $\mu$ g.L<sup>-1</sup> also significantly decreased RE of the rotifers (Fig. 1).

$\beta$ -HCH significantly influenced all the population parameters and RE of the rotifers ( $P < 0.05$ ). Compared with the control,  $\beta$ -HCH at 0.1–100  $\mu$ g.L<sup>-1</sup> decreased the  $r$ , but  $\beta$ -HCH at 1000  $\mu$ g.L<sup>-1</sup> markedly increased it.  $\beta$ -HCH at 0.001  $\mu$ g.L<sup>-1</sup> highly significantly increased the OF/NOF, but  $\beta$ -HCH at concentrations 0.1 and higher than 1  $\mu$ g.L<sup>-1</sup> significantly decreased it.  $\beta$ -HCH at 1 and 10  $\mu$ g.L<sup>-1</sup> highly significantly increased the MF/AF.  $\beta$ -HCH at 1  $\mu$ g.L<sup>-1</sup> highly significantly increased the MR, but  $\beta$ -HCH at 1000  $\mu$ g.L<sup>-1</sup> highly significantly decreased it.  $\beta$ -HCH at concentrations higher than 10 and 1  $\mu$ g.L<sup>-1</sup> highly significantly decreased the FR and RE, respectively (Fig. 1).

Chlordecone significantly affected  $r$ , OF/NOF, MR and RE ( $P < 0.05$ ), but had no significant effect on the MF/AF and FR of the rotifers ( $P > 0.05$ ). Compared with the control, chlordecone at 0.05 and 50  $\mu$ g.L<sup>-1</sup> significantly decreased  $r$ . Chlordecone at 0.005 and higher than 0.5  $\mu$ g.L<sup>-1</sup> markedly decreased the OF/NOF. Chlordecone at 0.005, 0.05 and 50.0  $\mu$ g.L<sup>-1</sup> all significantly decreased the MR. Furthermore, chlordecone at concentrations higher than 0.005  $\mu$ g.L<sup>-1</sup> markedly decreased RE of the rotifers (Fig. 1).

A clear dose–response relationship existed between RE and the concentration of dieldrin,  $\beta$ -HCH, chlordecone, and the OF/NOF and aldrin concentration (Table 1).

A comparison among NOEC, LOEC and EC<sub>50</sub> of all the parameters for each compound showed that both LOECs and EC<sub>50</sub>s of the RE were not more than those of the  $r$  for dieldrin,  $\beta$ -HCH and chlordecone, and those of OF/NOF were the lowest among all the parameters for the aldrin (Table 2).



**Fig. 1.** The effects of aldrin, dieldrin,  $\beta$ -HCH and chlordecone on the reproduction of *Brachionus calyciflorus* after 3-day or 4-day exposure. *r*: population growth rate; OF/NOF: ratio of ovigerous females to non-ovigerous females; MF/AF: ratio of mictic females to amictic females; FR: fertilization rate; RE: resting eggs; MR: mictic rate.

**Table 1.** Relationships between the OF/NOF and aldrin concentration, RE production and the concentration of dieldrin,  $\beta$ -HCH and chlordecone.

OCPs	Parameters	Regression equations	Significant tests
Aldrin	OF/NOF	$Y = -0.0427x^2 + 0.2641x + 0.5185$	$R^2 = 0.8447, P < 0.001$
Dieldrin	RE	$Y = -0.9871x^2 + 4.3442x + 57.08$	$R^2 = 0.8272, P < 0.001$
$\beta$ -HCH	RE	$Y = -1.2113x^2 + 7.0982x + 27.571$	$R^2 = 0.8151, P < 0.001$
Chlordecone	RE	$Y = -0.5357x^2 - 0.6071x + 33.429$	$R^2 = 0.9401, P < 0.001$

## Discussion

Halbach (1984) was among the first person to use population growth as a tool for evaluating the effects of toxicants. Several workers later have used this approach for many species of rotifers such as *B. plicatilis*, *B. calyciflorus* and *B. patulus* to quantify the effects of pesticides (*i.e.*, DDT, lindane, thiophanate-methyl, glyphosate, diazinon, fenitrothion, methoprene, isoprothiolane, cypermethrin, deltamethrin, dicofol, endosulfan, atrazine and carbaryl) (Rao and Sarma, 1986, 1990; Janssen *et al.*, 1994; Xi and Feng, 2004; Chu *et al.*, 2005; Marcial *et al.*, 2005; Xu *et al.*, 2005; Xi *et al.*, 2007; Huang *et al.*, 2011; Lu *et al.*, 2012) and other pollutants (*i.e.*, growth hormone, human chorionic gonadotropin,  $17\beta$ -estradiol, triiodothyronine, 20-hydroxyecdysone,

5-hydroxytryptamine,  $\gamma$ -aminobutyric acid, juvenile hormone, ethinylestradiol, nonylphenol, testosterone, phthalate acid esters and melamine) (Gallardo *et al.*, 1997; Radix *et al.*, 2002; Zhao *et al.*, 2007; Wen *et al.*, 2011).

In a previous study, we found that aldrin at 20–640  $\mu\text{g.L}^{-1}$  significantly increased the *r* by means of life-table techniques with *B. calyciflorus* (Huang *et al.*, 2007), which was absolutely similar to our present results that aldrin at 10  $\mu\text{g.L}^{-1}$  significantly increased the *r*. Another study results showed that dieldrin at 100  $\mu\text{g.L}^{-1}$  significantly decreased *r* of *B. calyciflorus* (Huang *et al.*, 2012), which was similar to our present results that dieldrin at 0.001 and 0.1  $\mu\text{g.L}^{-1}$  significantly decreased it, but dieldrin at 100  $\mu\text{g.L}^{-1}$  did not affect it significantly. Furthermore, chlordecone at 0.05  $\mu\text{g.L}^{-1}$  significantly increased the *r* of *B. calyciflorus*, which was different from



**Table 2.** Assessments of chronic toxicity of aldrin, dieldrin,  $\beta$ -HCH and chlordecone on the rotifer *Brachionus calyciflorus*.

Parameters	Aldrin			Dieldrin			Chlordecone			$\beta$ -HCH		
	NOEC	LOEC	EC <sub>50</sub>	NOEC	LOEC	EC <sub>50</sub>	NOEC	LOEC	EC <sub>50</sub>	NOEC	LOEC	EC <sub>50</sub>
<i>r</i>	1	10	–	0	0.001	–	0.005	0.05	> 50	0.01	0.1	–
OF/NOF	0.01	0.1	31.12	0.001	0.01	≥ 1000	0.0005	0.005	–	0	0.001	> 1000
MF/AF	–	–	–	0	0.001	≥ 1000	–	–	–	0.1	1	> 1000
MR	1	10	44.56	0	0.001	≥ 1000	0.0005	0.005	> 50	0.1	1	> 1000
FR	–	–	–	100	1000	≥ 1000	–	–	–	10	100	–
RE	10	100	> 100	0	0.001	> 1000	0.005	0.05	0.57	0.001	0.01	31.63

*r*: population growth rate; OF/NOF: ratio of ovigerous females to non-ovigerous females; MF/AF: ratio of mictic females to amictic females; FR: fertilization rate; RE: resting eggs; MR: mictic rate. NOEC: no-observed-effect concentration; LOEC: lowest-observed-effect concentration; EC<sub>50</sub>: median effective concentration. All values are in  $\mu\text{g.L}^{-1}$ .

the results obtained by [Zha \*et al.\* \(2007\)](#) that chlordecone did not significantly influence the *r*. From the aforementioned results, we can find an increase in the *r* of *B. calyciflorus* with low dose of aldrin and chlordecone. This, phenomenon known as hormesis, has been reported for many species of zooplankton ([Calabrese and Baldwin, 2003](#); [Gama-Flores \*et al.\*, 2007](#); [Guo \*et al.\*, 2012b](#); [Rumengan and Ohji, 2012](#)). However, dieldrin promoted the decrease in the *r*. There were two possible reasons for the disparity between these results. One might be the difference in toxicity test methods such as life table demography and population growth studies. The life table method does not provide information on the possible adaptation of offspring born under the toxicant treatments, but the population growth method can do. The other might be different from pesticide species and sensitivity of the rotifer *B. calyciflorus* to different pesticides. However, the detailed reasons for their different effects need further research in the future.

[Radix \*et al.\* \(2002\)](#) found that nonylphenol at concentrations from 129.94 to 599.05  $\mu\text{g.L}^{-1}$  significantly increased the OF/NOF in *B. calyciflorus* population, but the reverse was true for ethinylestradiol at concentrations higher than 201.55  $\mu\text{g.L}^{-1}$ . Meanwhile, testosterone did not significantly influence it, which was similar to butyl benzyl phthalate, but both 5000  $\mu\text{g.L}^{-1}$  di-*n*-butyl phthalate and 5–5000  $\mu\text{g.L}^{-1}$  di (2-ethylhexyl) phthalate significantly increased the OF/NOF ([Zhao \*et al.\*, 2007](#)). Furthermore, lindane at 7000  $\mu\text{g.L}^{-1}$  significantly increased the OF/NOF, but the reverse was true for DDT at concentrations higher than 240  $\mu\text{g.L}^{-1}$ , dicofol at 1200  $\mu\text{g.L}^{-1}$  and endosulfan at 7000  $\mu\text{g.L}^{-1}$  ([Xi \*et al.\*, 2007](#)). In a previous study, results showed that cypermethrin at 500  $\mu\text{g.L}^{-1}$  significantly increased the OF/NOF, but deltamethrin at each concentration did not significantly affect it ([Huang \*et al.\*, 2011](#)). In the present study, we found that both aldrin at 0.1 and  $\beta$ -HCH at 0.001  $\mu\text{g.L}^{-1}$  highly significantly increased the OF/NOF, but aldrin at concentrations higher than 1, dieldrin at 0.01 and 1000,  $\beta$ -HCH at concentrations 0.1 and higher than 1 and chlordecone at 0.005 and higher than 0.5  $\mu\text{g.L}^{-1}$  all significantly decreased it. From the results stated above, it might be concluded that the effect of pollutants on the OF/NOF in a rotifer population depended on the pollutant species. Sensitivity of the rotifer *B. calyciflorus*

to different pollutants might be different. A similar conclusion was obtained by an environmental pollutant including the present four pesticides influenced the MF/AF of rotifers ([Radix \*et al.\*, 2002](#); [Xi \*et al.\*, 2007](#); [Zhao \*et al.\*, 2007](#); [Huang \*et al.\*, 2011](#)).

In the present study, dieldrin and  $\beta$ -HCH at higher concentrations decreased significantly the FR, which was similar to the result of DDT ([Xi \*et al.\*, 2007](#)), but aldrin and chlordecone had no significant effect on it, which was similar to the results of pentachlorophenol, cadmium and naphthol ([Snell and Carmona, 1995](#)), dicofol, lindane and endosulfan ([Xi \*et al.\*, 2007](#)), chlorpyrifos, flutamide, testosterone, nonylphenol, diazinon, fenitrothion, methoprene and isoprothiolane ([Preston \*et al.\*, 2000](#); [Marcial \*et al.\*, 2005](#)), and cypermethrin and deltamethrin ([Huang \*et al.\*, 2011](#)). It is possible that lower production of males is one of the reasons which decreased the FR of rotifers.

The general hypothesis that repression of sexual reproduction was a general response to environmental stresses of many types ([Lubzens \*et al.\*, 1985](#); [Snell, 1986](#); [Snell and Boyer, 1988](#); [Snell and Carmona, 1995](#); [Preston \*et al.\*, 2000](#); [Xi and Feng, 2004](#); [Marcial \*et al.\*, 2005](#); [Xi \*et al.\*, 2007](#)). Similar results were obtained in the present study for dieldrin, chlordecone and  $\beta$ -HCH. The comparison among NOEC, LOEC and EC<sub>50</sub> of all the parameters for each compound showed that both LOECs and EC<sub>50</sub>s of the RE were not more than those of the *r* for all the tested compounds except aldrin ([Table 2](#)), indicating that sexual reproduction was more sensitive than asexual reproduction to those compounds, meaning that the RE was a suitable endpoint for assessing the effects of these three pesticides, which agreed with the results obtained by [Snell and Carmona \(1995\)](#), [Xi and Feng \(2004\)](#) and [Xi \*et al.\* \(2007\)](#). Meanwhile, the OF/NOF in *B. calyciflorus* population was a suitable endpoint for assessing the effects of aldrin, which was similar to the results of [Radix \*et al.\* \(2002\)](#) on ethinylestradiol and nonylphenol. Furthermore, the MR was a secondary sensitive endpoint for assessing the effects of aldrin and chlordecone. The other endpoints were not suitable for assessing the effects of these OCPs in the present study. However, asexual reproduction parameters such as *r* sometimes are more sensitive than sexual reproduction parameters such as RE to some pesticides ([Zhao \*et al.\*, 2007](#); [Huang \*et al.\*, 2011](#)). The important

reasons are possible differences between rotifer species or strains or contaminant categories.

## Conclusion

Organochlorine pesticides tested in this study such as aldrin, dieldrin,  $\beta$ -HCH and chlordecone affected the asexual and sexual reproductions of *B. calyciflorus*, and changed the population structure. The assessment of a 3-day population growth or 4-day RE production test might be used to quantitatively monitor the effects of the four organochlorine pesticides on the reproduction of rotifers, and RE production and the ratio of ovigerous females to non-ovigerous females in rotifer populations might be suitable endpoints for monitoring the low concentration of the three organochlorine pesticides (dieldrin,  $\beta$ -HCH and chlordecone) and aldrin in natural water bodies, respectively.

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## References

- An N., Fu Y.Q., Wang Y.M., Chen C., Niu F., Han Y. and Dong S.Y., 2010. Residual levels of HCH and DDT in Songhua river water, soil and foodstuff (in Chinese with an English abstract). *J. Environ. Heal.*, 27, 335–338.
- Birky C.W. and Gilbert J.J., 1971. Parthenogenesis in rotifers: the control of sexual and asexual reproduction. *Am. Zool.*, 11, 245–246.
- Calabrese E.J. and Baldwin L.A., 2003. Inorganics and hormesis. *Crit. Rev. Toxicol.*, 33, 215–304.
- Cheng X., 1990. The pesticides production of China Going to a period of higher efficient and more secure (in Chinese with an English abstract). *Pesticides*, 29, 15–19.
- Chu Z.X., Xi Y.L. and Xu X.P., 2005. Effect of glyphosate on the life history characteristics of freshwater rotifer *Brachionus calyciflorus* pallas (in Chinese with an English abstract). *Chin. J. Appl. Ecol.*, 16, 1142–1145.
- Dodson S.I. and Frey D.G., 2000. Cladocera and other branchiopoda. In: Thorp J.H. and Covich A.P. (eds), *Ecology and Classification of North American Freshwater Invertebrates*, Academic Press, London, 850–914.
- Fernandez-Casalderrey A., Ferrando M.D. and Andreu-Moliner E., 1992. Acute toxicity of several pesticides to rotifer *Brachionus calyciflorus*. *Bull. Environ. Contam. Toxicol.*, 48, 14–17.
- Gallardo W.G., Hagiwara A., Tomita Y., Soyano K. and Snell T.W., 1997. Effect of some vertebrate and invertebrate hormones on the population growth, mictic female production, and body size of the marine rotifer *Brachionus plicatilis* Müller. *Hydrobiologia*, 358, 113–120.
- Gama-Flores J.L., Castellanos-Paez M.E., Sarma S.S.S. and Nandini S., 2007. Effect of pulsed exposure to heavy metals (copper and cadmium) on some population variables of *Brachionus calyciflorus* Pallas (Rotifera: Brachionidae: Monogononta). *Hydrobiologia*, 593, 201–208.
- Guo R.X., Ren X.K. and Ren H.Q., 2012a. Assessment the toxic effects of dimethoate to rotifer using swimming behavior. *Bull. Environ. Contam. Toxicol.*, 89, 568–571.
- Guo R.X., Ren X.K. and Ren H.Q., 2012b. Effects of dimethoate on rotifer *Brachionus calyciflorus* using multigeneration toxicity tests. *J. Environ. Sci. Heal. B*, 47, 883–890.
- Halbach U., 1984. Population dynamics of rotifers and its consequences for ecotoxicology. *Hydrobiologia*, 109, 79–96.
- Heberer T., 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicol. Lett.*, 13, 15–17.
- Huang L., Xi Y.L., Zha C.W. and Zhao L.L., 2007. Effect of aldrin on life history characteristics of rotifer *Brachionus calyciflorus* Pallas. *Bull. Environ. Contam. Toxicol.*, 79, 524–528.
- Huang L., Liu C.L., Wei C.B. and Xia L., 2011. Effects of cypermethrin and deltamethrin on reproduction of *Brachionus calyciflorus* (in Chinese with an English abstract). *Acta Ecol. Sin.*, 31, 7632–7638.
- Huang L., Xi Y.L., Zha C.W., Zhao L.L. and Wen X.L., 2012. Effects of dieldrin and 17 $\beta$ -estradiol on life history characteristics of freshwater rotifer *Brachionus calyciflorus* Pallas. *J. Freshwat. Ecol.*, 27, 381–392.
- Janssen C.R., Ferrando M.D. and Persoone G., 1993. Ecotoxicological studies with the freshwater rotifer *Brachionus calyciflorus*: I. Conceptual framework and application. *Hydrobiologia*, 255/256, 21–32.
- Janssen C.R., Persoone G. and Snell T.W., 1994. Cyst-based toxicity tests.VIII. Short-chronic toxicity tests with the freshwater rotifer *Brachionus calyciflorus*. *Aquat. Toxicol.*, 28, 243–258.
- King C.E. and Snell T.W., 1977. Sexual recombination in rotifers. *Heredity*, 39, 357–360.
- Koste W., 1978. Rotatoria. Die Rädertiere Mitteleuropas. Ein Bestimmungswerk begründet von Max Voigt, Bornträger, Stuttgart, Vol. 1: Textband 673 p., Vol. 2: Tafelband 234 p.
- Lampert W. and Sommer U., 1997. *Limnology. The Ecology of Lakes and Streams*, Oxford University Press, New York, 382 p.
- Li S., Zhu H., Xia Y., Yu M., Liu K., Ye Z. and Chen Y., 1959. The mass culture of unicellular green algae. *Acta Hydrobiol. Sin.*, 4, 462–472.
- Li Y.F., Cai D.J. and Singh A., 1998. Technical hexachlorocyclohexane use trends in China and their impact on the environment. *Arch. Environ. Contam. Toxicol.*, 35, 688–697.
- Li Y.F., Macdonald R.W., Jantunen L.M.M., Harner T., Bidleman T.F. and Strachan W.M.J., 2002. The transport of  $\beta$ -hexachlorocyclohexane to the western Arctic Ocean: a contrast to  $\alpha$ -HCH. *Sci. Total Environ.*, 291, 229–246.
- Lin Y.S., Gong R.Z. and Zhu Z.L., 2000. *Pesticides and Eco-Environmental Protection*, Chemical Industry Press, Beijing, China.
- Lu Z.H., Zhao B.K., Yang J.X. and Snell T.W., 2012. Effects of atrazine and carbaryl on growth and reproduction of the

- rotifer *Brachionus calyciflorus* Pallas. *J. Freshwat. Ecol.*, 27, 527–537.
- Lubzens E., Minkoff G. and Marom S., 1985. Salinity dependence of sexual and asexual reproduction in the rotifer *Brachionus plicatilis*. *Mar. Biol.*, 85, 123–126.
- Mangas-Ramírez E., Sarma S.S.S. and Nandini S., 2004. Recovery patterns of *Moina macrocopa* exposed previously to different concentrations of cadmium and methyl parathion: life table demography and population growth studies. *Hydrobiologia*, 526, 255–265.
- Marcial H.S., Hagiwara A. and Snell T.W., 2005. Effect of some pesticides on reproduction of rotifer *Brachionus plicatilis* Muller. *Hydrobiologia*, 546, 569–575.
- Pennak R.W., 1989. Freshwater Invertebrates of the United States, The Ronald-Press Co., New York.
- Pourriot R. and Snell T.W., 1983. Resting eggs of rotifers. *Hydrobiologia*, 104, 213–224.
- Preston B.L. and Snell T.W., 2001. Full life cycle toxicity assessment using rotifer resting egg production: implication for ecological risk assessment. *Environ. Pollut.*, 114, 399–406.
- Preston B.L., Snell T.W., Roberston T.L. and Dingmann B.J., 2000. Use of freshwater rotifer *Brachionus calyciflorus* in screening assay for potential endocrine disruptors. *Environ. Toxicol. Chem.*, 19, 1097–1101.
- Radix P., Severin G., Schamm K.W. and Kettrup A., 2002. Reproduction disturbances of *Brachionus calyciflorus* (rotifer) for the screening of environmental endocrine disruptors. *Chemosphere*, 47, 1097–1101.
- Rao T.R. and Sarma S.S.S., 1986. Demographic parameters of *Brachionus patulus* Müller (Rotifera) exposed to sublethal DDT concentration at low and high food levels. *Hydrobiologia*, 139, 193–200.
- Rao T.R. and Sarma S.S.S., 1990. Interaction of Chlorella density and DDT concentration on the population dynamics of the rotifer, *Brachionus patulus* (Rotifera). *Indian J. Environ. Health*, 32, 157–160.
- Rumengan I.F.M. and Ohji M., 2012. Ecotoxicological risk of organotin compounds on zooplankton community. *Coastal Marine Science*, 35, 129–135.
- Sarma S.S.S. and Nandini S., 2001. Life table demography and population growth of *Brachionus variabilis* Hampel, 1896 in relation to algal (*Chlorella vulgaris*) density. *Hydrobiologia*, 446/447, 75–83.
- Snell T.W., 1986. Effect of temperature, salinity and food level on sexual and asexual reproduction in *Brachionus plicatilis* (Rotifera). *Mar. Biol.*, 92, 157–162.
- Snell T.W. and Boyer E.M., 1988. Thresholds for mictic female production in the rotifer *Brachionus plicatilis* (Müller). *J. Exp. Mar. Biol. Ecol.*, 124, 73–85.
- Snell T.W. and Carmona M.J., 1995. Comparative toxicant sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflorus*. *Environ. Toxicol. Chem.*, 14, 415–420.
- Snell T.W. and Janssen C.R., 1995. Rotifers in Ecotoxicology: a review. *Hydrobiologia*, 313/314, 231–247.
- Snell T.W. and Moffat B.D., 1992. A 2-d life cycle test with rotifer *Brachionus calyciflorus*. *Environ. Toxicol. Chem.*, 11, 1249–1257.
- Stephan C.E. and Rogers J.R., 1985. Advantages of using regression analysis to calculate results of chronic toxicity test. In: Bahner R.C. and Hansen D.J.H. (eds.), Aquatic Toxicology and Hazard Assessment: Eight Symposium, American Society for Testing and Materials, Philadelphia, 328–339.
- USEPA, 1985. In: Peltier, W.H. and Weber, C.I. (eds.) Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. EPA/600/4-85/013, US Environmental Protection Agency, Washington, DC, 216 p.
- Wallace R.L., Snell T.W., Ricci C. and Nogrady T., 2006. Rotifera Part 1: Biology, Ecology and Systematics. Guides to the Identification of the Microinvertebrates of the Continental Waters of the World (Zooplankton Guides). Kenobi Productions, Ghent, Belgium/Backhuys Publishers, The Hague, The Netherlands.
- Wen X.L., Xi Y.L., Yang Y.F., Zhang G., Zhang Q. and Hu Y.H., 2011. Effect of melamine on the reproductive strategy of rotifer *Brachionus calyciflorus*. *Chin. J. Zool.*, 46, 62–67.
- Wong C.K.C., Leung K.M., Poon B.H.T., Lan C.Y. and Wong M.H., 2002. Organochlorine hydrocarbons in human breast milk collected in Hong Kong and Guangzhou. *Arch. Environ. Contam. Toxicol.*, 43, 364–372.
- Xi Y.L. and Feng L.K., 2004. Effects of thiophanate-methyl and glyphosate on asexual and sexual reproduction in the rotifer *Brachionus calyciflorus* pallas. *Bull. Environ. Contam. Toxicol.*, 73, 644–651.
- Xi Y.L., Chu Z.X. and Xu X.P., 2007. Effect of four organochlorine pesticides on the reproduction of freshwater rotifer *Brachionus calyciflorus* pallas. *Environ. Toxicol. Chem.*, 26, 1695–1699.
- Xu X.P., Xi Y.L., Chu Z.X. and Chen F., 2005. The effect of deltamethin on experimental population dynamics of freshwater rotifer *Brachionus calyciflorus* (in Chinese with an English abstract). *Acta Zool. Sin.*, 51, 251–256.
- Xue N.D., Xu X.B. and Jin Z.L., 2005. Screening 31 endocrine-disrupting pesticides in water and surface sediment samples from Beijing Guanting Reservoir. *Chemosphere*, 61, 1594–1606.
- Zar J.H., 1999. Biostatistical Analysis, Vol. 4: Prentice Hall, Upper Saddle River, NJ, USA.
- Zha C.W., Xi Y.L., Huang L. and Zhao L.L., 2007. Effect of sublethal exposure to chlordecone on life history characteristics of freshwater rotifer *Brachionus calyciflorus* Pallas. *Bull. Environ. Contam. Toxicol.*, 78, 79–83.
- Zhao L.L., Xi Y.L., Huang L. and Zha C.W., 2007. Effects of phthalate acid esters on population growth and sexual reproduction of rotifers *Brachionus calyciflorus* (in Chinese with an English abstract). *Acta Zool. Sin.*, 53, 250–256.
- Zhong W.K., Hao J., Sun M.X. and Chen Y.L., 2000. Pesticides residues in food in China. *Pesticide*, 39, 1–4.
- Zhou R.B., Zhu L.Z. and Chen Y.Y., 2008. Levels and source of organochlorine pesticides in surface waters of Qiantang River, China. *Environ. Monit. Assess.*, 136, 277–87.
- Zou E.M., 2003. Current status of environmental endocrine disruption in selected aquatic invertebrates. *Acta Zool. Sin.*, 49, 551–565.