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Effect of Sublethal Exposure to Chlordecone on Life History Characteristics of Freshwater Rotifer *Brachionus calyciflorus* Pallas

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Various chemicals such as pesticides, plasticizers, and persistent pollutants are highly suspected to display endocrine-disrupting effects in animals and humans. Many studies have shown that environmental endocrine-disrupting chemicals could be the cause of reproductive disorders, endometriosis, and testicular cancer in animals and humans (Scippo et al., 2004).

The organochlorine pesticide chlordecone belongs to persistent organic pollutants, and demonstrates estrogenic activity by a series of assays such as MCF-7 cell proliferation and binding capacity to recombinant human steroid receptors (Hammond et al.,1979; Okubo et al., 2004; Scippo et al., 2004). Because it has been used in agriculture, a great quantity of chlordecone will eventually enter water bodies and affect aquatic organisms, including rotifers.

Rotifers are extremely important in freshwater ecosystems because their reproductive rates are the fastest among all the metazoan. They can convert primary (algal and bacterial) production into a form usable for secondary consumers (e.g., insect larvae, fish fry) (Nogrady et al., 1993). To date, some studies have been designed to investigate the effects of sublethal exposure to environmental endocrine-disrupting chemicals on the reproduction of rotifers (Ferrando et al., 1993; Ferrandez-Casalderrey et al., 1991, 1993; Gallardo et al., 1997, 2000; Janssen et al., 1994; Marcial et al., 2005; Preston et al., 2000; Preston and Snell, 2001; Radix et al., 2002; Rao and Sarma, 1986, 1990; Snell and Carmona, 1995; Xi and Feng, 2004), but none have dealt with chlordecone.

The main purpose of the current study was to assess the effects of sublethal exposure to chlordecone on development, survival, and reproduction of the freshwater rotifer *Brachionus calyciflorus*.

Materials and Methods

The rotifer *B. calyciflorus* used in this experiment was obtained by hatching resting eggs collected from sediments of Lake Jinghu and then clonally culturing them under controlled laboratory conditions. Stock rotifer cultures were kept at 25° ± 1°C on natural light and daily fed on 3×10^6 cells/mL of *Scenedesmus obliquus.* For mass cultures and for experiments, reconstituted hard water (Environment Protect Agency, EPA medium, prepared by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$, and 4 mg KCl in 1 L of distilled water) (USEPA, 1985) was used as the medium. Algae were grown in a semicontinuous culture using HB-4 medium (Xi et al., 2001) renewed daily at 20%. Algae in exponential growth were centrifuged, then resuspended in EPA medium.

The pesticide chlordecone (standard grade, 99.8%; Sigma-Aldrich, Munich, Germany) was used as the toxicant. Stock solution of 1,000.0 mg/L was prepared by dissolving chlordecone in 100% acetone, then diluting it to the desired concentrations using EPA medium.

A preliminary range-finding test showed that 500 μg/L of chlordecone led to 100% mortality of the rotifers during

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Table 1 Mean values ± standard error of durations of embryonic development (ED), juvenile period (JP), reproductive period (RP), postreproductive period (PP), life span (ML) and lifetime egg production (NE) of *Brachionus calyciflorus* exposed to sublethal concentrations of chlordecone

Toxicant (μg/L)	ED (h)	JP (h)	RP (h)	PP (h)	ML (h)	NE (ind)
Blank control	11.81 ± 0.33	17.14 ± 0.41	57.50 ± 4.05	25.78 ± 2.12	100.42 ± 4.48	15.11 ±1.36
Solvent control	11.74 ± 0.27	17.06 ± 0.40	60.21 ± 4.21	23.53 ± 1.79	100.79 ± 3.93	16.74 ± 1.33
0.0005	11.87 ± 0.25	16.48 ± 0.38	63.97 ± 5.53	24.00 ± 2.19	104.45 ± 5.56	17.03 ± 1.59
0.005	12.19 ± 0.32	17.22 ± 0.63	62.69 ± 4.15	21.00 ± 2.12	100.91 ± 3.17	17.69 ± 1.54
0.05	11.36 ± 0.32	17.39 ± 0.57	57.29 ± 3.81	18.29 ± 1.83[a]	92.96 ± 3.98	15.46 ± 1.39
0.5	11.50 ± 0.37	18.42 ± 0.57	67.27 ± 3.98	22.46 ± 2.17	108.15 ± 4.25	20.15 ± 1.36[b]
5.0	10.22 ± 0.52[a]	20.89 ± 0.60[a]	65.56 ± 3.58	19.85 ± 1.31[b]	106.30 ± 3.26	19.07 ± 1.14[b]
50.0	10.47 ± 0.26[a]	19.88 ± 0.45[a]	69.88 ± 2.61[b]	22.75 ± 2.25	112.50 ± 3.13[b]	22.88 ± 1.23[a]

Ind individual

[a, b] Significant difference with the blank controls ([a] $p < 0.05$, [b] $p < 0.01$ one-way ANOVA and Dunnett's test), ($n = 40$)

the initial 24-h exposure, but that 50 μg/L of chlordecone did not cause any mortality. Therefore, we selected six toxicant concentrations (0.0005, 0.005, 0.05, 0.5, 5.0, and 50.0 μg/L), a blank control agent (EPA medium), and a solvent control agent (EPA medium and 0.5‰ [v/v] acetone), each comprising four replicates of 10 rotifers for this study.

The experiments were conducted in 24-well tissue culture plates and started by introduction of one neonate (<2 h old) into each well, which contained 0.5 mL of test solution with 3.0×10^6 cells/mL of *S. obliquus*. The rotifers were checked every 3 h during the initial 48 h, and the time of the first egg and neonate produced was recorded. Thereafter, the number of eggs and neonates produced and the number of original test individuals alive were recorded, and neonates were removed every 8 h. The original rotifers alive were transferred into freshly prepared test solution every 24 h. The life table experiments were conducted in darkness at 25° ± 1°C until each individual of every cohort had died.

On the basis of the data collected, the durations of principal development stages, mean life span, and lifetime egg production of the rotifers were calculated. Survivorship (l_x) and fecundity (m_x) were constructed for each cohort (replicate) using conventional life table techniques (Poole, 1974). Intrinsic rate of population increase (r_m), net reproductive rate (R_0), generation time (T), and life expectancy at hatching (e_0) were calculated according to Krebs (1985) and Lotka (1913).

One-way analysis of variance (ANOVA), with concentration of chlordecone as the independent variable and each of the life history parameters as the dependent variable, followed by Dunnett's test was conducted for pair-wise comparisons of each concentration of chlordecone and the solvent control agent relative to the blank control agent (Zar, 1999).

Results and Discussion

Chlordecone significantly influenced the durations of embryonic development, the juvenile period, the reproductive and postreproductive periods, the mean life span, and the lifetime egg production of the rotifers ($p < 0.05$, one-way ANOVA) (Table 1). As compared with the blank control agent, acetone at 0.5‰ had no marked effects on the durations of principal development stages, the mean life span, or the lifetime egg production of the rotifers ($p > 0.05$), but chlordecone at 5 and 50 μg/L shortened the duration of embryonic development ($p < 0.01$), exactly resembling the effects of glyphosate at 3 and 8 mg/L (Chu et al., 2005), but different from those of thiophanate-methyl and deltamethrin, which had no effect on the duration of embryonic development (Xi and Hu, 2003; Xu et al., 2005). Chlordecone at 5 and 50 μg/L prolonged the juvenile period of the rotifers ($p < 0.01$), similar to the effects of thiophanate-methyl at 1.2 and 1.8 mg/L, deltamethrin at 0.6 and 1.2 mg/L, and glyphosate at concentrations ranging from 3 to 10.5 mg/L (Chu et al., 2005; Xi and Hu, 2003; Xu et al., 2005), but contrary to those of deltamethrin at 2.4 and 3 mg/L (Xu et al., 2005).

Chlordecone may directly or indirectly affect the duration of the juvenile period of the rotifers by altering the algal quality because the algal quality affects the duration of the juvenile period of the rotifers (Xi and Huang, 1999; Xi et al., 2001). However, chlordecone may directly affect only the embryonic development of the rotifers because the algal quality does not affect the duration of embryonic development of the rotifers (Xi and Huang, 1999). Further research should seek do discover how chlordecone directly affects the embryonic development time.

Chlordecone at 50 μg/L significantly prolonged the reproductive period of the rotifers ($p < 0.05$) (Table 1), exactly resembling the effects of deltamethrin at 0.6 and

Table 2 Intrinsic rate of population increase (r_m), net reproductive rate (R_0), generation time (T) and life expectancy at hatching (e_0) of *Brachionus calyciflorus* exposed to sublethal concentrations of chlordecone (mean ± standard error)

Toxicant (μg/L)	r_m/h	R_0 (ind)	T (h)	E_0 (h)
Blank control	0.0515 ± 0.0033	12.76 ± 2.62	55.69 ± 1.48	96.00 ± 5.98
Solvent control	0.0545 ± 0.0019	15.76 ± 0.79	59.83 ± 1.42	97.53 ± 2.35
0.0005	0.0543 ± 0.0011	15.90 ± 1.70	60.37 ± 3.20	99.90 ± 5.97
0.005	0.0530 ± 0.0006	16.80 ± 1.01	61.08 ± 2.06	98.72 ± 6.34
0.05	0.0538 ± 0.0016	14.36 ± 0.85	56.80 ± 0.51	88.95 ± 1.27
0.5	0.0535 ± 0.0021	19.22 ± 1.01[a]	64.84 ± 2.86[a]	104.81 ± 1.41
5.0	0.0555 ± 0.0026	18.21 ± 2.38[b]	59.81 ± 1.34	102.08 ± 3.42
50.0	0.0572 ± 0.0007	22.40 ± 0.15[a]	64.26 ± 0.59[a]	108.75 ± 4.28

Ind individual

[a, b] Significant difference with the blank controls ([a] $p < 0.05$, [b] $p < 0.01$ one-way ANOVA and Dunnett's test), ($n = 4$)

1.2 mg/L, but contrary to those of deltamethrin at 2.4 and 3 mg/L (Xu et al., 2005), and different from those of glyphosate at 0.1 to 10.5 mg/L, which had no effect on the duration of the reproductive period of the rotifers (Chu et al., 2005). Chlordecone at 50 μg/L also prolonged the mean life span of the rotifers ($p < 0.05$) (Table 1), similar to the effects of glyphosate at 3 mg/L (Chu et al., 2005), but contrary to those of thiophanate-methyl at 0.6 to 4.2 mg/L and deltamethrin at 1.2 to 3.6 mg/L (Xi and Hu, 2003; Xu et al., 2005).

Chlordecone at 0.05 and 5 μg/L shortened the postreproductive period of the rotifers ($p < 0.05$) (Table 1), similar to the effect of deltamethrin at 1.2 to 3.6 mg/L (Xu et al., 2005), but different from that of glyphosate at 0.1 to 10.5 mg/L, which had no effect on the duration of the rotifers' reproductive period (Chu et al., 2005).

Chlordecone at concentrations ranging from 0.5 to 50.0 μg/L increased the lifetime egg production of the rotifers ($p < 0.05$) (Table 1), in contrast to the effect of deltamethrin at 1.2 to 3.6 mg/L (Xu et al., 2005).

Based on the age-specific survival and fertility of the rotifers exposed to sublethal concentrations of chlordecone (Fig. 1), we calculated the intrinsic rate of population increase, the net reproductive rate, the generation time, and the life expectancy at hatching of the rotifers (Table 2). In contrast to the effects of all the other tested compounds such as pentachloro-phenol, 3,4-dichloroaniline, DDT, endosulfan, Cu, lindane, methylparathion, thiophanate-methyl, glyphosate, deltamethrin (Chu et al., 2005; Ferrandez-Casalderrey et al., 1991, 1993; Ferrando et al., 1993; Halbach, 1984; Janssen et al., 1993, 1994; Kooijman and Metz, 1984; Rao and Sarma, 1986; Xi and Hu, 2003; Xu et al., 2005), chlordecone did not significantly influence the intrinsic rate of population increase or the life expectancy at hatching of the rotifers ($p > 0.05$, one-way ANOVA).

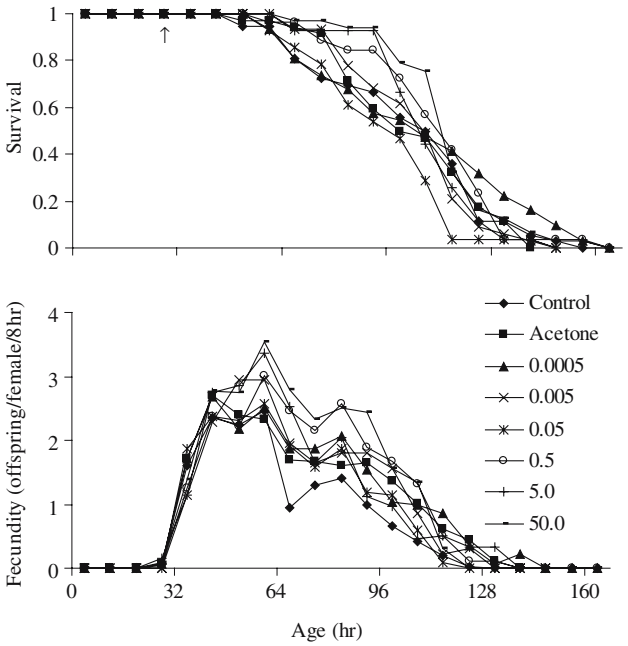

Fig. 1 The effect of sublethal exposure to chlordecone on the age-specific survivorship and fecundity of *Brachionus calyciflorus*. The arrow indicates the age at which the rotifers became reproductive

The reason for the disparity between our results and those of the aforementioned authors may be the difference in the concentration range of the tested compounds. In all of the aforementioned studies, the concentrations near to the LC50s were chosen to be the highest concentrations for all the compounds. In this study, however, considering the nominal concentration of chlordecone in natural water bodies, the concentration that did not result in mortality of rotifers during the initial 24-h exposure (50 μg/L) was chosen to be the highest concentration.

Chlordecone markedly influenced the net reproductive rate and the generation time of the rotifers ($p < 0.05$, one-

way ANOVA). Compared with the blank control agent, acetone at 0.5‰ had no effect on the rotifers, but chlordecone at 0.5 to 50 μg/L increased the net reproduction rate ($p < 0.05$), which was identical to its effect on the lifetime egg production (Table 1), but contrary to the effects of pentachloro-phenol, 3,4- dichloroaniline, DDT, endosulfan, Cu, lindane, methylparathion, thiophanate-methyl, glyphosate, or deltamethrin on the net reproduction rates of *B. calyciflorus* and *Brachionus patulus* (Chu et al., 2005; Ferrandez-Casalderrey et al., 1991, 1993; Ferrando et al., 1993; Halbach, 1984; Janssen et al., 1993, 1994; Kooij-man and Metz, 1984; Rao and Sarma, 1986; Xi and Hu, 2003; Xu et al., 2005). In addition, chlordecone at 0.5 and 50 μg/L prolonged the generation time ($p < 0.01$), which contributed to the prolonged duration of the juvenile period (Table 1).

In conclusion, chlordecone at 5 and 50 μg/L shortened the duration of embryonic development, but prolonged the juvenile period and the generation time. Chlordecone at 50 μg/L prolonged the reproductive period and the mean life span. Chlordecone at 0.05 and 5 μg/L shortened the postreproductive period. Chlordecone at 0.5 to 50 μg/L increased the lifetime egg production and the net reproduction rate. However, chlordecone at 0.0005 to 50 μg/L did not significantly influence the intrinsic rate of population increase or the life expectancy at hatching of the rotifers, indicating that chlordecone at 0.0005 to 50 μg/L will not induce any significant effects on the growth of the natural *B. calyciflorus* population.

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References

Chu ZX, Xi YL, Xu XP (2005) Effect of glyphosate on the life history characteristics of freshwater rotifer *Brachionus calyciflorus* pallas (in Chinese with an English abstract). Chin J Applied Ecol 16:1142–1145

Fernandez-Casalderrey A, Ferrando MD, Andreu-Moliner E (1991) Demographic parameters of *Brachionus calyciflorus* pallas (rotifers) exposed to sublethal endosulfan concentrations. Hydrobiologia 226:103–110

Ferrandez-Casalderrey A, Ferrando MD, Andreu-Moliner E (1993) Chronic toxicity of methylparathion to the rotifer *Brachionus calyciflorus* fed on *Nannochloris oculata* and *Chlorella pyrenoidosa*. Hydrobiologia 255/256:41–49

Ferrando MD, Janssen CR, Andreu E, Persoone G (1993) Ecotoxicological studies with the freshwater rotifer *Brachionus calyciflorus:* II. An assessment of the chronic toxicity of lindane and 3,4-dichloroaniline using life tables. Hydrobiologia 255/256:33–40

Gallardo WG, Hagiwara A, Tomita Y, Snell TW (2000) Effect of juvenile hormone and serotonin (5-HT) on mixis induction of the rotifer *Brachionus plicatilis* Müller. J Exp Mar Biol Ecol 252:97–107

Gallardo WG, Hagiwara A, Tomita Y, Soyano K, Snell TW (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

Halbach U (1984) Population dynamics of rotifers and its consequences for ecotoxicology. Hydrobiologia 109:79–96

Hammond PB, Katzenellenbogen BS, Krauthammer N, et al (1979) Estrogenic activity of the insecticide chlordecone (Kepone) and interaction with uterine estrogen receptors. Proc Natl Acad Sci USA 76:6642–6645

Janssen CR, Ferrando MD, Persoone G (1993) Ecotoxicological studies with the freshwater rotifer *Brachionus calyciflorus:* I. Conceptual framework and application. Hydrobiologia 255/256:21–32

Janssen CR, Persoone G, Snell TW (1994) Cyst-based toxicity test: VIII. Short-chonic toxicity test with the freshwater rotifer *Brachionus calyciflorus*. Aquatic Toxicology 28:243–258

Kooij- man S, Metz J (1984) On the dynamics of chemically stressed populations: The deduction of population consequences from effects on individuals. Ecotoxicol Environ Safety 13:88–91

Krebs CJ (1985) Ecology: The experimental analysis of distribution and abundance. Harper & Row, New York, 800 pp

Lotka AJ (1913) A natural population norm. J Washington Acad Sci 3:241–248, 532 pp

Marcial SH, Hagiwara A, Snell TW (2005) Effect of some pesticides on reproduction of rotifer *Brachionus plicatilis*. Hydrobiologia 546:569–575

Nogrady T, Wallace RL, Snell TW (1993) Morphology and internal organization. In: Rotifera Vol 1: Biology, ecology, and systematics. SPB Academic Publishing, The Hague, 142 pp

Okubo T, Yokoyama Y, Kano K, Soya Y, Kano I (2004) Estimation of estrogenic and antiestrogenic activities of selected pesticides by MCF-7 cell proliferation assay. Arch Environ Contam Toxicol 46:445–453

Poole RW (1974) An introduction to quantitative ecology. McGraw-Hill, New York, 532 pp

Preston BL, Snell TW (2001) Full-life cycle toxicity assessment using rotifer resting egg production: Implications for ecological risk assessment. Environ Pollut 114:399–406

Preston BL, Snell TW, Roberston TL, Dingmann BJ (2000) Use of freshwater rotifer *Brachionus calyciflorus* in screening assay for potential endocrine disruptors. Environ Toxic Chem 19:2923–2928

Radix P, Severin G, Schamm KW, Kettrup A (2002) Reproduction disturbances of *Brachionus calyciflorus* (rotifer) for the screening of environmental endocrine disruptors. Chemosphere 47:1097–1101

Rao TR, Sarma SSS (1986) Demographic parameters of *Brachionus patulus* Muller (Rotifera) exposed to sublethal DDT concentrations at low and high food levels. Hydrobiologia 139:193–200

Rao TR, Sarma SSS (1990) Interaction of *chlorella* density and DDT concentration on the population dynamics of the rotifer *Brachionus patulus* (Rotifera). Ind J Environ Health APR 32:157–160

Scippo ML, Argiris C, Weerdt CVA, Muller M, Willemsen P, Martial J, Maghuin-Rogister G (2004) Recombinant human estrogen, androgen, and progesterone receptors for detection of potential endocrine disruptors. Anal Bioanal Chem 378:664–669

Snell TW, Carmona MJ (1995) Comparative toxicity sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflrorus*. Environ Toxicity Chem 14:415–420

USEPA (1985) Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. In: Peltier WH, Weber CI (eds) EPA/600/4-85/013, U.S. Environment Protect Agency, Washington DC, 216 pp

Xi YL, Feng LK (2004) Effect of thiophanate-methyl and glyphosate on asexual and sexual reproduction in the rotifer *Brachionus calyciflorus* pallas. Bull Environ Contam Toxicol 73:644–651

Xi YL, Hu HY (2003) Effect of thiophanate-methyl on the reproduction and survival of the freshwater rotifer *Brachionus calyciflorus* pallas. Bull Environ Contam Toxicol 71:722 – 728

Xi YL, Huang XF (1999) Effect of food supply in both food quality and quantity on the population dynamics of *Brachionus urceolaris* (in Chinese with an English abstract). Acta Hydrobiol Sin 23:227–234

Xi YL, Huang XF, Jin HJ (2001) Life history of three types of females in *Brachionus calyciflorus* pallas (Rotifera) fed different algae. Hydrobiologia 446/447:95–98

Xu XP, Xi YL, Chu ZX, 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

Zar JH (1999) Biostatistical analysis. Prentice Hall Upper Saddle River. NJ