

Effect of Aldrin on Life History Characteristics of Rotifer *Brachionus calyciflorus* Pallas

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Abstract The organochlorine insecticide aldrin is commonly used in intensive agriculture, and demonstrates estrogenic activity. Rotifers such as *Brachionus calyciflorus* are favored test animals in aquatic toxicology because of their more sensitivity to most toxicants. In the tested concentration range of 0.04–1.28 mg/L, aldrin shortened significantly the durations of embryonic development. Lower concentrations of aldrin had an intriguing effect on the reproduction of the rotifers and are beneficial to their survival. Different endpoints of both development and reproduction had different sensitivity to aldrin. The reproductive endpoint of the rotifers is more sensitive to aldrin than the developmental endpoint.

Keywords Rotifer · Aldrin · Life history characteristics

The organochlorine insecticide aldrin is commonly used to kill soil pests, preserve wood and treat seeds, although it has been banned in technologically advanced countries. Aldrin can persist in the environment, and is susceptible to bio-magnification. Aldrin is readily absorbed into the circulating blood from the gastrointestinal tract, through the skin or by inhalation, and is rapidly converted to dieldrin through a mixed function monooxygenase-dependent

pathway (aldrin epoxidase) (Hayes 1982). Chronic exposure of animals and human beings to aldrin or dieldrin has resulted in dose-related hepatomegaly and histological changes (Jager 1970; Edwards and Priestly 1994; Høyer 1998). Aldrin demonstrates estrogenic activity by a series of assays such as increase in the weights of uteri in immature and ovariectomized mature rats and binding capacity to recombinant human steroid receptors (Chatterjee et al. 1992; Scippo et al. 2004). Like other organochlorine insecticides, aldrin can enter into aquatic environment by direct or indirect routes. Thus, it is important to evaluate the effect of aldrin on aquatic animals.

Zooplankton is frequently used to detect anthropogenic contamination because of their sensitivity to various toxicants and their important role in the ecosystem. Among the zooplanktons, rotifers, especially *Brachionus calyciflorus* and *B. plicatilis*, are favored test animals in aquatic toxicology because of their global distribution, small size, simple life cycle, rapid reproduction, short generation time, more sensitivity to most toxicants, simplicity of culture and the commercial availability of resting eggs (Snell and Moffat 1992; Janssen et al. 1993; Snell and Janssen 1995). As test animals, rotifers have been extensively used to monitor the acute and chronic toxicities of heavy metals, pesticides and other pollutants (Snell and Janssen 1995). By chronic toxicity tests, some researchers investigated the effects of toxicants on the survival and reproduction of rotifers, but few researchers dealt with the effects on the development, including embryonic and juvenile developments of rotifers (Xi and Hu 2003; Chu et al. 2005; Xu et al. 2005; Huang et al. 2006; Zha et al. 2007).

The main purpose of the present study was to assess the effects of different concentrations of aldrin on the development, survival and reproduction of freshwater rotifer

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B. calyciflorus, and detect the relative sensitivity of developmental and reproductive endpoints to aldrin exposure.

Materials and Methods

The rotifer *B. calyciflorus* used in this experiment was obtained by hatching resting eggs collected from sediments of Lake Jinghu (31°33'N, 118°37'E) and then clonally culturing under controlled laboratory conditions. Stock rotifer cultures had been kept under static-renewal conditions with a 16:8 h light:dark photoperiod at 130 l_x at (25 ± 1)°C in an illumination incubator for over 2 months, and with the rotifer culture medium of Gilbert (1963) and the green alga *Scenedesmus obliquus* as food. Before the experiments commenced, rotifers were cultured in EPA medium (USEPA 1985) and fed on 3.0×10^6 cells/mL of *S. obliquus* for at least 2 weeks. Algae were grown in a semi-continuous culture using HB-4 medium (Li et al. 1959) renewed daily at 20%. Algae in exponential growth were centrifuged and resuspended in EPA medium.

The pesticide aldrin (standard grade, 99.7%; Supelco company, USA; product number: LB17243) was used as the toxicant. Stock solution of 1,000 mg/L was prepared by dissolving aldrin in 100% acetone, then diluted to the desired concentrations using EPA medium.

In order to choose appropriate toxicant concentrations for life-table experiments, six concentrations of aldrin (0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mg/L) with a control and a solvent control (containing 0.16% acetone) were used in the acute toxicity test. Each treatment had four replicates. Rotifers with amictic eggs were randomly removed from the stock rotifer cultures (rotifer populations in exponential phases at densities of 50–80 ind./mL and mixis rates of 2%–5%) and placed into a glass dish containing 10 mL of EPA medium with 3.0×10^6 cells/mL of *S. obliquus*. After 2 h, ten neonates (<2 h old) for each replicate were collected and transferred into a 5-mL glass cup containing 2.5 mL of test solution with 3.0×10^6 cells/mL of *S. obliquus*. After 24 h, the number of rotifers alive was counted for each cup. The LC₅₀-value was derived following the probit method (Finley 1971).

Based on the LC₅₀-value, we selected seven toxicant concentrations (0.02, 0.04, 0.08, 0.16, 0.32, 0.64, and 1.28 mg/L), a control and a solvent control (containing 0.128% acetone) for the life-table experiments, each treatment consisting of four replicates of ten rotifers. Life-table experiments were conducted in 24-well tissue culture plates and started by introducing one neonate (<2 h old) into each well which contained 0.5 mL 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 then neonates were eliminated 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 died.

Based on the data collected, the durations of embryonic development, juvenile period, reproductive period and post-reproductive period, and mean lifespan of the rotifers were calculated. Survivorship and fecundity were constructed for each cohort using conventional life-table techniques (Poole 1974), and intrinsic rate of population increase, net reproductive rate, generation time and life expectancy at hatching of the rotifers were calculated according to Krebs (1985) and Lotka (1913).

One-way analysis of variance (ANOVA), with the concentration of aldrin as the independent variable, and each of the durations of principal developmental periods, the mean lifespan and the life-table demographic parameters as the dependent variable, followed by Dunnett's test was conducted for pair-wise comparisons of each concentration of test chemicals and the solvent control relative to the control (Zar 1999).

Results and Discussion

The durations of principal development stages except post-reproductive stage, and the mean lifespan of the rotifers were significantly influenced by aldrin (one-way ANOVA, $p < 0.05$). Compared to the controls, acetone at 0.128% did not markedly influence the duration of embryonic development of rotifers. However, aldrin at 0.04–1.28 mg/L shortened the duration of embryonic development of rotifers by as much as 18.9%–28.7% (Table 1), which was identical to the effects of dieldrin at 0.01, 0.1 and 10.0 µg/L, 17β-estradiol at 0.001–0.1 µg/L and 10.0 µg/L, and chlordecone at 5.0 and 50.0 µg/L (Huang et al. 2006; Zha et al. 2007). Because the algal quality does not affect the duration of embryonic development of the rotifers (King 1967; Xi and Huang 1999), aldrin at 0.04–1.28 mg/L may directly affect the embryonic development of the rotifers. Interestingly, dieldrin, chlordecone and aldrin all have estrogenic activity (Chatterjee et al. 1992; Scippo et al. 2004). However, whether their significant effects on the duration of embryonic development of rotifers are attributed to their endocrine disrupting activity or structure similarity needs further researching, because information on the endocrinology of rotifer reproduction is still scarce.

Aldrin at 0.16–1.28 mg/L prolonged the duration of juvenile period of rotifers by 7.2%–26.6% (Table 1), which was similar to the effects of 17β-estradiol at 0.01, 0.1 and

Table 1 Mean values (\pm SE) of durations of embryonic development (ED), juvenile period (JP), reproductive period (RP), post-reproductive period (PP) and lifespan (ML) of *B. calyciflorus* exposed to different concentrations (mg/L) of aldrin

| Toxicant | ED (h) | JP (h) | RP (h) | PP (h) | ML (h) |
|----------|-------------------|-------------------|-------------------|------------------|--------------------|
| Control | 14.09 \pm 0.87 | 16.36 \pm 0.28 | 56.52 \pm 3.61 | 20.85 \pm 1.55 | 107.82 \pm 4.48 |
| Acetone | 12.17 \pm 0.60 | 15.80 \pm 0.33 | 60.89 \pm 2.83 | 19.66 \pm 1.62 | 108.51 \pm 3.47 |
| 0.02 | 15.24 \pm 0.83 | 16.11 \pm 0.23 | 67.54 \pm 3.01 | 29.41 \pm 2.50 | 128.30 \pm 4.25* |
| 0.04 | 11.42 \pm 0.68* | 14.67 \pm 0.24 | 71.36 \pm 3.59* | 19.33 \pm 1.54 | 116.78 \pm 4.04 |
| 0.08 | 10.15 \pm 0.34* | 16.55 \pm 0.32 | 75.34 \pm 3.42* | 27.57 \pm 2.74 | 129.62 \pm 5.51* |
| 0.16 | 10.73 \pm 0.43* | 18.50 \pm 0.39* | 72.30 \pm 2.95* | 28.88 \pm 3.46 | 130.40 \pm 5.18* |
| 0.32 | 10.05 \pm 0.45* | 17.53 \pm 0.35* | 64.40 \pm 2.82 | 26.00 \pm 2.76 | 117.98 \pm 4.77 |
| 0.64 | 10.65 \pm 0.43* | 18.73 \pm 0.28* | 60.85 \pm 2.87 | 22.40 \pm 1.86 | 112.63 \pm 3.78 |
| 1.28 | 10.82 \pm 0.56* | 20.71 \pm 0.38* | 42.50 \pm 2.39* | 21.89 \pm 1.27 | 95.92 \pm 2.70 |

* Significant difference with the controls (one-way ANOVA and Dunnett's test, $p < 0.05$), $n = 40$

10.0 μ g/L, and chlordecone at 5.0 and 50.0 μ g/L (Huang et al. 2006; Zha et al. 2007). Aldrin at 0.16–1.28 mg/L may directly affect the duration of juvenile period of the rotifers, or/and indirectly affect it by altering the algal quality, because the algal quality affects the duration of juvenile period of the rotifers (Xi and Huang 1999; Xi et al. 2001).

Aldrin at 0.04–0.16 mg/L prolonged the duration of reproductive period of rotifers by as much as 21.3%–26.3% (Table 1), which was similar to the effects of dieldrin at 0.001–100.0 μ g/L, 17 β -estradiol at 0.001, 0.01, 1.0, 100.0 and 1,000.0 μ g/L, and chlordecone at 50.0 μ g/L (Huang et al. 2006; Zha et al. 2007). However, aldrin at 1.28 mg/L shortened the duration of reproductive period of rotifers by 24.8% (Table 1), identical to the effect of deltamethrin at 2.4 and 3.0 mg/L (Xu et al. 2005). The above stated results indicate that lower concentrations of pollutants with endocrine disrupting activity might have an intriguing effect on the lengthening of reproductive period, but higher concentrations of them might have a toxic effect, or the toxic effect might be higher than the intriguing effect.

Aldrin did not influence the duration of post-reproductive period of the rotifers (Table 1), identical to the effect of glyphosate at 0.1–10.5 mg/L (Chu et al. 2005), but different from the effects of deltamethrin at 1.2–3.6 mg/L, and chlordecone at 0.05 and 5.0 μ g/L which shortened the duration of post-reproductive period of the rotifers (Xu et al. 2005; Zha et al. 2007), and those of dieldrin at 0.001 μ g/L and 10.0–1,000.0 μ g/L, and 17 β -estradiol at 100.0 and 1,000.0 μ g/L which prolonged the duration of post-reproductive period of the rotifers (Huang et al. 2006). It might be possible that the effects of pollutants on the duration of post-reproductive period of rotifers depend on their species and concentration.

Aldrin at 0.02, 0.04 and 0.16 mg/L prolonged the mean lifespan of rotifers by 18.9%–20.9% (Table 1), which was similar to the effects of glyphosate at 3.0 mg/L, deltamethrin at 1.2 mg/L, dieldrin at 0.001–1,000 μ g/L, 17 β -estradiol at 0.001–1.0 μ g/L, 100.0 and 1,000.0 μ g/L, and chlordecone at 50.0 μ g/L (Chu et al. 2005; Xu et al. 2005; Huang et al. 2006; Zha et al. 2007). The above stated

results indicate that lower concentrations of pollutants with endocrine disrupting activity might have an intriguing effect on the lengthening of not only the reproductive period but also on the lifespan of rotifers.

Based on the age-specific survival and fertility of the rotifers exposed to different concentrations of aldrin (Fig. 1), we calculated intrinsic rate of population increase, net reproductive rate, generation time and life expectancy at hatching (Table 2). All the above life-table demographic parameters were significantly influenced by aldrin (one-way ANOVA, $p < 0.05$). Compared to the controls, acetone at 0.128% did not markedly influence all the life-table demographic parameters of rotifers. However, aldrin at 0.02–0.64 mg/L increased the intrinsic rate of population increase of rotifers by as much as 13.2%–23.7% (Table 2), which was similar to the effects of certain concentrations of pollutants with hormonal activity (Gallardo et al. 1997; Xi and Feng 2004; Huang et al. 2006). Similarly, aldrin at 0.02–0.32 mg/L increased the net reproduction rate of rotifers by as much as 50.6%–79.8% (Table 2), which was

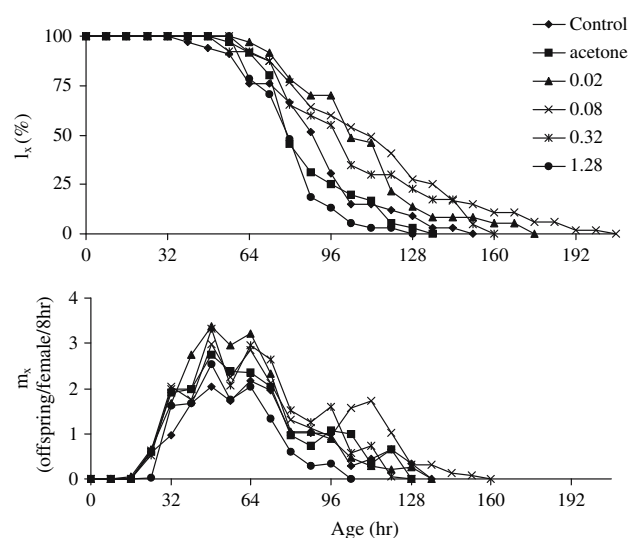


Fig. 1 Age-specific survivorship (l_x) and fecundity (m_x) of *B. calyciflorus* exposed to different concentrations (mg/L) of aldrin

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 *B. calyciflorus* exposed to different concentrations (mg/L) of aldrin

| Toxicant | r_m (/d) | R_0 (ind.) | T (h) | e_0 (h) |
|----------|------------------|---------------|---------------|----------------|
| Control | 1.1958 ± 0.0380 | 11.97 ± 2.28 | 56.49 ± 2.77 | 87.15 ± 6.30 |
| Acetone | 1.2614 ± 0.0090 | 14.47 ± 0.16 | 53.55 ± 1.03 | 87.86 ± 0.75 |
| 0.02 | 1.4797 ± 0.0757* | 19.38 ± 0.85* | 56.77 ± 1.75 | 104.71 ± 1.76* |
| 0.04 | 1.4762 ± 0.0207* | 18.08 ± 0.12* | 56.49 ± 1.10 | 96.89 ± 0.80 |
| 0.08 | 1.4054 ± 0.0269* | 18.76 ± 1.17* | 63.10 ± 0.92* | 111.67 ± 2.83* |
| 0.16 | 1.4131 ± 0.0127* | 21.52 ± 0.92* | 62.76 ± 1.20* | 114.92 ± 3.51* |
| 0.32 | 1.4141 ± 0.0096* | 18.03 ± 1.45* | 58.16 ± 1.94 | 100.80 ± 6.34 |
| 0.64 | 1.3537 ± 0.0086* | 15.27 ± 0.67 | 55.45 ± 1.06 | 95.20 ± 3.41 |
| 1.28 | 1.1894 ± 0.0504 | 10.44 ± 0.99 | 51.18 ± 0.62 | 79.22 ± 2.01 |

* Significant difference with the controls (one-way ANOVA and Dunnett's test, $p < 0.05$), $n = 4$

identical to the effects of dieldrin at 0.001 µg/L, 17β-estradiol at 100.0 and 1,000.0 µg/L, and chlordecone at 0.5–50.0 µg/L (Huang et al. 2006; Zha et al. 2007). The above stated results indicate that lower concentrations of pollutants with endocrine disrupting activity might have an intriguing effect on the reproduction of the rotifers, but the mechanism of their intriguing effects needs further researching.

Aldrin at 0.08 and 0.16 mg/L prolonged significantly the generation time of rotifers by 11.7% and 11.1%, respectively (Table 2), which was similar to the effects of dieldrin at 0.001–1,000 µg/L, 17β-estradiol at 0.01, 100.0 and 1,000.0 µg/L, and chlordecone at 0.5 and 50.0 µg/L (Huang et al. 2006; Zha et al. 2007). However, aldrin at 1.28 mg/L shortened the generation time of rotifers (Table 2), which was identical to the effect of thiophanate-methyl at 1.2 and 1.8 mg/L, and deltamethrin at 2.4 and 3.0 mg/L (Xu et al. 2005).

Aldrin at 0.02, 0.08 and 0.16 mg/L increased the life expectancy at hatching of rotifers by 20.1%, 28.1% and 31.7% (Table 2), which was similar to the effects of dieldrin at 0.001–1,000.0 µg/L, and 17β-estradiol at 0.01, 1.0, 100.0 and 1,000.0 µg/L (Huang et al. 2006), but different from the effect of chlordecone at 0.0005–50.0 µg/L which did not significantly influence the life expectancy at hatching of the rotifers (Zha et al. 2007). Aldrin at 0.02, 0.08 and 0.16 mg/L increased the life expectancy at hatching of rotifers, which was identical to its effect on the mean lifespan.

Among all the developmental and reproductive parameters, the net reproductive rate was most significantly affected by aldrin, and the intrinsic rate of population growth was affected as much as the durations of all the developmental period. Different endpoints of both development and reproduction of the rotifers had different sensitivity to aldrin. Among the developmental endpoints, embryonic development time was the most sensitive; and among the reproductive endpoints, intrinsic rate of population increase was the most sensitive. Compared with the embryonic development time, the intrinsic rate of population increase is more sensitive.

Snell et al. (1991a) compared the sensitivity of the acute *B. plicatilis* test with four marine tests for five metals and pentachlorophenol, and found that the rotifer had comparable sensitivity to most compounds, but no single species was consistently the most sensitive to all compounds. A similar comparative study of *B. calyciflorus*, *Daphnia magna* and *Pimephales promelas* (fathead minnow) revealed that the LC50s of the two invertebrates were within one order of magnitude for 9 of 12 compounds (Snell et al. 1991b). Based on the test of 99 environmental samples (effluents, solid wastes and sediments), Persoone et al. (1993) showed that the cyst-based acute test with *B. calyciflorus* was in 45% of the cases as sensitive and in 17% more sensitive than the conventional *D. magna* test. A similar comparison between the rotifer test and the bacterial Microtox test made for 250 samples showed that the latter was more sensitive than the former in 68% of the cases and as sensitive in 10% of the cases (Persoone and Janssen 1993). In the present study, LC50 of aldrin to *B. calyciflorus* was 1.52 mg/L. Compared with all the tested species of freshwater organisms, *B. calyciflorus* was less sensitive to aldrin than fishes (48–96 h LC50 of 0.9–53 µg/L), nine species of freshwater crustaceans (48–96 h LC50 of 0.1–50 µg/L) and six species of freshwater insects (48–96 h LC50 of 1–42 µg/L), but as sensitive as two species of freshwater amphibians (48–96 h LC50 of 68–2,400 µg/L), and more sensitive than one species of freshwater molluscs (48–96 h LC50 of 2,035 µg/L) (US Department of Interior, Fish and Wildlife Service 1980).

In conclusion, aldrin at 0.04–1.28 mg/L, 1.28, 1.28 mg/L shortened significantly the durations of embryonic development and reproductive period, and the generation time of *B. calyciflorus*, respectively. However, aldrin at 0.16–1.28 mg/L, 0.04–0.16 mg/L, 0.02, 0.04 and 0.16 mg/L, and 0.08 and 0.16 mg/L prolonged the durations of juvenile and reproductive periods, the mean lifespan and the generation time of rotifers, respectively. Aldrin at 0.02–0.64 mg/L, 0.02–0.32 mg/L, and 0.02, 0.08 and 0.16 mg/L increased the intrinsic rate of population increase, the net reproduction rate and the life expectancy at hatching of rotifers, respectively.

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