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Life Table Evaluation of Chronic Exposure of *Eurytemora affinis* (Copepoda) to Kepone

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Abstract

Life table estimates of intrinsic rate of natural increase (r) were used to assess chronic toxicity of Kepone to the copepod *Eurytemora affinis* (Poppe). The acute toxicity (48-h LC 50) was determined to be 40 $\mu g\, l^{-1}$ (95% CL 33.9–47.2). A reduction in r was observed at all concentrations above 5 $\mu g\, l^{-1}$, and r was near zero at 20 $\mu g\, l^{-1}$. This was due to the combination of lowered survivorship, delayed onset of reproduction and reduced fecundity. We discuss the value of the life table approach both as an experimental protocol and an ecologically realistic bioassay of chronic effects, and document that as few as 21 d are sufficient as an adequate test duration.

Introduction

It is generally recognized that chronic exposure to toxins may be deleterious at concentrations well below those identified as lethal in shortterm, acute toxicity tests (Sprague, 1976). One attempt to predict long-term chronic responses from short-term acute effects is the "application factor", obtained from the ratio between the concentration causing a chronic response and the LC 50 (2 d–2 wk) (Eaton, 1973). A value of 0.01 has been suggested as general for this application factor (NAS-NAE, 1972), but in specific instances 0.01 may be too high or too low (Hansen *et al.*, 1977; Nimmo *et al.*, 1977). Clearly results will depend at least on the type of chronic test, the duration of the acute test and the mode of action of the toxicant.

In a previous paper (Daniels and Allan, 1981), we hypothesized that the intrinsic rate of natural increase, r, calculated from the life table equation of Lotka (1925):

$$\Sigma\, l_x\, m_x\, e^{-rx} = 1 \qquad (1)$$

is an ecologically meaningful bioassay and a useful protocol for toxicity tests. The statistic r is based on both survivorship (l_x) and fecundity (m_x), and is a measure of a population's capacity to increase in an unlimited environment (cf. Ricklefs, 1973). We suggest that the relationship between a chronic effect and the LC 50 could be treated as three alternative hypotheses. These are (1) no chronic effect until very near the LC 50, (2) impairment of ecological growth potential in direct proportion to increasing toxicity, and (3) severe reduction in ecological growth potential at concentrations well below the LC 50. Clearly a continuum exists between (1) and (3), however.

In the present study we use life table estimates of r to assess the effects of chronic exposure to Kepone® for the estuarine copepod *Eurytemora affinis*. This copepod and *Acartia* spp. are the dominant zooplankters of the Chesapeake Bay (Heinle and Flemer, 1975) and are important food for fish.

Kepone is a highly toxic and stable organochlorine pesticide which bioaccumulates through the food chain (Hansen *et al.*, 1976; Bell *et al.*, 1979). It is a contaminant of the James River, lower Chesapeake Bay due to uncontrolled discharges from a manufacturing plant, and is also a degradation product of Mirex (Carlson *et al.*, 1976). The effects of Kepone on aquatic biota has been studied for microbes (Orndorff and Colwell, 1980), algae (Walsh *et al.*, 1977), decapod crustaceans (Schimmel and Wilson, 1977; Schimmel *et al.*, 1979) and fish (Schimmel and Wilson, 1977; Hansen *et al.*, 1977). Studies by Hansen *et al.* (1977), Nimmo *et al.* (1977) and Bahner *et al.* (1977) have included chronic toxicity testing and food chain bioaccumulation. However, to our knowledge, no previous study has examined the effects of Kepone on zooplankton, nor utilized the life table approach.

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Materials and Methods

Eurytemora affinis (Poppe) cultures were collected from the upper reaches of the Patuxent River at Lower Marlboro, Md. The copepods were maintained in filtered (0.45 μm Millipore), autoclaved Chesapeake Bay water (salinity 8–10‰S) at 20 °C (± 1 C°) in a 12-h photoperiod. Stock and experimental copepods were fed *Pseudoisochrysis* sp. and *Isochrysis* sp., two yellow-brown algae obtained from Horn Point Biological Laboratory, Horn Point, Maryland. The algae were grown in full-strength Provasoli's E.S. medium (Stein, 1973) at 20 °C (± 1 C°) on a 16 h L : 8 h D light-dark cycle. The algae were maintained in log phase growth by transferring cultures to new medium every week.

Both acute and chronic bioassays were begun with newborn (± 24 h) nauplii. Forty-eight hour acute bioassays were initiated with 30 nauplii at concentrations of 10, 15, 20, 30, 40, 60, and 80 $\mu g\, l^{-1}$. Five nauplii each were placed in six 62 × 26 mm stendor dishes containing 20 ml of bay water. Nauplii used in acute tests were not fed. Nauplii were considered dead if no movement was observed at a magnification of 25×. Each chronic bioassay was initiated with 72 nauplii, six individuals in each of 12 stendor dishes containing 20 ml of bay water. Kepone concentrations used were 0 (control), 5, 10, 15, 20, 25 and 30 $\mu g\, l^{-1}$. Because acetone was required as a carrier for Kepone an additional control, acetone only at 30 $\mu g\, l^{-1}$, was included. Thus a total of 8 tests were conducted. Until an age of 10 to 13 d, sexes could not be distinguished and survivorship was based on both males and females. After Day 12, a mating pair of copepods was isolated in one dish and thereafter survivorship was based on females only. Individuals were transferred to freshly made up Kepone solutions every other day and fed $3 \cdot 5–5 \times 10^5$ cells · ml^{-1}. Surplus algal cells were always observed still in suspension when the medium was changed. Algal concentrations were determined using a Klett Sommerson colorimeter with a No. 42 blue filter. The algae were concentrated by centrifugation at 200 rpm for 10 min and resuspended in the appropriate Kepone solution.

The Kepone used in these experiments was 9% H_2O and was obtained from the Toxicology Laboratory, Department of Entomology, University of Maryland. A stock solution of 25 mg Kepone in 25 ml of reagent-grade acetone was used to make all experimental solutions. Five and 10 μl (± 0.1 μl) Hamilton syringes were used to make all experimental concentrations from the stock solution. The stock solution was checked at the end of the experiments using gas chromatography and no change in concentration was observed. It was determined by hexane extraction and analysis on a Hewlett Packard 5 840 A gas chromatograph that a 5 $\mu g\, l^{-1}$ Kepone concentration remained in solution over a 3-d period.

Results

The 48-h LC 50 value for one-day-old nauplii was found to be 40 $\mu g\, l^{-1}$ (95% confidence intervals 33 · 9–47 · 2). The method of Litchfield and Wilcoxon (1949) was used to determine the LC 50 value, confidence limits and chi-square goodness of fit.

Survivorship

Increasing concentration of Kepone clearly resulted in reduced survivorship. The proportion of individuals surviving as a function of age (l_x) is graphed for each Kepone

Table 1. *Eurytemora affinis.* Demographic trends under various Kepone treatments. Standard error where indicated

Kepone concentration $\mu g\, l^{-1}$	Mean longevity (d)	Mean day of 1st reproduction	Max longevity (d)	Total number of young	Total number of broods	Mean brood size	Numbers of females in life table
Blank control	50.2 ± 3.89	12.45 ± 0.44	64	9 278	224	41.4 ± 1.2	22
Acetone control	42.5 ± 2.56	11.75 ± 0.57	63	20 163	298	67.7 ± 1.2	20
5	36.7 ± 2.82	13.21 ± 0.20	53	8 519	202	42.2 ± 1.1	19
10	41.88 ± 3.58	14.94 ± 0.51	59	5 542	232	23.9 ± 0.7	17
15	30.5 ± 2.66	17.07 ± 0.58	51	3 033	93	31.6 ± 1.4	19
20	27.7 ± 1.83	20.22 ± 1.27	38	187	19	9.8 ± 1.4	17
25	17.0 ± 0.97	19.0 ± 2.0	22	25	3	8.3 ± 3.2	9
30	0	0	4	0	0	0	0

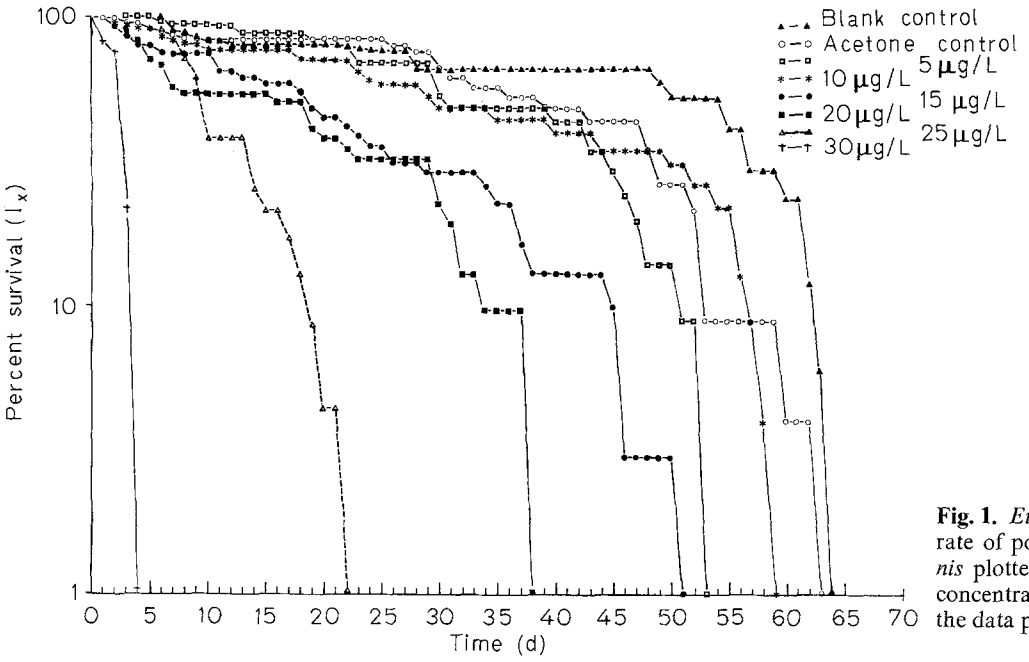

Fig. 1. *Eurytemora affinis.* The intrinsic rate of population increase, *r*, for *E. affinis* plotted against experimental Kepone concentrations. The line was fit visually to the data points

treatment in Fig. 1. We performed contingency table analysis (Sokal and Rohlf, 1969) of numbers surviving *vs* numbers dead at two ages: 12 d to represent the average age at which reproduction began in the control treatments, and 21 d to represent the mid-point of the reproduction period. At 12 d, survivorship did not differ significantly between control through 15 $\mu g\, l^{-1}$, nor between 20 and 25 $\mu g\, l^{-1}$, but did differ ($P < 0.05$) between those two groupings. At 21 d, blank and acetone controls, and 5 and 10 $\mu g\, l^{-1}$ treatments, did not differ from one another but did differ significantly from 15 and 20 $\mu g\, l^{-1}$, which also differed from 25 $\mu g\, l^{-1}$. Mean and maximum longevity also showed a response to Kepone level (Table 1). Adult survivorship (from Day 12) was further analyzed using the Kruskal-Wallis test with day of death as the ranked observation. *A posteriori* comparison of survivorship curves was accomplished using ranked sums (Hollander and Wolfe, 1973). Based on day at death, control individuals (blank and acetone) differed significantly ($\alpha = 0.10$) from those exposed to 15, 20 and 25 $\mu g\, l^{-1}$. Individuals at 5 and 10 $\mu g\, l^{-1}$ did not differ from control or 20 $\mu g\, l^{-1}$, but did differ from the 25 $\mu g\, l^{-1}$ treatment. Thirty $\mu g\, l^{-1}$ was not included in this analyses since individuals in this concentration never reached adulthood.

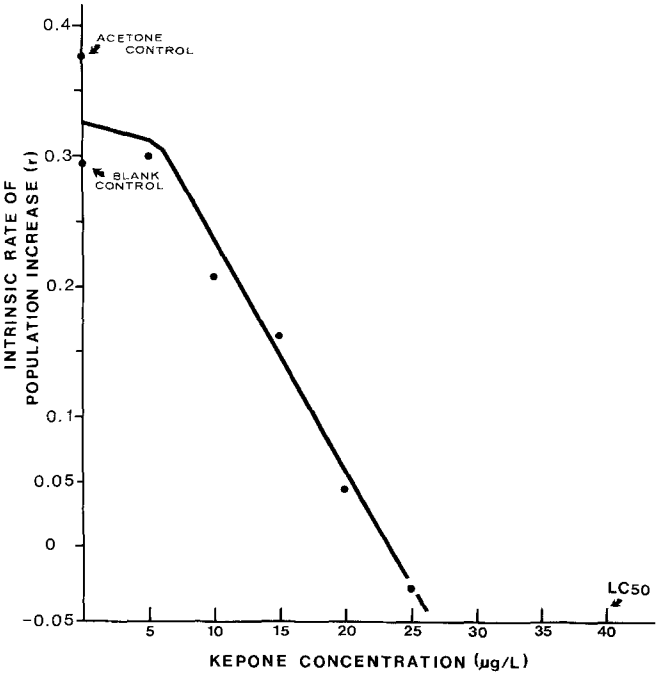

Fig. 2. *Eurytemora affinis.* Survivorship curves (l_x) for *E. affinis* in six concentrations of Kepone and two controls

Reproduction

Fecundity, or number of female offspring per female of a given age (m_x), was calculated by assuming a 1 : 1 sex ratio of nauplii. Fecundity appeared to be enhanced in acetone compared to blank control, and was not adversely affected at 5 $\mu g\, l^{-1}$ Kepone. Copepods at higher Kepone concentrations had reduced reproduction (Table 2). Mean brood size was significantly higher in acetone control than all other treatments (Table 1, $P < 0.05$, Kruskal-Wallis *a posteriori* test). Blank control and 5 $\mu g\, l^{-1}$ did not differ from one another but did from all others. Brood size was significantly greater at 15 than at 10 and 20 $\mu g\, l^{-1}$, an inexplicable result. Small sample size precluded testing brood size at 25 $\mu g\, l^{-1}$ against other treatments. No individuals survived to reproductive age at 30 $\mu g\, l^{-1}$ (Table 1).

The mean first day of reproduction increased significantly with increasing Kepone concentration, based on a Kruskal-Wallis rank test and multiple comparisons between treatments. Females at 5, 10, 15 and 20 $\mu g\, l^{-1}$ reproduced significantly ($\alpha = 0.10$) later than in acetone control, while only the 15 and 20 $\mu g\, l^{-1}$ treatments showed

Table 2. *Eurytemora affinis.* Age-specific fecundity (M_x) in various kepone treatments. Observations are female offspring per female per day

(Days)	Blank control	Acetone control	Kepone concentration				
			5 $\mu g\ l^{-1}$	10 $\mu g\ l^{-1}$	15 $\mu g\ l^{-1}$	20 $\mu g\ l^{-1}$	25 $\mu g\ l^{-1}$
10	0	5.88	0.00	0.00	0.00	0.00	0.00
11	4.68	15.88	1.22	0.28	0.00	0.00	0.00
12	8.85	24.60	19.19	2.05	0.00	0.00	0.00
13	9.25	19.30	0.00	3.03	2.37	0.00	0.00
14	6.51	22.85	9.92	4.08	0.00	0.00	0.00
15	8.96	31.98	14.38	5.11	1.72	0.00	0.00
16	10.57	13.90	8.30	4.20	1.31	0.00	0.00
17	7.79	22.45	4.30	3.54	1.92	0.00	1.75
18	12.26	16.81	7.26	7.31	5.29	0.44	0.00
19	2.10	37.02	6.76	3.58	1.07	0.42	0.00
20	19.68	15.47	21.59	4.87	3.43	2.71	0.00
21	0.82	23.68	2.76	6.10	6.14	0.00	5.50
22	16.04	20.03	17.53	3.88	0.00	0.00	
23	4.38	8.08	1.68	7.52	6.04	0.00	
24	3.88	19.76	15.07	5.76	0.45	0.60	
25	12.96	4.82	9.21	3.03	5.09	0.00	
26	2.70	25.53	16.25	3.35	1.90	0.05	
27	3.07	8.61	20.18	1.92	9.85	0.25	
28	2.57	32.68	1.96	2.28	12.55	0.00	
29	7.54	12.50	28.38	1.71	1.55	1.75	
30	3.83	25.47	7.80	2.91	11.50	1.21	
31	3.29	13.79	12.20	6.32	5.33	1.00	
32	8.23	12.57	5.00	0.00	9.22		
33	1.27	28.31	12.00	2.04	3.00		
34	6.19	9.11	16.00	4.23	4.69		
35	2.05	14.88	2.60	0.75	9.21		
36	7.05	4.83	10.25	2.85	2.14		
37	2.44	17.46	0.00	0.35	9.40		
38	3.29	7.21	7.45	2.65	0.00		
39	9.29	10.36	0.00	0.85	9.75		
40	4.91	15.04	2.78	5.61	0.00		
41	7.94	11.04	2.65	8.33	5.13		
42	1.88	7.45	0.00	0.00	7.75		
43	5.79	0.00	0.50	1.50	0.00		
44	1.91	11.75	1.50	0.00	10.13		
45	1.19	2.10		0.81	11.50		
46	5.06	7.75					
47	0.97	0.00					
48	0.90	0.00					
49	4.25	5.21					
50	2.00	0.00					
51	5.91	0.50					
52	0.00						
53	2.91						
54	0.00						
55	3.14						
56	0.70						
57	3.00						
58	0.63						
59	0.00						
60	0.00						

significantly later reproduction than blank control. Females at 5 and 10 $\mu g\ l^{-1}$ reproduced significantly earlier than did those at 20 $\mu g\ l^{-1}$. There is a strong trend for increased time to onset of reproduction with increasing Kepone concentration (Table 1). Total number of broods declined dramatically at concentrations above 10 $\mu g\ l^{-1}$ (Table 1), due to the combined effects of reduced survivorship and delayed onset of reproduction.

The intrinsic rate of natural increase (r) was calculated for each treatment using (1), and plotted against Kepone concentrations (Fig. 2). The acetone control had the highest r, due to enhanced fecundity (Table 2). A Kepone concentration of 5 $\mu g\ l^{-1}$ had no apparent depressive effect on r, but this statistic declined steadily with concentrations of 10 and 15 $\mu g\ l^{-1}$. At 20 $\mu g\ l^{-1}$ potential growth rate was near zero, while at 25 $\mu g\ l^{-1}$ r was negative.

Discussion

Clearly Kepone is lethal to the copepod *Eurytemora affinis* at low concentrations (40 $\mu g\, l^{-1}$). *E. affinis* was similarly sensitive to dieldrin, for which the LC 50 was 23 $\mu g\, l^{-1}$ (Daniels and Allan, 1981).

The acute toxicity of Kepone to grass shrimp (*Palaemonetes pugio*) and blue crab (*Callinectes sapidus*) was estimated to be 121 and >210 $\mu g\, l^{-1}$ respectively (Schimmel and Wilson, 1977). In contrast, Nimmo *et al.* (1977) using a mysid (*Mysidopsis bahia*) found the 96-h LC 50 to be 10.1 $\mu g\, l^{-1}$, and the 19-d LC 50 to be $1 \cdot 4\ \mu g\, l^{-1}$, values lower than we observed for *E. affinis*. The sheepshead minnow (*Cyprinodon variegatus*) had a 96-h LC 50 of 70 $\mu g\, l^{-1}$. However, a 36-d LC 50 for juveniles gave a value of $6 \cdot 7\ \mu g\, l^{-1}$, and the 28-d LC 50 for adults was $1 \cdot 3\ \mu g\, l^{-1}$. Clearly Kepone is toxic at low concentrations, especially when chronic effects are considered.

Ecological sensitivity to chronic exposure may be indicated by reduced survivorship, impaired reproduction or some combination thereof. Survivorship data alone require the least effort to collect, and certainly reveal a response of *Eurytemora affinis* to Kepone at 10 and 15 $\mu g\, l^{-1}$. However, one could conclude that this copepod was not seriously affected until 20 $\mu g\, l^{-1}$ was reached. Brood size declined sharply between 5 and 10 $\mu g\, l^{-1}$, while number of broods declined sharply between 10 and 15 $\mu g\, l^{-1}$ (Table 1). While these several measures are not seriously inconsistent, it is clear that survivorship and reproductive characteristics may indicate different critical levels for chronic effects.

Life table estimates of intrinsic rate of natural increase have been used by ecologists since Birch (1948) introduced the approach to assess the growth rate potential of a population in various environments. It has been used as a toxicity bioassay in few instances (Marshall 1962, 1978; Hummon, 1974; Winner and Farrell, 1976; Daniels and Allan, 1981). The method has two main advantages: it is a convenient experimental protocol, and it provides a single statistic *r*, which integrates survivorship and fecundity information and assesses the population's capacity for increase under given environmental conditions. As Fig. 2 shows, any concentration of Kepone above 5 $\mu g\, l^{-1}$ impairs growth potential; 20 $\mu g\, l^{-1}$ (50% of the 48-h LD 50) reduces *r* to nearly zero. In nature, populations grow at rates approximating the laboratory estimate of growth potential only when conditions are especially favorable and mortality very low. A population whose maximum attainable *r* was reduced to near-zero by chronic stress would rapidly disappear.

The manner by which various components of survivorship and fecundity contribute to the observed (Fig. 2) decline in *r* is summarized in Table 3. Theoretical analyses of the importance of these components (Allan, 1976) suggest that delay in onset of reproduction can be more critical than reduction in number of young produced, but clearly all are contributing here.

Life table evaluation of the response of *Eurytemora affinis* to dieldrin (Daniels and Allan, 1981) revealed a similar pattern of reduction in *r*, at an even lower pesticide concentration relative to the 48-h LC 50. In that study the principal effect of chronic exposure was on survivorship; copepods which survived evidenced slightly lengthened pre-reproductive time at higher dieldrin concentrations, but no reduction in brood size.

A possible objection to this approach is the length of time required for complete life table analysis (up to 60 d). Daniels and Allan (1981) showed that accurate estimates of *r* could be obtained with experiments of shorter duration due to the greater contribution to *r* made by reproduction early in life (Fisher, 1958). For *Eurytemora affinis* raised at 18 °C in various dieldrin concentrations, the first 30 d of the life tables were sufficient. This was true for the present experiments as well. Because reproduction commences earlier at 20 °C, the temperature used here, we also calculated *r* based on the first 21 d of life, and regressed this on *r* calculated from the entire data set. The equation was not significantly different from $Y = X$, with $r^2 = 0.996$. Thus the experiments could have been terminated after 21 d, with no significant, or even discernible, difference in results.

Of the three hypotheses suggested by Daniels and Allan (1981) concerning the effects of a toxin on intrinsic rate of natural increase, the results (Fig. 2) are most consistent with the second and third hypotheses. There was a substantial sub-lethal impairment of ecological

Table 3. Contribution of several life table components to decline in intrinsic rate of natural increase at higher Kepone concentration

Increase in Kepone concentration ($\mu g\, l^{-1}$)	% of LC50	l_x-Effect on *r*			
			Age at first reproduction	Brood size	Number of broods
0→5	12.5	none	none	none	none
5→10	25	none	small delay	large reduction	none
10→15	37.5	reduced	delayed	none (reversed)	large reduction
15→20	50	minor reduction	delayed	large reduction	large reduction
20→25	62.5	reduced	none	none	large reduction

growth potential. The interpretation depends on the exact position of the LC 50 value, which in turn may be affected by the length of the acute test. We used a 48-h period; had we chosen a 72 or 96-h acute test time the LC 50 would have been reduced. However, inspection of survivorship curves (Fig. 1) shows that even at 96 h the LC 50 is between 25 and 30 $\mu g\, l^{-1}$. Hence our conclusion of a reduction in r well below the LC 50 remains valid.

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