213932,213929
RECORD NO.

109301
SHAUGHNESSEY NO.

REVIEW NO.

EEB REVIEW

DATE: IN 02/10/88 OUT 02/26/88

FILE OR REG. NO 352-485, 352-502

PETITION OR EXP. NO

DATE OF SUBMISSION: 01/25/88

DATE RECEIVED BY HED: 02/05/88

RD REQUESTED COMPLETION DATE: 04/05/88

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RD ACTION CODE/ TYPE OF REVIEW: 570

TYPE PRODUCT(S): Synthetic pyrethroid

ACCESSION NUMBER(S): 404440-01-02-03

PRODUCT MANAGER: G. Larocca (15)

PRODUCT NAME(S): Asana/Pydrin/Fenvalerate

COMPANY NAME: E.I. DuPont De Nemours

PURPOSE OF SUBMISSION: Submission of Daphnid studies
CHRONIC
ACUTE

SHAUGHNESSEY NO.	CHEMICAL AND FORMULATION	%A.I.
109301	Fenvalerate	98.6%

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of fenvalerate toxicity studies on Daphnia

FROM: David Johnson, Ph.D.
Fishery Biologist
Ecological Effects Branch

David Johnson 7 March 88

THROUGH: Otto Gutenson, Acting Head-Section 4
Ecological Effects Branch
Hazard Evaluation Division (TS-769C)

3/8/88

THROUGH: Henry Craven, Acting Chief
Ecological Effects Branch
Hazard Evaluation Division (TS-769C)

Henry T. Craven

TO: G. Larocca, Product Manager 15
Registration Division

E.I. Dupont De Nemours and Company, Inc. has submitted four Daphnid studies for review. Our reviews of these studies, and our conclusions are summarized below.

1. Hutton, D.G., 1987. Daphnia magna static acute 48-hour EC50 of technical Asana insecticide. Report number: 402-87, Prepared by E.I. du Pont de Nemours and Company, Inc., Haskell Laboratory, Newark, DE. Submitted by E.I. du Pont de Nemours and Company, Inc. Newark, DE. Assession No: 404440-02.

The technical grade of Asana^{tm} (fenvalerate) was found to be extremely toxic to Daphnia, with a 48h LC50 of 0.9 ppb. This study is scientifically sound.

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2. Hutton, D.G., 1987. Chronic toxicity of technical Asana insecticide to Daphnia magna. Report number: 589-87, Prepared by E.I. du Pont de Nemours and Company, Inc., Haskell Laboratory, Newark, DE. Submitted by E.I. du Pont de Nemours and Company, Inc. Newark, DE. Assession No: 404440-01.

The study is judged to be core, but the deviations in dissolved oxygen are noted to be a significant shortcoming. The NOEL (52 nanograms/L) and the MATC ($\geq$52ng/L & $\leq$79ng/L) indicate that fenvalerate is extremely toxic to daphnids on a chronic basis.

3. Hutton, D. G. 1987. Fed Daphnia magna static acute 48-hour EC50 of technical asana insecticide. Prepared by E.I. du Pont de Nemours and Company, Inc., Haskell Laboratory, Newark, DE. Submitted by E.I. du Pont de Nemours and Company, Inc. Newark, DE. Assession No: 404440-03.

This study does not address any current Guideline standard, and therefore was not reviewed.

4. McKee, M.J. and C.O. Knowles. 1986. Effects of fenvalerate on biochemical parameters, survival, and reproduction of Daphnia magna. Ecotoxicology and Environmental Safety 12: 70-84. Submitted by E.I. du Pont de Nemours and Company, Inc. Newark, DE. Assession No: 404493-01.

This study does not address any current Guideline standard, and therefore was not reviewed. EEB will retain a copy of this report as supplemental data for our files.

B:fenval.der

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DATA EVALUATION RECORD

1. CHEMICAL: fenvalerate (Asana) SN:109301

2. TEST MATERIAL: TGAI 98.6%

3. STUDY/ACTION TYPE: Acute Toxicity on Freshwater Invertebrate species: Daphnia magna

4. STUDY IDENTIFICATION:

Hutton, D.G., 1987. Daphnia magna static acute 48-hour EC50 of technical Asana insecticide. Report number: 402-87, Prepared by E.I. du Pont de Nemours and Company, Inc., Haskell Laboratory, Newark, DE. Submitted by E.I. du Pont de Nemours and Company, Inc. Newark, DE. Assession No: 404440-02.

5. REVIEWED BY:

David Johnson, Fishery Biologist
Ecological Effects Branch

Signature: David Johnson
Date: 7 March 88

6. APPROVED BY:

Otto Gutenson, Acting Head Section
Ecological Effects Branch
Hazard Evaluation Division

Signature: Otto Gutenson
Date: 3/8/88

7. CONCLUSIONS:

The technical grade of Asana^{tm} (fenvalerate) was found to be extremely toxic to Daphnia, with a 48h LC50 of 0.9 ppb. This study is scientifically sound and satisfies Guideline requirements.

8. RECOMMENDATION: N/A

9. BACKGROUND: N/A

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10. DISCUSSION OF INDIVIDUAL STUDIES OR TESTS: N/A

11. METHODS AND MATERIALS:

Species. Daphnia magna

Size/Age/Physical Condition. Daphnids less than 24h in age were selected from an established culture.

Source. The Daphnids were cultured from laboratory stock.

Food prior to the test. not specified

Test water

Temperature: 20°C

Water source and chemistry: reconstituted hard water

The properties of the water are: Hardness- 177mg/L $CaCO_3$, pH- 8.3

Aeration: Test solutions were not aerated.

Solvent: acetone

Controls: Controls were run concurrent with the test.

Test System.

Vessel Size/Volume: 250ml/200ml of test solution

Vessel Construction: Glass

Photoperiod: 16h-light/8h-dark

Loading: ≈1 daphnid/20ml solution

Number of Daphnids/concentration.10/vessel x 2 rep. = 20

Test Levels:
nominal:4.0, 2.4, 1.4, .86, .52, .31, .19, .11 µg/L
measured: not measured

Toxic signs. mortality, inability to swim

Statistical analysis.
Probit analysis was used to estimate the EC50 and confidence interval.

12. REPORTED RESULTS:

Chemical analysis of dilution water included

Data
The data were included with the study report.

Analysis of Test Concentrations Chemical analyses of the test concentrations were not performed.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

Technical Asana was found to exhibit very high acute toxicity.

48h EC50(95%CL): 0.9 µg/L (0.7-1.2) Slope: not specified
NOEL: 48h EC0: 0.11 µg/L

24h EC50: 3.7 ppb NOEL 24h: .52 ppb

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY:

A. Test Procedure.
This study was performed under conditions that generally comply with current Guideline standards.

B. Statistical Analysis.

EEB agrees with the statistical method. The study author's calculations match the reviewer's. The calculations are appended.

C. Results/Discussion.

The study is judged to be scientifically sound and acceptable for use in a hazard assessment.

D. Adequacy of the Study.
1. Category: core

2. Rationale: N/A

3. Remedy: N/A

15. COMPLETION OF ONE LINER 26 February 1988

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d.johnson FENVALERATE DAPHNIA 02-26-88

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
4	20	20	100	9.536742E-05
2.4	20	15	75	2.069473
1.4	20	12	60.00001	25.17223
.86	20	11	55	41.19014
.52	20	7	35	13.1588
.31	20	3	15	.1288414
.19	20	1	5	2.002716E-03
.11	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .31 AND 2.4 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .7592603

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
7	5.135018E-02	.8496861	.688847	1.075918

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	6.654026E-02	1	.638863

SLOPE = 2.355707

95 PERCENT CONFIDENCE LIMITS = 1.748043 AND 2.963371

LC50 = .8925751

95 PERCENT CONFIDENCE LIMITS = .7012163 AND 1.149892

LC10 = .2579458

95 PERCENT CONFIDENCE LIMITS = .1586533 AND .3556047

****

TABLE 1

RESULTS OF A 48-HOUR STATIC ACUTE TOXICITY TEST WITH UNFED Daphnia magna EXPOSED TO TECHNICAL ASANA® INSECTICIDE IN ACETONE (H-16,626) (MR 4581-474)

Nominal Test Concentrations (mg/L)	Acetone added mL/L	Cumulative Observed Immobility (%) 24 Hours A*	24 Hours B*	48 Hours A*	48 Hours B*
0.0040	0.060	70	40	100	100
0.0024	0.036	30	20	70	80
0.00144	0.0215	10	30	60	60
0.00086	0.0128	10	20	60	50
0.00052	0.0078	0	0	50	20
0.00031	0.0048	0	0	10	20
0.00019	0.0028	0	0	10	0
0.00011	0.0018	0	0	0	0
Acetone Control	0.06	0	0	0	0
H_2O Control	-	0	0	0	0

* Replicate exposure chambers containing ten daphnids each.

EC50 Calculations

	EC50 mg/L	95% Confidence Limits, mg/L Lower	Upper
24 Hrs.	0.0037	0.0027	0.0071
48 Hrs.	0.00090	0.00070	0.00116

TABLE 2

RESULTS OF PHYSICAL AND CHEMICAL PARAMETERS
MEASURED DURING A 48-HOUR STATIC ACUTE TOXICITY TEST WITH UNFED
Daphnia magna EXPOSED TO TECHNICAL ASANA® INSECTICIDE IN ACETONE (H-16,626)
(MR 4581-474)

Physical/Chemical Parameters	Nominal Test Concentrations (mg/L) 0.004 (High)	0.00086 (Medium)	0.00011 (Low)	Controls H_2O	Controls Acetone
Dissolved Oxygen (mg/L)					
0 Hr.	8.3	8.3	8.3	8.4	8.3
48 Hr.	8.4	8.4	8.3	8.4	8.3
pH					
0 Hr.	8.3	8.3	8.3	8.3	8.3
48 Hr.	8.2	8.2	8.2	8.2	8.2

Water Chemistry on the H_2O Control at 0 Hours

Total Alkalinity (mg/L as $CaCO_3$)	EDTA Hardness (mg/L as $CaCO_3$)	Conductivity (µmhos/cm)
114	177	560

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DATA EVALUATION RECORD

1. CHEMICAL: fenvalerate (Asana) SN:109301

2. TEST MATERIAL: TGAI 98.6%

3. STUDY/ACTION TYPE: 21-day Chronic Life-cycle Test
species: Daphnia magna

4. STUDY IDENTIFICATION:

Hutton, D.G., 1987. Chronic toxicity of technical Asana insecticide to Daphnia magna. Report number: 589-87, Prepared by E.I. du Pont de Nemours and Company, Inc., Haskell Laboratory, Newark, DE. Submitted by E.I. du Pont de Nemours and Company, Inc. Newark, DE. Assession No: 404440-01.

5. REVIEWED BY:

David Johnson, Fishery Biologist
Ecological Effects Branch

Signature: David Johnson
Date: 8 March 88

6. APPROVED BY:

Otto Gutenson, Acting Head Section
Ecological Effects Branch
Hazard Evaluation Division

Signature: Otto Gutenson
Date: 3/25/88

7. CONCLUSIONS: The study is judged to be scientifically sound, but the deviations in dissolved oxygen are noted to be a significant shortcoming. The NOEL (52 nanograms/L) and the MATC ($\geq$52ng/L & $\leq$79ng/L) indicate that fenvalerate is extremely toxic to daphnids on a chronic basis.

8. RECOMMENDATION: N/A

9. BACKGROUND: N/A

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10. DISCUSSION OF INDIVIDUAL STUDIES OR TESTS: N/A

11. METHODS AND MATERIALS:

Species. Daphnia magna

Size/Age/Physical Condition. Daphnids less than 24h in age were selected from an established culture.

Source. The Daphnids were cultured from laboratory stock.

Food during the test. trout chow, yeast, and alfalfa

Test water

Temperature: 20°C

Water source and chemistry: reconstituted hard water

The properties of the water are:
Hardness- 179mg/L $CaCO_3$
pH- 8.5(new solution), 7.6(old solution)
Dissolved Oxygen- 90%(new solution)
48%(old solutions with acetone)
range 3.0-7.1 mg/L

Aeration: Test solutions were not aerated.

Solvent: acetone

Controls: Controls were run concurrent with the test.

Test System.

Vessel Size/Volume: 250ml/200ml of test solution

Vessel Construction: Glass

Photoperiod: 16h-light/8h-dark

Ten 250ml beakers are used for each toxicant concentration: (a) 7 beakers contain 1 daphnid for collection of data on survival, growth, and reproduction; (b) three beakers at each concentration contain five daphnids each for collection of data on survival only.

Test Levels:
nominal: 30, 60, 120, 250, 500, 1000 nanograms/L
measured: 25, 52, 79, 150, 450, 1200 nanograms/L

Toxic signs. variations in survival, growth, and reproduction

Statistical analysis.
For the chronic toxicity test results, the data from each test concentration were compared to the solvent control using Dunett's test.

12. REPORTED RESULTS:

Chemical Parameters of the Test Solutions included

Data
The data were included with the study report.

Analysis of Test Concentrations Chemical analyses of the test concentrations were performed.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:
At measured concentrations of 52 ng/L and below, there were no statistically significant differences between control and treatment groups for any measured parameter. At 79 ng/L measured concentration, the total number of young, young/day, and growth, were significantly less than control values. At 150 ng/L all test parameters were significantly different from control values.

NOEL: EC0: 52 nanograms/L $52ng/L \leq MATC \leq 79ng/L$

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY:

A. Test Procedure.
This study was performed under conditions that generally comply with current Guideline standards, with the one exception that Dissolved Oxygen (DO) was low.

B. Statistical Analysis.

EEB agrees with the statistical method. The study author's calculations match the reviewer's.

C. Results/Discussion.

The study is judged to be sound, although DO was low in several test solutions.

D. Adequacy of the Study: Category: core

15. COMPLETION OF ONE LINER 26 February 1988

Pydrin ecological effects review

Page _____ is not included in this copy.

Pages 13 through 22 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

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ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 12, 70–84 (1986)

Effects of Fenvalerate on Biochemical Parameters, Survival, and Reproduction of *Daphnia magna*

MICHAEL J. MCKEE AND CHARLES O. KNOWLES[1]

Department of Entomology, University of Missouri, Columbia, Missouri 65211

Received February 10, 1986

Daphnia magna were exposed to fenvalerate at nominal concentrations of 0.5, 0.25, 0.13, 0.06, and 0.03 μg/liter for 21 days. On Days 7 and 21 of exposure, levels of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), adenosine diphosphate (ADP), adenosine triphosphate (ATP), glycogen, and lipid were measured, and the results were related to survival and reproduction during the 21-day test period. Survival was not significantly ($\alpha = 0.05$) affected by the 21-day exposure; however, reproduction was reduced at fenvalerate concentrations of 0.25 and 0.5 μg/liter. On Day 7, protein, RNA, ADP, caloric equivalents, and glycogen were also significantly reduced at fenvalerate concentrations of 0.25 and 0.5 μg/liter. Thus, these biochemical parameters identified the same no observable effect concentration (NOEC) as did reproduction. Variables derived from biochemical parameters were related to reduced protein growth and reproduction. Decreases in protein/RNA and glycogen/lipid ratios and increases in ADP/DNA, ADP/ATP, protein/RNA/DNA, and lipid/DNA ratios were observed at Day 7 for those daphnia exposed to 0.5 μg/liter of fenvalerate. The only derived variable that reflected the reduced protein growth at Day 7 in daphnia exposed to 0.25 μg/liter of fenvalerate was the glycogen/lipid ratio. Biochemical determinations at Day 21 indicated that the organisms exposed to 0.25 μg/liter of fenvalerate were not different from controls, whereas those exposed to 0.5 μg/liter were still affected.

INTRODUCTION

In the past decade a considerable amount of research has been devoted to studying biochemical indicators of stress in aquatic organisms (Barrett and Rosenberg, 1981; Pickering, 1981; Ivanovici and Wiebe, 1981; Livingstone, 1982; Giesy *et al.*, 1983; Sheehan, 1984; Neff, 1985). One goal of this research is to develop biochemical indicators that aid in understanding and predicting chronic toxicant effects on survival, growth, and reproduction of aquatic organisms in the laboratory. Another goal is to use the biochemical indicators to assess the "health" of aquatic organisms *in situ*. The laboratory component of biochemical indicator research may provide short-term alternatives to chronic toxicity tests currently required by regulatory agencies and, in addition, provide basic information that will be helpful in assessing their utility in field applications. The present study was directed at the laboratory component of bioindicator research.

Although many laboratory studies of the biochemical effects of toxicants in aquatic organisms have been conducted, the relationship between these effects and whole organism responses such as survival, growth, and reproduction need further elucidation (Mehrle and Mayer, 1980, 1985). Barron and Adelman (1984) found that

[1] To whom correspondence should be addressed.

0147-6513/86 $3.00

changes in nucleic acid and protein content were significantly correlated with reduced growth in fathead minnows (*Pimephales promelas*) exposed to several different toxicants. With regard to aquatic invertebrates, McKee and Knowles (1986) showed that reduced protein growth was associated with increased protein/RNA/DNA ratios in *Daphnia magna* during chronic exposure to chlordecone. They also found increases in the concentration of adenylate nucleotides relative to the concentration of DNA. The present investigation reports the relationships among total protein, RNA, DNA, adenosine diphosphate (ADP), adenosine triphosphate (ATP), glycogen, lipid, survival, and reproduction in *D. magna* exposed to the pyrethroid insecticide fenvalerate for 21 days.

MATERIALS AND METHODS

Organisms. Daphnia magna were cultured in well water at 22°C + 2°, 16:8 light: dark photoperiod, approximately 80 ft-candle lighting and at a density of 20 adults/liter. The chemistry of the well water was: hardness (as $CaCO_3$), 300 mg/liter; alkalinity (as $CaCO_3$), 250 mg/liter; pH, 7.9; conductivity, 473 μmho/cm; dissolved oxygen, saturation. The daphnia were fed approximately 1.2×10^{-7} cells of *Selenastrum capricornutum* and 10 mg of PR-11/yeast mix each day. The culture was cleaned three times each week.

Test system. The test system was patterned after the procedure recommended by the Society of American Standards for Testing and Materials (ASTM, 1985). A Micromedic pumping system was used to meter the toxicant to a mixing box, and a proportional diluter system was used to deliver the mixture of toxicant and well water to the test chambers (Mount and Brungs, 1967). The stock solution was prepared in acetone, and control daphnia were exposed to a level of acetone equivalent to the amount of acetone in the highest concentration. Solvent levels were maintained below 50 μl/liter, and a separate experiment was conducted to ensure that the acetone produced no observable effects on survival, reproduction, or the biochemical parameters.

The test system consisted of five toxicant concentrations and one solvent control. The test was conducted under conditions similar to the laboratory culture with the exception that flow-through rather than static conditions were used. The water was from a deep well and was passed through a physical aeration apparatus prior to use. Each test concentration was comprised of four replicate 1-liter beakers which received 3.6 turnovers of test solution per day. The study was initiated by adding 20 first instar daphnia (<24 hr old) to each replicate. The daphnia were fed 1.1×10^{7} cells of *Selenastrum capricornutum* and 5 mg of a mixture of yeast and PR-11 trout chow twice a day. The system was cleaned, and reproduction assessed three times each week. Adult daphnia were sampled for biochemical analysis on Days 7 and 21.

Fenvalerate analysis. A composite 100-ml sample from each test concentration was collected on Days 0, 7, 14 and 21. Three samples of control water were taken, one for background and two for quality control fortifications. The water was extracted three times with 5 ml of *n*-hexane, and the extract was dried with anhydrous sodium sulfate. The hexane extract was passed through a column packed with activated florisil, and the fenvalerate in the hexane was analyzed by gas chromatography using electron capture detection. The conditions were as follows:

Detector	ECD at 320°C
Injector	220°C
Column	10-m × 0.25-mm i.d. DBS
Flow	H_2 at 12 psi
Program	150°–270°C
	at 8°/min
	Hold 10 min
Attenuation	32×10^{-11}

Isolation of biomolecules. Day 7 samples were collected by pipetting 10 adult daphnia from each of four replicates per concentration making a total of 24 samples (control + five treatments). Samples on Day 21 were comprised of remaining adult daphnia. Daphnia from each replicate were placed in a 2-ml Wheaton homogenizing tube, and the water was removed by pipet. One hundred microliters of 1 *N* perchloric acid (PCA) were added, and the mixture was immediately diluted to a volume of 0.5 ml with deionized water and homogenized with a motor-driven Teflon pestle. The sample was placed in a microcentrifuge tube (1.5 ml), capped, and kept ice cold throughout subsequent procedures. Following homogenization, a 50-μl aliquot was removed and added to 950 μl of 1 *N* NaOH. It was stored at −20°C and was analyzed for total protein as described later.

To isolate the other biomolecules, the Schmidt–Thannhauser method described by Munroe and Fleck (1966) and modified by Shibko *et al.* (1967) and Vytasek (1982) was used. The homogenate was centrifuged for 10 min at 10,000 × *g* at 4°C. The supernatant, which contained the adenylate nucleotides (Munroe and Fleck, 1966) and glycogen (Shibko *et al.*, 1967), was neutralized with $KHCO_3$ and stored at −20°C. The pellet was washed twice with 0.2 *N* PCA, and the supernatants were discarded. It was resuspended in 0.5 ml of 0.3 *N* NaOH and stored overnight at −20°C. The following day the sample was incubated at 37° for 60 min and adjusted to about 0.2 *N* PCA by adding 25 μl of 10 *N* PCA. It was centrifuged as described above, and the pellet was washed twice with 0.5-ml aliquots of 0.2 *N* PCA. The supernatants were combined and comprised the RNA fraction. Lipid material in the pellet was extracted with ethanol–ether (3:1) (Vytasek, 1982). The pellet remaining contained the DNA (Vytasek, 1982).

Experiments were performed to ensure the validity of this protocol, and the results indicated no significant differences between frozen and fresh samples.

Analysis of biomolecules. The protein samples in 1 *N* NaOH were resolubilized by incubating at 100°C for 60 min prior to analysis. Protein was analyzed by the method of Lowry *et al.* (1951) as modified by Miller (1959) using bovine serum albumin as standard (Sigma Chemical Co., St. Louis, Mo.). Initially an experiment was performed to determine the amount of protein present in the various fractions. The results expressed as mean percent and standard deviation of the total homogenate were: nucleotide fraction, 1.1 ± 0.5; RNA fraction, 6.2 ± 0.7; DNA fraction, 10.1 ± 2.5; and final extracted pellet, 84.4 ± 6.8. Summation of these fractions was within 2% of the value obtained by assaying the initial total homogenate.

RNA was initially quantified by UV detection at 260 nm (Dagg and Littlepage, 1972) and by the orcinol reaction (Schneider, 1957). Since preliminary experiments indicated less than 5% difference between the two assays, all subsequent studies were

performed using the UV detection method. RNA from calf liver (Sigma) was used as the standard.

Deoxyribonucleic acid was determined by the fluorometric method of Kissane and Robbins (1958) as modified by Vytasek (1982). The pellet that remained in the microcentrifuge tube following lipid extraction was resuspended in 0.2 ml of 0.5 *N* PCA and hydrolyzed by incubation at 80°C for 15 min. After cooling, 0.2 ml of the 3,5-diaminobenzoic acid reagent were added, and the reaction mixture was incubated at 37°C for 60 min (Vytasek, 1982). The sample was centrifuged for 5 min at 10,000 × *g* in a microcentrifuge (Fisher Scientific, Inc., St. Louis, Mo.). The supernatant was collected, and 2.5 ml of 1 *N* HCl were added. Fluorescence was measured on a Model 110 Turner Fluorometer (G. K. Turner Associates, Inc., Palo Alto, Calif.) using a No. 405 excitation filter and a No. 8 yellow emission filter. DNA from calf thymus was used as standard (sodium salt, Sigma).

Glycogen present in the neutralized nucleotide fraction was isolated by precipitation using 5 vol of absolute ethanol. The excess solvent was evaporated in a vacuum desiccator, and the glycogen pellet was resuspended in 0.2 ml of water. The carbohydrate content was determined with the anthrone reagent as described by Roe and Dailey (1966) using purified glycogen from oysters (Sigma) as standard.

Lipid material was extracted twice with 0.5 ml of a mixture of ethanol and ether (3:1), and the combined extract was stored at −20°C until assayed. A 0.2-ml aliquot of the extract was assayed using the micromethod described by Pande *et al.* (1963). Standard and spiking solutions were made using cholesterol (Sigma).

Nucleotides were separated by a modification of the hplc method described by Folley *et al.* (1983) using triethylamine (TEA) as the ion-pairing agent. Solvent A was prepared in deionized double distilled water by adding 1.37 g Na_2HPO_4, 0.54 g NaH_2PO_4, and 10 g of TEA (1%) per liter. The solution was titrated to pH 5.5 using phosphoric acid. Solvent B was similar to solvent A, except that it contained 5 m*M* magnesium sulfate. All buffers were filtered through a 0.4-μm membrane filter and degassed under pressure while emersed in a sonicating bath.

Analysis of the nucleotides was accomplished with a Varian LC 5021 pumping system (Varian Associates, Inc., Palo Alto, Calif.), a MCH 10 Micropak C_{18} reverse phase column (Varian), and Schoeffel Spectroflow Monitor SF770 UV detector (Kratos, Inc., Westwood, N.J.). The conditions for hplc were as follows:

Sample injection: 50 μl
Solvents A: 1% TEA phosphate buffer, pH 5.5
B: 1% TEA phosphate buffer, pH 5.5
5 m*M* $MgSO_4$
Gradient 0–5 min: 100% A, 0% B, flow 1.5 ml/min
5–15 min: 0–50% B (5%/min)
15–16 min: 50–0% B (50%/min)
16–24 min: 100% A, 0% B, flow 2.0 ml/min
24 min: 100% A, 0% B, flow 1.5 ml/min

Detectable limits were in the low nanomolar range as described by Folley *et al.* (1983).

Sodium salts of AMP, ADP, and ATP (Sigma) were used as standards and for spikes. Because of the small sample size involved in the present study and the relatively low amount of AMP present, AMP concentrations, and recoveries were not performed.

TABLE 1

SURVIVAL OF ADULT DAPHNIA AFTER 21 DAYS EXPOSURE TO FENVALERATE

Concentration (μg/liter)	Survival (%)
0	97 (4.7)
0.03	99 (2.2)
0.06	93 (3.9)
0.13	94 (7.4)
0.25	94 (11.3)
0.50	80 (18.7)

Note. Mean and standard deviation of 4 replicates.
* Significantly different from control at $\alpha = 0.05$ using ANOVA and Tukey's HSD test. Analysis was performed on arcsine transformed numbers.

Recovery of biomolecules. Recoveries were determined by spiking three samples with about 50% of the expected tissue concentration with a fourth sample serving as a nonspiked control. Each recovery sample was split into six subsamples containing 10 daphnia each. Recoveries were calculated as a mean and standard deviation of the three original spikes (i.e., the six subunits constituted an experimental unit). Analytical variability was calculated as the mean and standard deviation of the coefficient of variation determined for the six subsamples from each of the four recovery samples. Recovery and analytical variation as the coefficient of variation were 99.1 ± 9.0% and 3.6 ± 1.0% for protein, 88.7 ± 3.0% and 3.6 ± 1.0% for RNA, 86.3 ± 6.5% and 6.6 ± 1.5% for DNA, 84.9 ± 17.0% and 14.3 ± 7.4% for glycogen, 93.3 ± 5.0% and 9.0 ± 2.5% for lipid, 90.0 ± 4.0% and 5.0 ± 2.0% for ADP, and 91.0 ± 4.0% and 4.0 ± 2.0% for ATP.

Statistical analysis. One way analysis of variance was used to determine treatment effects on the various parameters. Mean separation was accomplished with Tukey's honestly significant difference test (Tukey, 1949). The significance level in all instances was probability = 0.05. All statistical calculations were performed by the statistical software package, Systat (Systat, Inc., Evanston, Ill.). Each sample value reported is the mean of 4 replicates except Day 7 at 0.03 and 0.5 μg/liter for ADP and ATP, $n = 3$; Day 21 at 0 and 0.5 μg/liter for all parameters, $n = 3$; Day 21 at all concentrations for ADP and ATP, $n = 3$.

RESULTS

Numerous attempts were made to clean up the extracted fenvalerate samples for glc analysis. The 100-ml samples contained a maximum of 50 ng of fenvalerate. Although the detectable limits were in the low nanogram range, background interference precluded detection of fenvalerate. Therefore, subsequent discussion will refer to the nominal concentrations of fenvalerate in the water which were 0.5, 0.25, 0.13, 0.06, and 0.03 μg/liter.

Survival of *Daphnia magna* was not significantly decreased by fenvalerate at any of the concentrations when compared to controls (Table 1). The highest concentration had more mortality than the others, but the variation between replicates pre-

cluded statistical detection. Reproduction as cumulative young per adult was significantly reduced at 0.25 and 0.5 μg/liter on Days 13 and 21 of fenvalerate exposure (Fig. 1).

Levels of biomolecules per daphnid were affected by exposure to fenvalerate on Days 7 and 21 (Figs. 2–5). Daphnia exposed to 0.25 and 0.5 μg/liter of fenvalerate for 7 days had significantly decreased levels of protein (Fig. 2), RNA (Fig. 3), ADP (Fig. 4), and glycogen (Fig. 5) when compared to control animals. ATP (Fig. 4) and lipid (Fig. 5) levels were significantly reduced only in the 0.5 μg/liter fenvalerate exposure group. DNA levels were decreased at 0.13 and 0.5 μg/liter of fenvalerate but not at the intermediate dose of 0.25 μg/liter (Fig. 3).

The effect of fenvalerate on the content of biomolecules in daphnia was not as pronounced on Day 21 as it was on Day 7 (Figs. 2–5). The 0.5-μg/liter fenvalerate exposure resulted in decreased levels of protein (Fig. 2), RNA (Fig. 3), DNA (Fig. 3), glycogen (Fig. 5), and lipid (Fig. 5) per daphnid. The 0.25-μg/liter fenvalerate exposure did not significantly influence any biochemical parameters at Day 21. Moreover, no effects were observed on ADP and ATP content in any of the test concentrations after 21 days of exposure (Fig. 4).

Fenvalerate exposure effected significant changes in some of the variables derived from protein, nucleic acids, and adenylates on Day 7 (Table 2). The ratio of protein to RNA was lower than control values in daphnia exposed to 0.5 μg/liter, while the ratios of ADP/DNA, ADP/ATP, and protein/RNA/DNA were higher than controls. A significant increase in the RNA/DNA ratio was observed at 0.13 μg/liter of fenvalerate but not at higher concentrations. No significant effects were observed for protein/DNA and ATP/DNA. Variables derived from macromolecular and adenylate content on Day 21 were not significantly affected by fenvalerate exposure (Table 2).

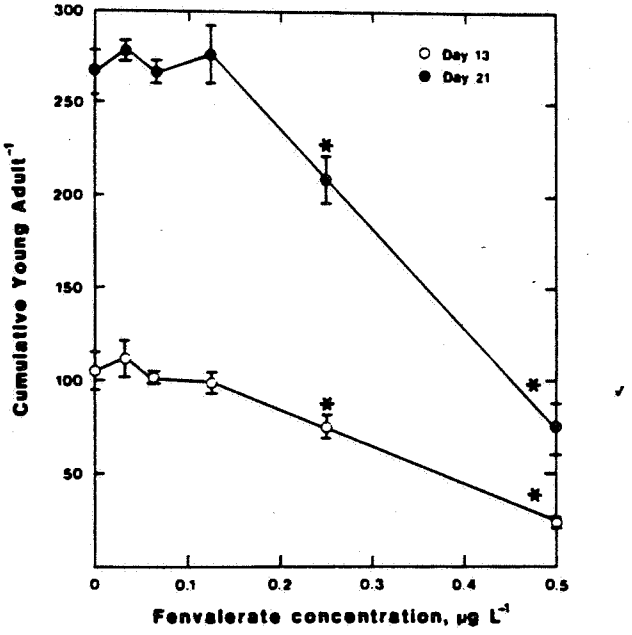

FIG. 1. Effects of fenvalerate on reproduction of *Daphnia magna* after 13 and 21 days of exposure. Mean (±S.D.), $n = 4$, * = significantly different from control.

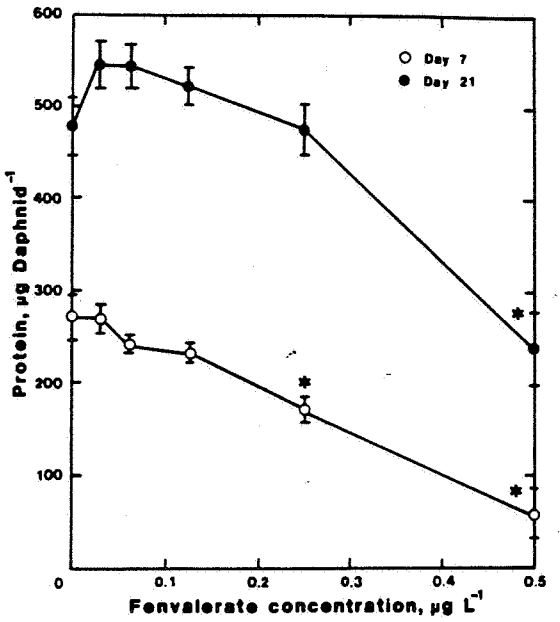

FIG. 2. Effects of fenvalerate on protein levels in *Daphnia magna* after 7 and 21 days of exposure. See Fig. 1 for legend.

Variables derived from glycogen and lipid content of daphnia exposed to fenvalerate are presented in Table 3. On Day 7 glycogen content was significantly decreased relative to lipid content in daphnia exposed to 0.25 and 0.5 µg/liter of fenvalerate. No change was observed for glycogen content relative to DNA; however, an increase in lipid levels relative to DNA was found in the highest test concentration. The glycogen/lipid ratio on Day 21 remained depressed in daphnia exposed to 0.5 µg/liter of fenvalerate but not to 0.25 µg/liter. The glycogen/DNA ratio was also lower than controls at the highest test concentration after 21 days exposure. Lipid to DNA ratios were not significantly different from controls at Day 21 (Table 3).

The caloric equivalent was calculated based on lipid, glycogen, and protein content (Mann and Gallager, 1985). The caloric values used were 9.45 cal/mg of lipid, 4.1 cal/mg of glycogen, and 5.65 cal/mg of protein. Caloric content per daphnid was significantly lower than controls at 0.25 and 0.5 µg/liter of fenvalerate at Day 7 but was significantly reduced only at 0.5 µg/liter at Day 21 (Table 3). No change was observed in caloric content relative to DNA levels at any of the test concentrations for Day 7 or Day 21.

The relative sensitivity of the biochemical parameters, derived variables, and reproduction in daphnia exposed to fenvalerate was determined by recalculating the data as a percent of the control values. The most sensitive parameters are illustrated in Fig. 6. The decrease observed in reproduction over the 21-day period was similar in sensitivity to the decrease observed for protein, RNA, DNA, and glycogen after 7 days of exposure. These parameters along with Day 7 glycogen/lipid ratio and caloric equivalent content all identified the same no observable effect concentration (NOEC) of 0.13 µg/liter of fenvalerate.

DISCUSSION

The toxicity of fenvalerate insecticide to mammals and birds is relatively low (Casida *et al.*, 1983); however, it is toxic to some aquatic organisms (Khan, 1983) with crustaceans being particularly sensitive (McKenney and Hamaker, 1984). The acute toxicity of fenvalerate to *Daphnia magna* and *Gammarus pseudolimnaeus* is 2.1 and 0.43 μg/liter, respectively (Mayer and Ellersieck, 1986). Chronic investigations with fenvalerate and the estuarine grass shrimp, *Palaemonetes pugio*, demonstrated that larval development was significantly affected at a nominal fenvalerate concentration of 1.6 ng/liter (McKenney and Hamaker, 1984). Reproduction of *D. magna* in the present study was sensitive to fenvalerate exposure with a NOEC of 0.13 μg/liter.

As mentioned earlier, one purpose of this study was to determine the utility of monitoring levels of biomolecules as a bioindicator of chronic toxicant effects in the laboratory. Interpretation of these data can be facilitated by separating the effects on

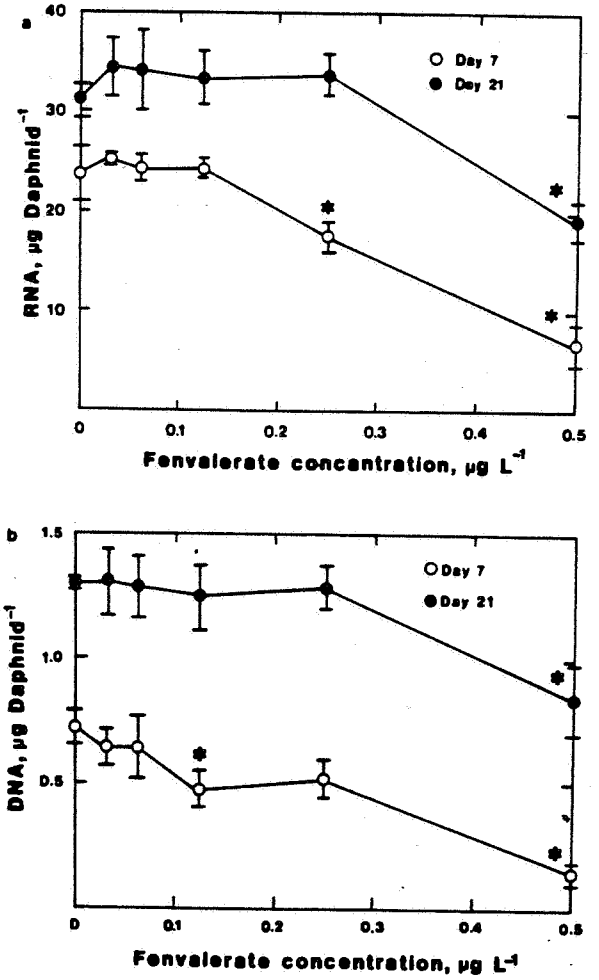

FIG. 3. Effects of fenvalerate on RNA and DNA levels in *Daphnia magna* after 7 and 21 days of exposure. See Fig. 1 for legend.

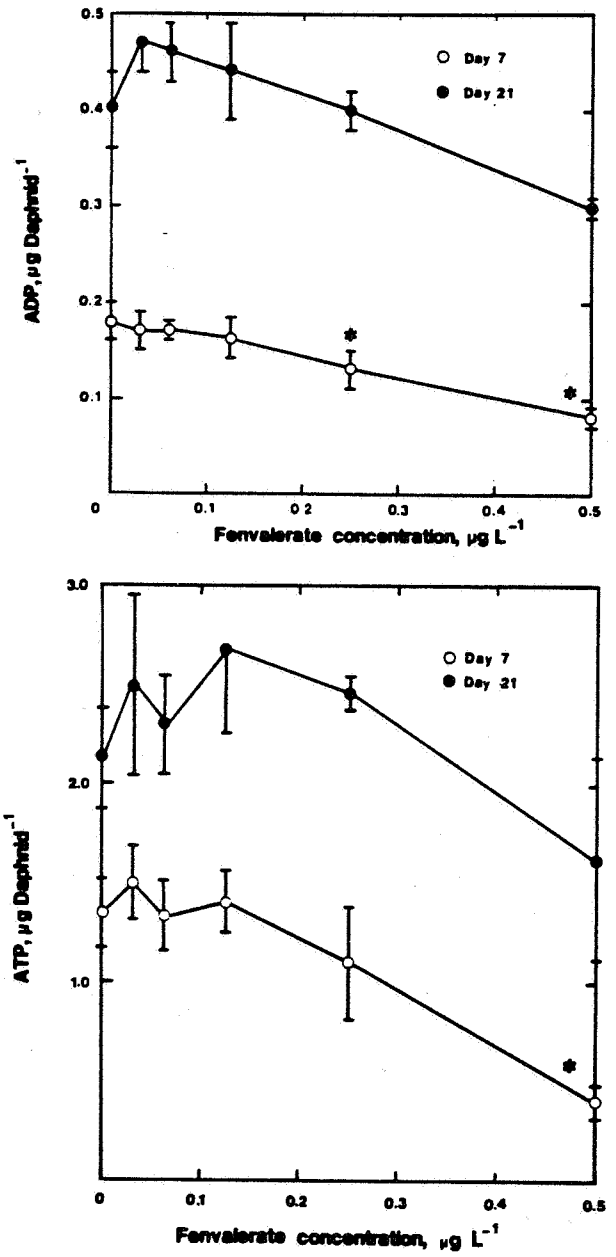

FIG. 4. Effects of fenvalerate on ADP and ATP levels in *Daphnia magna* after 7 and 21 days of exposure. See Fig. 1 for legend.

biomolecules into static and dynamic components. The static component refers to the biomolecular content relative to the individual daphnid. Static indicators are related to the absolute growth of the animal (i.e., larger daphnia have more protein, RNA, etc.), and in some cases can be used to predict biomass. For example, predic-

tion of planktonic biomass using biomolecules has been investigated for protein and nucleic acids (Sutcliff, 1965; Holm-Hansen *et al.*, 1968; Dagg and Littlepage, 1972; Dortch *et al.*, 1983; Bamstedt, 1983) and adenylates (Banse, 1980). The dynamic component refers to the derived variables which may be indicative of the growth rate of an organism. Protein/RNA ratios (Lang *et al.*, 1965) and RNA/DNA ratios (Bulow, 1970; Dortch *et al.*, 1983; Buckley *et al.*, 1985) are examples of dynamic indicators since they have been shown under certain conditions to correlate with growth rate.

The static indicators in the present study were affected more severely at Day 7 than at Day 21. Protein, RNA, ADP, ATP, glycogen, and caloric content were reduced at 0.25 and 0.5 μg/liter of fenvalerate. DNA was significantly reduced at 0.13 μg/liter of fenvalerate, but these data are difficult to interpret since DNA content at 0.25 μg/liter was reduced but not significantly. The reduction in static bioindicators at the two highest concentrations of fenvalerate are probably indicative of reduced growth. Although growth was apparently reduced at 0.25 μg/liter of fenvalerate at Day 7, there

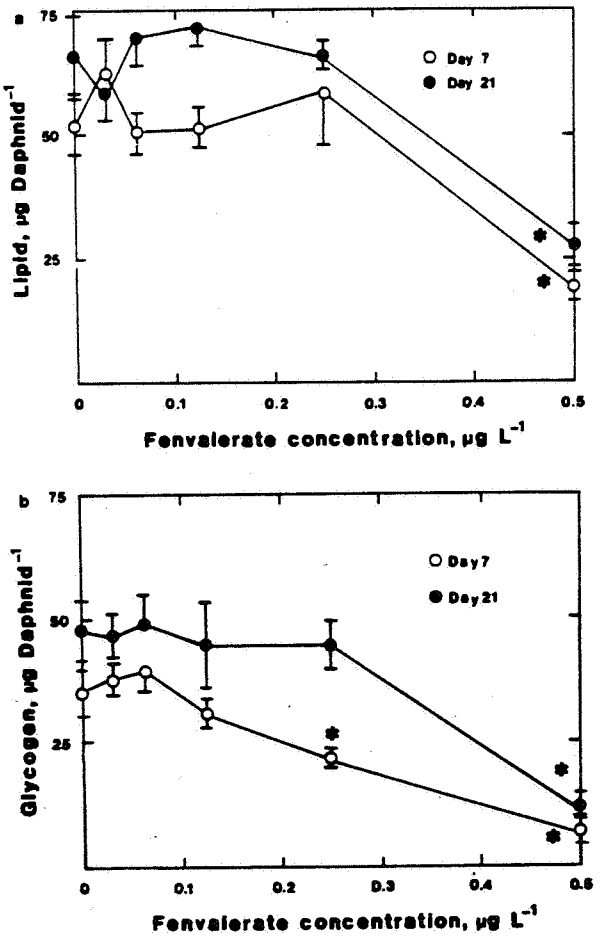

FIG. 5. Effects of fenvalerate on lipid and glycogen levels in *Daphnia magna* after 7 and 21 days of exposure. See Fig. 1 for legend.

TABLE 2

DERIVED VARIABLES FOR PROTEIN, NUCLEIC ACIDS, AND ADENYLATES IN *Daphnia magna* EXPOSED TO FENVALERATE

Fenvalerate concentration (μg/liter)	Derived variables						
	Protein/RNA	Protein/DNA	RNA/DNA	ADP/DNA	ATP/DNA	ADP/DNA	PRO/RNA/DNA
			Day 7				
0.0	11.6 (1.2)	379.40 (27.68)	39.11 (7.42)	0.29 (0.04)	2.21 (0.17)	0.13 (0.10)	18.85 (1.39)
0.03	10.7 (0.9)	422.13 (31.88)	46.71 (8.38)	0.32 (0.05)	2.87 (0.61)	0.11 (0.01)	19.65 (0.86)
0.06	10.0 (0.6)	402.84 (122.70)	46.82 (12.61)	0.33 (0.08)	2.62 (0.74)	0.13 (0.02)	19.78 (6.91)
0.13	9.6 (0.5)	503.08 (92.48)	61.18 (10.38)*	0.41 (0.04)	3.52 (0.22)	0.12 (0.01)	24.47 (4.89)
0.25	9.9 (0.4)	343.74 (67.84)	41.15 (9.14)	0.32 (0.08)	2.57 (0.94)	0.13 (0.02)	22.80 (2.91)
0.50	8.1 (1.1)*	404.85 (98.70)	58.77 (7.99)	0.71 (0.21)*	3.39 (0.35)	0.20 (0.04)*	72.80 (15.80)*
			Day 21				
0.0	15.4 (1.0)	429.96 (37.01)	27.89 (1.43)	0.37 (0.04)	1.90 (0.23)	0.19 (0.01)	13.88 (1.19)
0.03	16.0 (1.9)	494.50 (84.43)	31.07 (5.17)	0.43 (0.07)	2.16 (0.21)	0.20 (0.04)	14.46 (2.89)
0.06	16.1 (1.4)	498.37 (31.77)	30.99 (1.51)	0.44 (0.01)	2.13 (0.30)	0.21 (0.04)	14.90 (2.77)
0.13	15.9 (1.4)	497.30 (65.72)	31.33 (2.02)	0.42 (0.03)	2.46 (0.34)	0.17 (0.01)	15.17 (3.42)
0.25	14.2 (0.5)	432.97 (32.98)	30.58 (3.49)	0.36 (0.04)	2.16 (0.17)	0.17 (0.01)	12.88 (0.25)
0.50	12.4 (1.2)	333.62 (38.17)	27.11 (2.98)	0.49 (0.04)	2.74 (0.68)	0.18 (0.03)	17.63 (3.24)

Note. Mean and standard deviation in micrograms of 4 replicates.
* Significantly different from controls using one-way ANOVA and Tukey's HSD test with $\alpha = 0.05$.

TABLE 3

DERIVED VARIABLES FOR GLYCOGEN, LIPID, AND CALORIC EQUIVALENTS IN *Daphnia magna* EXPOSED TO FENVALERATE

Concentration (μg/liter)	Derived variables[a]				
	Gly/Lip (μg/μg)	Gly/DNA (μg/μg)	Lip/DNA (μg/μg)	CE/individual (cal/daphnid)	CE/DNA (cal/μg)
			Day 7		
0.0	0.68 (0.14)	57.77 (13.41)	84.84 (11.22)	2.17 (0.17)	3.55 (0.29)
0.03	0.61 (0.08)	69.61 (7.86)	114.32 (33.63)	2.27 (0.16)	4.16 (0.24)
0.06	0.78 (0.11)	76.26 (21.02)	98.72 (29.35)	2.00 (0.08)	3.91 (1.17)
0.13	0.60 (0.06)	77.64 (9.85)	129.67 (17.80)	1.93 (0.02)	4.87 (0.78)
0.25	0.38 (0.08)*	50.58 (10.23)	139.48 (48.10)	1.62 (0.12)*	3.81 (0.89)
0.50	0.34 (0.07)*	58.79 (16.43)	176.40 (44.56)*	0.53 (0.16)*	4.58 (0.77)
			Day 21		
0.0	0.74 (0.18)	42.79 (6.51)	59.08 (9.04)	3.52 (0.13)	3.16 (0.15)
0.03	0.80 (0.09)	42.06 (10.45)	52.20 (8.41)	3.83 (0.17)	3.46 (0.59)
0.06	0.71 (0.14)	44.36 (2.73)	64.20 (11.78)	3.94 (0.12)	3.61 (0.27)
0.13	0.63 (0.15)	42.08 (8.67)	67.45 (7.29)	3.82 (0.12)	3.62 (0.45)
0.25	0.68 (0.11)	40.55 (6.86)	59.56 (0.93)	3.50 (0.16)	3.18 (0.21)
0.50	0.36 (0.05)*	16.24 (1.00)*	45.73 (6.02)	1.70 (0.28)*	2.39 (0.27)

Note. Mean and standard deviation of 4 replicates.
* Significantly different from controls using one-way ANOVA and Tukey's HSD test with $\alpha = 0.05$.
[a] Gly, glycogen; Lip, total lipid; cal, calories; CE, caloric equivalents.

was complete recovery by Day 21. This is in agreement with McKenney and Hamaker (1984) who noted that the early larval stages of the grass shrimp were the most sensitive to fenvalerate.

Several of the derived variables appeared to be dynamic indicators of the reduced growth rate observed in daphnia exposed to 0.5 μg/liter of fenvalerate. Reductions were observed at Day 7 of exposure for protein/RNA and glycogen/lipid ratios. The decreased ratio of protein/RNA probably reflects decreased protein synthesis relative to RNA content. These results are in contrast to those of Barron and Adelman (1984) who found that decreased growth rates in fathead minnows exposed to toxicants were associated with an increase in protein/RNA ratio. Glycogen content was significantly reduced relative to lipid content at 0.25 and 0.5 μg/liter of fenvalerate. This was the only derived variable that detected the reduced growth rate at 0.25 μg/liter of fenvalerate. Although glycogen stores were depleted relative to lipid content, the ratio of glycogen to DNA remained unchanged. This indicates that the decreased glycogen/lipid ratio is primarily a result of increased relative amounts of lipid. The glycogen/lipid ratio was significantly lower than controls at 0.5 μg/liter of fenvalerate at Day 21 as was glycogen content relative to DNA. These decreased ratios represent a depletion of glycogen stores during the chronic exposure. Depletion of glycogen has previously been suggested as a bioindicator of chronic stress in aquatic organisms (Carr and Neff, 1981).

Increases were observed for protein/RNA/DNA, ADP/DNA, and ADP/ATP ratios at the highest test concentration on Day 7. McKee and Knowles (1986) also

found that the protein/RNA/DNA ratio was elevated in daphnia exposed to chlordecone for 7 days. This ratio appears to be indicative of the protein synthesis activity since growth was reduced at the same exposure concentrations. ADP content was significantly elevated relative to DNA and ATP levels. Pyrethroids have been shown to affect ATPase activity in some organisms (Clark and Matsumura, 1982; El-Sebae *et al.*, 1981) suggesting that the increased concentration of ADP may be a result of decreased ATP synthesis under stressed conditions. Similar results were not observed in daphnia exposed to chlordecone (McKee and Knowles, 1986). In that study, ATP content relative DNA content was increased, but ADP/DNA and ADP/ATP ratios remained unchanged.

An overall aim for the laboratory component of bioindicator research is to relate toxicant effects to ecologically relevant whole organism responses such as survival, growth, and reproduction. The relationship between growth and the static biomolecular indicators is a close one as discussed earlier. The comparative sensitivity of the various toxicological endpoints as percent of control indicated that static indicators at Day 7 accurately predicted reproduction effects at Day 21 (Fig. 6). This is in agreement with a previous investigation with chlordecone and daphnia where protein content at Day 7 predicted Day 21 effects on reproduction (McKee and Knowles, 1986). The NOEC for fenvalerate based on reproduction of *D. magna* was 0.13 μg/liter. The same NOEC was identified by most static parameters and by the dynamic indicator of glycogen/lipid ratio. In addition to identifying the same NOEC, all of the parameters were similar in sensitivity (Fig. 6).

CONCLUSIONS

Levels of certain biomolecules in *Daphnia magna* can be used to predict chronic effects of fenvalerate. The static component of the research provides many parameters that are as sensitive as reproduction but not more sensitive. The only dynamic variable that had similar sensitivity was the ratio of glycogen to lipid. We are continuing this research with other chemical stressors.

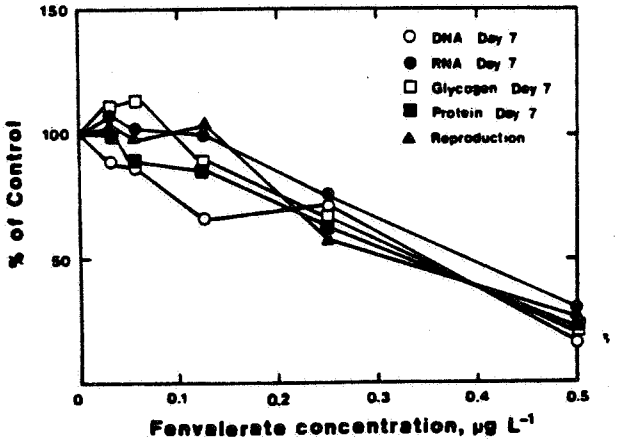

FIG. 6. Most sensitive parameters as percent of control for *Daphnia magna* during chronic exposure to fenvalerate.

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REFERENCES

AMERICAN SOCIETY FOR TESTING AND MATERIALS. (1985). Proposed standard practice for conducting chronic toxicity tests with *Daphnia magna* in a flow-through system. Amer. Soc. Test. Mater. ASTM E-35.21 Draft No. 5 (March 1985).

BAMSTEDT, V. (1983). RNA concentration in zooplankton: Seasonal variation in boreal species. *Mar. Ecol. Prog. Ser.* **11**, 291–297.

BANSE, K. (1980). Microzooplankton interference with ATP estimates of plankton biomass. *J. Plankton Res.* **2**, 235–238.

BARRETT, G. W., AND ROSENBERG, R., eds. (1981). *Stress Effects on Natural Ecosystems*, pp. 1–312. Wiley, New York.

BARRON, M. G., AND ADELMAN, I. R. (1984). Nucleic acid, protein content and growth of larval fish sublethally exposed to various toxicants. *Canad. J. Fish. Aquat. Sci.* **41**, 141–150.

BUCKLEY, L. J., HALAVIK, T. A., LAURENCE, G. C., HAMILTON, S. U., AND YEVICH, P. (1985). Comparative swimming stamina, biochemical composition, backbone mechanical properties, and histopathology of juvenile striped bass from rivers and hatcheries of the eastern United States. *Trans. Amer. Fish. Soc.* **114**, 114–124.

BULOW, F. J. (1970). RNA–DNA ratios as indicators of recent growth rates of a fish. *J. Fish. Res. Board Canad.* **27**, 2343–2349.

CARR, R. S., AND NEFF, J. M. (1981). Biochemical indices of stress in the sandworm *Neanthes virens* (Sars). I. Response to pentachlorophenol. *Aquatic Toxicol.* **1**, 313–327.

CASIDA, J. E., GAMMON, D. W., GLICKMAN, A. H., AND LAWRENCE, L. J. (1983). Mechanism of selective action of pyrethroid insecticides. *Ann. Rev. Pharmacol. Toxicol.* **23**, 413–438.

CLARK, J. M., AND MATSUMURA, F. (1982). Two different types of inhibitory effects of pyrethroids on nerve Ca^{-} and Ca^{+} Mg—ATPase activity in the squid, *Loligo paelei*. *Pestic. Biochem. Physiol.* **18**, 180–190.

DAGG, M. J., AND LITTLEPAGE, J. L. (1972). Relationships between growth rate and RNA, DNA, protein and dry weight in *Artemia salina* and *Euchaeta elongata*. *Mar. Biol.* **17**, 162–170.

DORTCH, Q., ROBERTS, T. L., CLAYTON, J. R., JR., AND AHMED, S. I. (1983). RNA/DNA ratios and DNA concentrations as indicators of growth rate and biomass in planktonic marine organisms. *Mar. Ecol. Prog. Ser.* **13**, 61–71.

EL-SEBAE, H., SHERBY, S. I., AND MANSOUR, N. A. (1981). Gossypol as an inducer or inhibitor in *Spodoptora littoralis* larvae. *J. Environ. Health* **B16**, 167–168.

FOLLEY, L. S., POWER, S. D., AND POYTON, R. O. (1983). Separation of nucleotides by ion-pair, reversed phase high-performance liquid chromatography. Use of Mg(II) and triethylamine as competing betaerons in the separation of adenine and guanine nucleotides. *J. Chromatog.* **281**, 199–207.

GIESY, J. R., DUKE, C. S., BINGHAM, R. D., AND DICKSON, G. W. (1983). Changes in phosphoadenylate concentrations and adenylate energy charge as an integrated biochemical measure of stress in invertebrates: The effects of cadmium on the freshwater clam *Corbicula fluminea*. *Toxicol. Environ. Chem.* **6**, 259–295.

HOLM-HANSEN, O., SUTCLIFFE, W. H., JR., AND SHARP, J. (1968). Measurement of the deoxyribonucleic acid in the ocean and its ecological significance. *Limnol. Oceanog.* **13**, 507–514.

IVANOVICI, A. M., AND WIEBE, W. J. (1981). Towards a working definition of "stress": A review and critique? In *Stress Effects on Natural Ecosystems* (G. W. Barrett and R. Rosenberg, eds.), pp. 13–27. Wiley, New York.

KHAN, N. Y. (1983). An assessment of the hazard of synthetic pyrethroid insecticides to fish and fish habitat. In *Pesticide Chemistry: Human Welfare and the Environment*, Vol. 3: *Mode of Action, Metabolism and Toxicology* (J. Miyamoto and P. C. Kearney, eds.), pp. 437–450. Pergamon, Oxford.

KISSANE, J. M., AND ROBBINS, E. (1958). Fluorometric measurement of deoxyribonucleic acid in animal tissues with special reference to the central nervous system. *J. Biol. Chem.* **233**, 184–188.

17

36

LANG, C. A., LAU, H. Y., AND JEFFERSON, D. J. (1965). Protein and nucleic acid changes during growth and aging in the mosquito. *Biochem. J.* **95**, 372–377.

LIVINGSTONE, D. R. (1982). General biochemical indices of sublethal stress. *Mar. Pollut. Bull.* **13**, 261–263.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **93**, 265–275.

MANN, R., AND GALLAGER, S. M. (1985). Physiological and biochemical energetics of larvae of *Teredo navalis* L. and *Bankia gouldi* (Bartsch) (Bivalvia: Teredinidae). *J. Exp. Mar. Biol. Ecol.* **85**, 211–218.

MAYER, F. L., AND ELLERSIECK, M. R. (1986). Manual of acute toxicity: Interpretation and data base for 410 chemicals and 66 species of freshwater animals. *Fish Wildlife Ser. Res. Pub. Ser. USA* pp. 1–507.

MCKEE, M. J., AND KNOWLES, C. O. (1986). Protein, nucleic acid and adenylate levels in *Daphnia magna* during chronic exposure to chlordecone. *Environ. Pollut. Ser. A.*, in press.

MCKENNEY, C. L., JR., AND HAMAKER, D. A. (1984). Effects of fenvalerate on larval development of *Palaemonetes pugi* (Holthius) and on larval metabolism during osmotic stress. *Aquatic Toxicol.* **5**, 343–355.

MEHRLE, P. A., AND MAYER, F. L. (1980). Clinical tests in aquatic toxicology: State of the art. *Environ. Health Perspect.* **34**, 139–143.

MEHRLE, P. M., AND MAYER, F. L. (1985). Biochemistry/physiology. In *Fundamentals of Aquatic Toxicology: Methods and Applications* (G. M. Rand and S. R. Petrocelli, eds.), pp. 264–282. Hemisphere, Washington, D.C.

MILLER, G. L. (1959). Protein determination for large numbers of samples. *Anal. Chem.* **31**, 964.

MOUNT, D. I., AND BRUNGS, W. A. (1967). A simplified dosing apparatus for fish toxicological studies. *Water Res.* **1**, 21–29.

MUNROE, H. N., AND FLECK, A. (1966). Recent developments in the measurements of nucleic acids in biological materials. A supplementary review. *Analyst* **91**, 78–88.

NEFF, J. M. (1985). Use of biochemical measurements to detect pollutant-mediated damage to fish. In *Aquatic Toxicology and Hazard Assessment: Seventh Symposium*, ASTM STP 854 (R. D. Cardwell, R. Purdy, and R. C. Bahner, eds.), pp. 155–183. Amer. Soc. Test. Mater., Philadelphia, Pa.

PANDE, S. V., KHAM, R. P., AND VENKITASUBRAMANIAN, T. A. (1963). Microdetermination of lipids and serum total fatty acids. *Anal. Biochem.* **6**, 415–423.

PICKERING, A. D., ed. (1981). *Stress and Fish*, pp. 1–330. Academic Press, New York.

ROE, J. H., AND DAILEY, R. E. (1966). Determination of glycogen with the anthrone reagent. *Anal. Biochem.* **15**, 245–250.

SCHNEIDER, W. C. (1957). Determination of nucleic acids in tissues by pentose analysis. In *Methods in Enzymology* (S. D. Colowick and N. O. Kaplan, eds.), Vol. 3, p. 680. Academic Press, New York.

SHEEHAN, P. J. (1984). Effects on individuals and populations. In *Effects of Pollutants at the Ecosystem Level* (P. J. Sheehan, D. R. Miller, G. C. Butler, and Ph. Bourdeau, eds.), pp. 23–54. Wiley, New York.

SHIBKO, S., KOIVISTOINEN, P., TRATNYEK, C. A., NEWALL, A. R., AND FRIEDMAN, L. (1967). A method for sequential quantitative separation and determination of protein, RNA, DNA, lipid, and glycogen from a single rat liver homogenate or from a subcellular fraction. *Anal. Biochem.* **19**, 514–528.

SUTCLIFFE, W. H., JR. (1965). Growth estimates from ribonucleic acid content in some small organisms. *Limnol. Oceanog.* (Suppl.) **10**, 253–258.

TUKEY, J. W. (1949). Comparing individual means in the analysis of variance. *Biometrics* **5**, 99–114.

VYTASEK, R. (1982). A sensitive fluorometric assay for the determination of DNA. *Anal. Biochem.* **120**, 243–248.