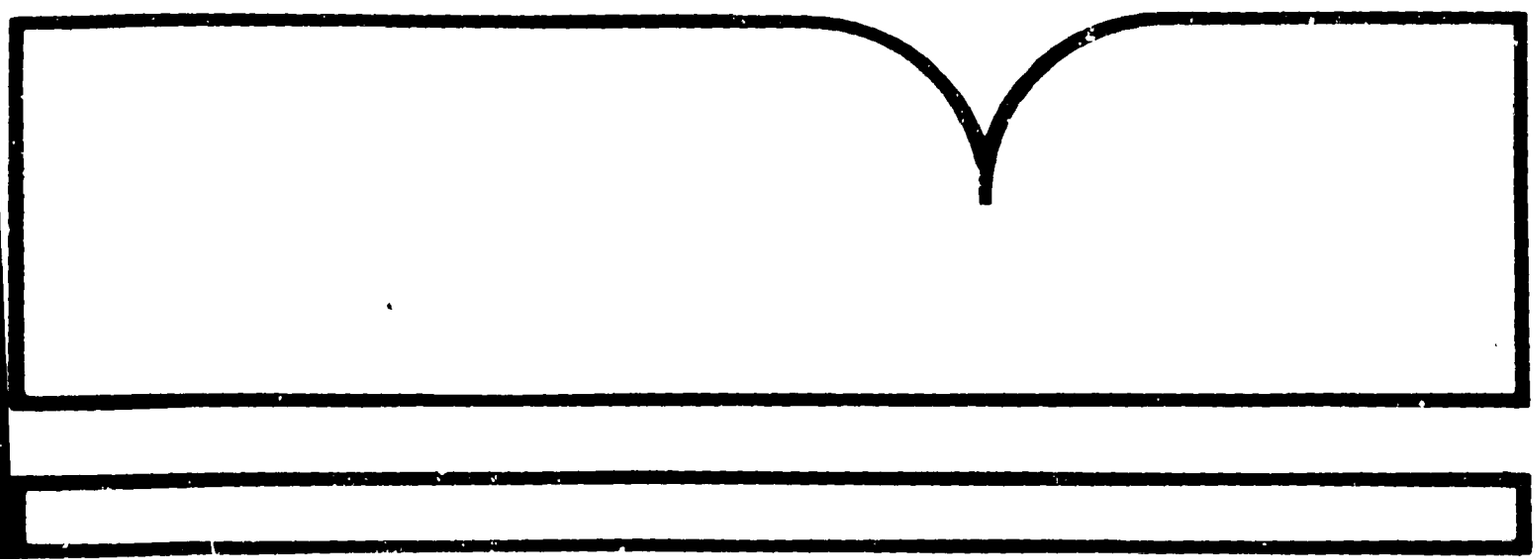
EPA 600-3-84-054

PB84-180025

Insect Interlaboratory Toxicity Test Comparison Study for the Chironomid ('Paratanytarsus sp.') Procedure

(U.S.) Environmental Research Lab.-Duluth, MN

Mar 84



U.S. Department of Commerce
National Technical Information Service
NTIS

EPA-600/3-84-054
March 1984

INSECT INTERLABORATORY TOXICITY TEST COMPARISON STUDY

for the

CHIRONOMID (*Paratanytarsus sp.*) PROCEDURE

by

Armond E. Lemke and Richard L. Anderson

U.S. Environmental Protection Agency

Environmental Research Laboratory-Duluth

6201 Congdon Boulevard

Duluth, Minnesota 55804

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/3-84-054		PB84 180025

4. TITLE AND SUBTITLE
Insect Interlaboratory Toxicity Test Comparison Study for the Chironomid (Paratanytarsus sp.) Procedure

5. REPORT DATE
March 1984

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Armond E. Lemke and Richard L. Anderson

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, Minnesota 55804

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
same as above

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA-600/03

15. SUPPLEMENTARY NOTES

16. ABSTRACT

A test method guideline for the chironomid Paratanytarsus sp. was evaluated. Six laboratories participated in the interlaboratory comparison study. Three items were compared, including start-up and maintenance of a rearing colony, a 48 hr acute test, and a 28 day life history chronic. All participating laboratories were able to start and maintain the rearing colonies. Chemicals used for testing were trichlorophenol and acenaphthene. Forty out of an expected total of 48 test results were reported.

The 17% failure rate appeared to be related to the volatility of the chemical in the acute tests and to an unexplained test water problem at specific laboratories in the chronic tests. All participants recommended the preparation of a set of forms for recording data and training rather than more detail in the guidelines would improve testing efficiency.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	Unclassified	18
	20. SECURITY CLASS (This page)	22. PRICE
	Unclassified	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Researchers in aquatic toxicology recognize that there are wide differences in aquatic species sensitivity to chemicals. Intuitively, the species dependent difference in sensitivity cannot be adequately measured with only a few test species. At this time, most of the test method development has been with fish. Only a few fish food organisms like Daphnia have had wide use as test subjects and, because of their use, testing methods and rearing procedures are available.

The need for invertebrate test species has lead to the development of the procedure evaluated in this report. The test species is a parthenogenetic insect Paratanytarsus sp. of the family Chironomidae in the insect Order Diptera. The insects are commonly called "midges". Members of this family are found in all freshwater and some saltwater habitats. Their usual mode of living is either on or below the surface of sediments or periphyton. The genus Paratanytarsus has been found in Australia, Japan and North America.

Objectives: The objective of this work was to evaluate the adequacy of the Paratanytarsus rearing and testing guidelines (Appendix A and B) by a group of laboratories that have a strong background in aquatic toxicity testing. Chironomids have not been tested extensively but all the selected laboratories had chironomid rearing and testing experience although not necessarily with Paratanytarsus. All the laboratories were requested to make comments on the rearing and testing guidelines, both as to operating problems and clarity. These comments would be incorporated into the guidelines.

Procedures

Before start: A Request for Project was developed, advertised, and the responses were evaluated by a panel at the Environmental Research Laboratory-Duluth (ERL-D). Five laboratories were chosen. A major part of the selection criteria was the suitability of the respondents test water. All the laboratories were asked to present information on heavy metals and pesticides in their water supplies and evidence that the water would support Daphnia cultures. All participants presented information of a satisfactory water supply. Common physical measurements for the test water from each of the selected laboratories are listed in Table 1.

A one-day informational conference was held at ERL-D and the principal investigator for each laboratory was present. One of the authors (RLA) described the toxicity test procedures and demonstrated the manipulative techniques that appeared in the guidelines. At the end of the day a question and answer session was held. After this meeting, the participants were told to use the rearing and testing guidelines and only minimum guidance was given. This was done to allow the method to stand by itself and to show guideline deficiencies. Also, at the close of the meeting each investigator was given stock supplies of the two chemicals that were to be tested and chironomid eggs from the ERL-D colony to start their own rearing colony. Chemicals were reagent grade trichlorophenol, and reagent grade acenaphthene. The chemicals were purchased from the manufacturer, Eastman Kodak Chemicals, and reported as 99% pure.

Testing

Each laboratory was to establish a culture and conduct two static 48 hr acute tests and two flow-through life tests with each of the chemicals for a total of 8 tests per participant. Analytical chemical methods were left to

the participants but those who requested help were assisted with methodology as the necessary analytical work was only an incidental part of the tested protocol. Methods used by each group are shown in Table 2.

Each laboratory was to analyze the chemical at the beginning of the acute tests and at the end of 48 hrs of exposure. In the life cycle tests, the chemical was analyzed at least twice each week with the samples being alternated to assure that all containers were analyzed.

Biological effects of the exposures were reported as LC50s for the acute tests and as effect and no effect concentrations for the life cycle tests. It was also required that a discussion of all problems, guideline ambiguities and a general commentary on the guidelines be produced.

No deadline was set for the completion of the tests and extensions were granted on an individual basis by specific request of the principal investigator to the project officer (AEL). If a principal investigator discovered and reported a problem which could affect all investigators, the project officer notified all.

Results

All laboratories reported success in establishing rearing colonies following the rearing guidelines. As evidence of their success, all participants were able to produce sufficient animals to use in the acute tests. Each acute test required at least 100 same age animals at one time (4 concentrations of 10 in duplicate and a 10 duplicate control). Several of the laboratories used up to 6 concentrations and a control to determine the LC50 rather than conducting a preliminary, range finder test.

The reported values for the static acute tests and flow-through life cycle tests are in Tables 3 and 4, respectively. Forty-eight tests were to

be run. Acceptable results were obtained in 40 tests and in two of the 40, the range numbers were not available because the required partial kills did not occur.

Acenaphthene was more difficult to test than trichlorophenol. All acute test failures and one of the life cycle failures were with this chemical. The only life cycle test failures occurred at OKS. The principal investigator reported non-toxicant related death at all concentrations and the control. OSU did not report an effect concentration in one set of life cycle tests with both chemicals. In each case, a very low replacement time in the test chambers was reported. Several of the laboratories repeated the experiments and the numbers reported to the Project Officer are those the principal investigator considered satisfactory.

Discussion

These tests, with the parthenogenetic chironomid Paratanytarsus sp., were done to determine the utility of the rearing and testing guidelines. A three part guideline was provided and each will be discussed separately.

Rearing: The first part of the guideline describes procedures for establishing a colony. The goal of the first section was to rear chironomids in sufficient numbers to provide larvae for the acute and eggs for the life cycle tests. This section had a two-fold purpose. First, it provided a check on the quality of the rearing water and, second, it provided an opportunity for the principal investigators to become familiar with the life cycle and biological requirements of the chironomid. This task was accomplished by all of the laboratories and no difficulties were reported with the rearing guideline as it was written.

Acute Tests: The second task was for each of the participants, by following the guideline, to conduct the acute 48 hr tests with the two chemicals. All laboratories were able to get an LC50 with trichlorophenol but two laboratories, OSU and CAL, did not get a 50% kill with acenaphthene although they tried several times. Of those completing the acenaphthene test one, the order, in increasing sensitivity, was 2.00 mg/l (NAS), 1.62 (OKS), 0.14 (ERL-D), and 0.06 (SRI). For test two the order was 2.09 mg/l (NAS), 1.65 (OKS), 0.47 (ERL-D) and 0.07 (SRI). Those laboratories which did not complete a successful acute test with acenaphthene reported that they were unable to get a kill with a saturated solution. All participants reported a loss of chemical during the 48 hr test period. This loss may explain the wide difference in LC50s between the laboratories. However, the interlaboratory results show that the LC50 values were similar. Only ERL-D showed a wide variance between the two tests. The maximum variance with the other three sets was 7% or less. The major problem in the acenaphthene tests was probably volatilization or breakdown of the chemical rather than the animals or guidelines.

The sensitivity order for the acute trichlorophenol test one was 43.0 (CAL), 41.9 (SRI), 23.6 (OKS), 21.8 (OSU), 10.3 (NAS) and 3.7 (ERL-D). For test two with trichlorophenol the order was 65.1 mg/l (SRI), 45.4 (OSU), 41 (CAL), 27.2 (OKS), 16.3 (NAS) and 2.5 (ERL-D). The variation between the high and low reported value is 11 for test one and 26 for test two. Of the 6 data sets, 3 of the intralaboratory data sets overlap in 95% confidence interval (C.I.) between test 1 and test 2, 2 do not overlap and 1 set does not have a confidence interval.

In summary, for all the acute tests, 10 data sets were reported and 6 of the 10 sets show an overlap of the 95% C.I between test 1 and test 2 for a

chemical. Of the remaining 4, 3 show no overlap and one set does not have a C.I. and cannot be judged although inspection of the single value and a comparison to the first test results indicates that an overlap of the C.I. is probable. In summary, the acute data show that in 60 or 70% of the tests, a laboratory will produce data that is internally consistent. This also shows that the guideline can produce data that is precise but, that other factors can impact the accuracy of toxicity test data. The factors which affect interlaboratory comparisons are discussed later in this report.

Chronic Tests: The life cycle values for the six laboratories (Table 4) are reported as effect and no effect concentrations. The reported effect and no effect concentrations are affected by the exposure concentrations selected by each laboratory. To facilitate comparison, geometric means of the effect and no effect concentrations were determined. For acenaphthene, the geometric mean ranges from 0.014 mg/l to 0.91 mg/l and 0.024 mg/l to 0.49 mg/l for test one and two. For trichlorophenol, the range of geometric means is between 0.31 mg/l to 5.0 mg/l and 0.38 mg/l to 2.6 mg/l. One laboratory, ORS, was unable to conduct the flow-through tests and OSU did not obtain a no effect value apparently because of extremely low flow in the test tanks and subsequent volatilization of toxicant from the tanks.

Summary

Sources of variation in toxicity tests with aquatic organisms can be grouped into five main areas. They are the dilution water, test organisms, chemical characteristics, test conditions, and chemical analysis. Variations in each of these 5 areas can result in little change in reported values between the laboratories if the factors balance each other or large variation if all of the factor effects are in a single direction. Therefore, a determination is needed of the problems found in this test series to distinguish errors in the method guideline from the 5 non-method type errors.

Forty-eight tests were to be completed in this project. Four acute values were not reported and one 95% confidence limit was not calculated. Four life cycle values were not reported and two did not develop effect concentrations and one did not develop an effect concentration. A failure rate of 12% is not unusual over a set of tests with biological organisms. Dilution water problems, chemical solubility, and exposures to an inappropriate concentration series all contribute to failures and data variability.

Examination of the individual laboratory reports reveal that the major difficulties were with the test chemical characteristics, dilution water or test animal selection. The low ERL-D trichlorophenol acute LC50 value was apparently caused by using fourth instar larva rather than third instar as suggested in the guidelines. All larva which molted into pupa died and this apparent life cycle related death skewed the data. If the low ERL-D trichlorophenol results are not considered the range of the extreme values is about six fold rather than 26.

Those laboratories having problems with acenaphthene reported that the chemical apparently volatilized from the test chambers. Losses up to 90% of initial concentration were reported. This chemical related problem would be particularly important in static tests. Losses up to 90% of initial concentration were reported. Volume to surface ratios probably added to the volatilization effects because test chamber shape was not specified.

The OKS laboratory could not complete a chronic test apparently because of an undetermined problem in their water supply. Static rearing and static acute tests were completed because they are "batch" events and are less dependent on variable water quality. The test water was suspected because in the longer flow-through tests even the controls did not survive.

Conclusions

The guidelines are in three sections: culture methods, acute test methods, and life cycle test methods.

The culture method was completed by all participants. The only difficulty reported was in selection of the correct instar for the acute tests in a crowded culture tank. More tanks with fewer animals would correct this problem. With this exception, the culture method guidelines appear satisfactory.

The acute test methods also appear to be satisfactory as those tests which were not completed were caused by the chemical being only sparingly soluble or volatile and and not by misstatements in the guidelines. All participants were able to show satisfactory survival of animals in the controls. It was reported that the protocol suggestion about using sand as a substrate during acute tests is not needed. Although the participants in this laboratory comparison series did not find the use of sand as a substrate to be important in these tests, others have found it useful and it should be included in the protocol as an alternative to the bare beaker method.

The life cycle exposure guideline should be modified to reflect the problems encountered by the participants. The following changes appear appropriate: (1) Start test by having all systems in operation; (2) take samples for chemical analysis; (3) shut down or divert flow and add eggs to test chamber; (4) run as static for 48 hrs; (5) take samples for chemical analysis; (6) start flow.

Recommendations of Participating Laboratories

The participating laboratories were asked to recommend changes or describe problem areas in the test guidelines. They had no difficulties in the rearing of the animals. It is recommended, however, to not overpopulate the culture tanks when planning for acute tests because the larger instars are more difficult to distinguish under crowded conditions.

The chronic tests gave the most problems to the participating laboratories. Their recommendations included a more detailed description of the chambers, a narrower range on the flow rate, treating the first 48 hrs of the test as a static to prevent loss of the pelagic larvae, and allowing more eggs to be used in starting tests. The use of more eggs would reduce egg manipulation time. Another suggestion was to use pupal cases from which the adults had emerged as a measure of reproduction which would eliminate the problems with retaining and capturing the adults in a flow-through system. If pupal cases are used, the effect of the toxicant on adult emergence could not be determined. This may be a sensitive endpoint and the researcher must make the decision about the loss of that data. It was also suggested that, rather than removing the adults, egg production in the original test chambers be monitored and compared with the control as this appeared to be a sensitive measure for the chemicals tested in this series.

Several of the laboratories are private testing organizations. Determining the cost of performing a test is an important problem in their bidding process. These laboratories recommended the following:

(1) The client organization is asked to produce a set of data forms which reflects the actual data needed during each day. These forms should include spaces for such items as animal numbers, number of routine chemistry analyses, number of analytical chemistry analyses, times for sample

collecting and any other data such as physical conditions of the test systems needed by the client. For an acute exposure, they recommend a three sheet set with one sheet for the initial day, 24 hr and 48 hrs of exposure.

(2) In the life cycle exposures, a set of data forms for each day plus an initial day and a final compilation sheet complete with the statistical formulas enumerated in such a way as to allow filling in the spaces with the required information and performing the associated arithmetic. Enumeration of the number and type of chemical analyses, the days the analyses should be done, and quality assurance needs such as slope and correlation (R^2) of the standard curves are also requested.

(3) All participants requested that some training should be given prior to the conducting of any test. All participants were of the opinion that if training and a set of data forms were prepared the guidelines were sufficient.

Table 1. Quality of Exposure Water for *Paratanytarsus* sp. Toxicity Tests

Test Facility	Temperature	pH	Hardness as $CaCO_3$	Alkalinity
OSU	19.9-22.1	7.6	650	127
OKS	19.4-20.5	8.0-8.2	148-170	150-161
NAS	20	6.5-7.7	40-60	20-30
SRI	19-22	7.6	46	37
CAL	19.3-22.4	7.2-7.75	640-735	120-205
ERL-D	20-21.5	7.6-7.8	47-48	43-44

Table 2. Methods of Toxicant Analysis

	Acenaphthene	Trichlorophenol
OSU	Fluorescence spectrophotometer	Gas chromatograph solvent extract
OKS	Fluorescence spectrofluorometer	Gas chromatograph solvent extract
NAS	Capillary gas chromatograph	Capillary gas chromatograph
SRI	High pressure liquid chromatography	High pressure liquid chromatography
CAL	Gas chromatograph	Gas chromatograph
ERL-D	Fluorescence spectrofluorometer	Auto analyzer direct colormetric

Table 3. LC50 Acute Values with a 95% Confidence Interval for the Chironomid *Paratanytarsus sp.*[a]

Test Facility	Acenaphthene		Trichlorophenol	
OSU	No Results	No Results	21.8 16.7-28.4	45.4 35.0-59.0
OKS	1.62* 1.45-1.81	1.65 1 49-1.86	23.6 15.2-41.5	27.2 6.9
NAS	2.00 1.69-2.36	2.09 1.83-2.39	10.3 8.4-12.7	16.3 13.1-20.7
SRI	.06 .05-.06	.07 .06-.07	41.9 37.9-59.8	65.1 52.0-81.4
CAL	No Results	No Results	43 30-61	41 29-58
ERL-D	.14 .10-.19	.47 .41-.53	3.7 2.6-5.4	2.5 .4-15.8

[a] Values presented by participants

* All values are mg/l

Table 4. Life Cycle Effect Values for the Chironomid *Paratanytarsus sp.*[a]

	Acenaphthene		Trichlorophenol	
OSU	>.8*	.49** .3-.8	5.0 2.5-10	>10
OKS	No Results		No Results	
NAS	.23 .18-.30	.035 .027-.044	.72 .55-.93	.69 .50-.94
SRI	.014 .01-.02	.024 .02-.03	<.93	.76 .49-1.18
CAL	.095 .06-.15	.057 .03-.11	1.49 1.3-1.7	2.6 1.6-4.2
ERL-D	.91 1.30-.64	.40 .27-.58	.31 .18-.53	.38 .22-.65

[a] Values presented by participants

* All values are mg/l

** Geometric mean of no effect/effect concentration