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PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION E

HAZARD EVALUATION:

WILDLIFE AND AQUATIC ORGANISMS

Series 72-1 to 72-5

Aquatic Testing for Marine/Estuarine and Freshwater Fish and Invertebrates

ADDENDUM 2 ON DATA REPORTING

Prepared by:

Curtis E. Laird
Ecological Effects Branch
Hazard Evaluation Division

Edited by:

Karen E. Warkentien
Hazard Evaluation Division

Project Manager:

Elizabeth M. K. Leovey, PhD
Hazard Evaluation Division

Office of Pesticide Programs
US Environmental Protection Agency

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Subdivision E - Aquatic Testing for Marine/Estuarine and Freshwater Fish and Invertebrates

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PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

AQUATIC TESTING FOR MARINE/ESTUARINE AND FRESHWATER FISH AND INVERTEBRATES

Subdivision E, Series 72-1 to 72-5

DATA REPORTING

INTRODUCTION

A. Purpose

To provide a data reporting format for fish and invertebrate studies.

B. Objective

These Data Reporting Guidelines are designed to aid the petitioner/registrant in generating reports which are compatible with the Agency's review process. Data submitters are encouraged to submit complete reports following this guidance for efficient review by the Agency. This guidance pertains to the substance of the data report. PR Notice 86-5, effective on November 1, 1986 (available from the Registration Support and Emergency Response Branch, Office of Pesticide Programs, US EPA), pertains to the physical formating of reports (which are referred to as "studies") and submittal packages. Some of the requirements in PR Notice 86-5 are mandatory.

RESPONSE TO PUBLIC COMMENTS

The purpose of this seciton is to acknowledge and address the concerns expressed in the letters of comments received by the Agency in response to the public notice in the FEDERAL REGISTER (51 FR 18660) of May 21, 1986.

This addendum to the Pesticide Assessment Guidelines [Subdivision E] is to be considered an all-encompassing document. The Ecological Effects Branch (EEB) has reviewed the ten (10) comments submitted by the registrants and/or committees in regard to the Data Reporting Guidelines (DRGs). These Guidelines are not intended to create new data requirements, but to provide for consistent reporting of the necessary aquatic data required to perform a scientifically sound hazard assessment. This approach should eliminate most, if not all, of the recycling of submissions between EEB scientists and the registrant.

The submitted comments pertain to either items common to all studies or a study specific item. Hence, this discussion has been arranged to consider the general comments first, followed by the specific comments.

General Comments

1. Location of a reporting item -

   Considering the need to maintain a consistent format, revisions were made to the DRGs in response to these comments whenever possible.

2. Composition of the test substance -

   EEB is asking for the "Identity and Composition" of the test substance instead of the "Identity and Composition" of the impurities. Under the present guidelines, these data are required under Subdivision E, Test Substance 70-4(2)(i).

3. Ingredient information from testing laboratories -

   It appears some sponsors do not make this information available to testing laboratories. In such a situation, the sponsor bears the responsibility since he is submitting the data for registration.

4. Telephone number of individual who can provide details of test -

   This item has been deleted from the DRG.

5. Time to implement new format -

   The registrant will be given sufficient time to implement the necessary changes in order to meet the recommended format standard.

6. Limited to DRG -

   Additional data can and should be reported, providing it is meaningful and useful in making a risk assessment.

7. Public comments -

   Ecological Effects Branch (EEB) is only addressing the comments relating to the nine aquatic studies.

Study-Specific Comments

1. The following were changed as a result of comments.

   A. The shell deposition value has been changed from LC_{50} to EC_{50} in ppm.

   B. The fish early life-cycle value has been changed from EC_{50} to MATC in ppm.

   C. The aquatic invertebrate life-cycle value has been changed from EC_{50} to MATC in ppm.

   D. Total and un-ionized ammonia has been deleted from fish early life-stage, aquatic invertebrate life-cycle, and fish life-cycle test.

2. The following were not changed as a result of comments.

   A. The water hardness, alkalinity, and conductivity are required under Subdivision E, Sections 70-4(6)(ii)(E)(F) which calls for "[t]he source of dilution water, its chemical characteristics, and description of any pretreatment" and the "[m]ethods used for, and results of, all chemical analysis of water and all toxicant concentrations at beginning, during, and at end of tests, including validation studies and reagent blanks, if there is reason to suspect that the concentrations administered to the test water do not approximate the actual concentrations."

   B. The no-observed-effect level (NOEL) for aquatic studies is required, except if the LC_{50} or EC_{50} has not been determined, as allowed by the specific testing requirement.

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Acute Toxicity Test for Freshwater Fish

Subdivision E, Series 72-1

DATA REPORTING

GUIDELINE

The following describes the order and format for a study report item by item.

TITLE/COVER PAGE

Refer to PR Notice 86-5.

TABLE OF CONTENTS

The Table of Contents should indicate the overall organization of the study, including tables and figures. It must follow the title, data confidentiality, and GLP (if appropriate) pages as described in PR Notice 86-5.

I. SUMMARY/INTRODUCTION

A. Sponsor: (name of study owner);

B. Name: (person(s) who can provide details of test procedures);

C. Location of study;

D. Location of raw data and final report;

E. Material: (common/trade name);

F. Subject: (final or draft report, acute 96-hour LC_{50}, and species tested);

G. Test Doses: (control, concurrent vehicle control, #, #, #, #, # ppm);

H. Test Dates: (initiation - date test started, termination and date of last day of observation);

I. Length of Study: (hours);

J. Results: (LC_{50} value, 95% confidence limits, and no-observed-effect level);

K. Tested Material: (chemical name, formulation, and percent active ingredient);

L. Test Species/Strain: (both common and scientific name);

M. Source of Organisms: (company and address);

N. Weight/Length and Physical Condition at the Initiation of Study.

II. MATERIAL/METHODS

This section is a narrative, which would include the following items.

A. Test Substance

1. Identification (this information may be provided by the laboratory or sponsor):

   a. Chemical name;
   b. Composition (qualitative and quantitative description);
   c. Percent active ingredient;
   d. Molecular structure;
   e. Source, lot number, or code;
   f. Identity and percent composition of impurities in the test substance.

2. Preparation of test solution (control/treatment):

   a. Any vehicle used to dissolve test material;
   b. Amount of vehicle added to control (if used);
   c. Description of method used to get test material into solution;
   d. Water source (reconstituted - if so, how prepared);
   e. Solubility of test material in ppm;
   f. Total amount of test material used;
   g. Temperature of water.

B. Test Fish

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E.

   a. Test species name (both scientific and common);
   b. History of test organisms (strain, diseases, and treatment).

2. Description of any pretest conditioning:

   a. Health:

      1. Sickness;
      2. Injuries;
      3. Abnormalities;
      4. Name of medication (if used);
      5. Pretest diet.

3. Size/age/physical condition:

   a. Age (if known);
   b. Size (weight in grams and length in mm).

4. Source/acclimation:

   a. Complete name and address of test fish supplier;
   b. Source of food and dilution water;
   c. Size of test container [in milliliters (mL)];
   d. Temperature, pH, and dissolved oxygen, and name of equipment used to measure water quality;
   e. Feeding schedule, holding, and acclimation period;
   f. Test organisms from the same source (yes or no);
   g. Number of days held.

C. Method

1. Test Vessels:

   a. Material type;
   b. Volume [in liters (L)];
   c. Depth of test solution [in centimeters (cm)];
   d. Size [in liters (L)].

2. Test system:

   a. Source of dilution water;
   b. If flow-through, description of system and flow rate/day;
   c. Procedures used to prepare toxicant stock solution;
   d. Criteria used to determine effects.

3. Test design:

   a. Method used in assigning test organisms to test and control groups, and the number of replicates used;
   b. Number of fish per dose level and control group;
   c. Name of protocol followed during this test;
   d. Loading (weight per unit volume of water);
   e. Number of treatment levels used (nominal or measured);
   f. Length of exposure period;
   g. Type of control (positive, negative, or solvent control);
   h. Temperature;
   i. pH (when checked - see table 1);
   j. Lighting (time in hours and intensity in footcandles);
   k. Dissolved oxygen (see table 1);
   l. Water hardness (expressed in mg/L as $CaCO_3$ - see table 1);
   m. Alkalinity (expressed in mg/L as $CaCO_3$ - see table 1);
   n. Conductivity (umhos/cm - see table 1);
   o. Range finding test results, concentrations, and mortality (if used);
   p. Aeration (yes or no);
   q. Water physical characteristic at the end of test:
      a. Water depth and volume;
      b. Temperature, pH, and dissolved oxygen;
   r. Concentration analysis (see table 1);
   s. Examination of fish physiology, locomotion, behavior and pathology;
   t. Mortalities during the test;

D. Statistical Analysis

1. Cite references (author, title, journal, number, page, etc.).

III. DISCUSSION AND RESULTS

A. Include LC_{50} value in ppm with 95% confidence limits (graphs, printouts, and other calculations should be attached to report). Provide raw mortality data (see table 2).

B. Discuss the relationship, if any, between the physical factors, toxicant, and observation, and describe any precipitation and solubility problems.

C. Provide statistical method used (cite references, author, etc.).

D. Observation - Provide data on the following:

1. Signs of intoxication - temporal onset, duration, and concentrations that showed effects;

2. The no-observed-effect level.

IV. CONCLUSIONS

This is a summation of the above section - Discussion/Results.

TABLE 1

WATER QUALITY PARAMETERS

Client :
Test Material:
Test Organism:
Test Water :

WATER QUALITY

pH	Conductivity umhos/cm	Hardness mg/L as $CaCO_3$	Alkalinity mg/L as $CaCO_3$	Temperature °C
X	X	X	X	X

Parameter	Time	Control	Nominal Concentration (mg/L) X	X	X	X	X
			High		Medium		Low
Dissolved Oxygen (mg/L)	Initial	X	X		X		X
	48 hours	X	X		X		X
	96 hours	X	X		X		X
pH	Initial	X	X		X		X
	96 hours	X	X		X		X

TABLE 2

PERCENT MORTALITIES AND LC_{50} VALUES

Client:
Test Material:
Test Organism:
Test Water:

PERCENT MORTALITY

TEST MATERIAL NOMINAL CONCENTRATION (mg/L)

	Control	X	X	X	X	X
24 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)
48 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)
96 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)

		24 hour	48 hour	96 hour
LC_{50} (mg/L)		X	X	X
95% Confidence Limits	Low	X	X	X
	High	X	X	X

Observed Effects

[a] floating
[b] swimming at surface
[c] lying on bottom
[d] turned dark
[e] loss of equilibrium
[f] gulping of air
[g] etc.

1/ How the percent of mortality table works.

1. #(#/#)[a].
2. # = the total number of organisms tested per dose level.
3. #(#/#) = % mortality [No. dead/No. treated, e.g., 10% (1/10)]
4. a = the observed effects.

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Acute Toxicity Test for Freshwater Aquatic Invertebrates

Subdivision E, Series 72-2

DATA REPORTING

GUIDELINE

The following describes the order and format for a study report item by item.

TITLE/COVER PAGE

Refer to PR Notice 86-5.

TABLE OF CONTENTS

The Table of Contents should indicate the overall organization of the study, including tables and figures. It must follow the title, data confidentiality, and GLP (if appropriate) pages as described in PR Notice 86-5.

I. SUMMARY/INTRODUCTION

A. Sponsor: (name of study owner);

B. Name: (person(s) who can provide details of test procedures);

C. Location of study;

D. Location of raw data and final report;

E. Material: (common/trade name);

F. Subject: (final or draft report, 48-hour LC_{50}, and species tested);

G. Test Doses: (control, concurrent vehicle control, #, #, #, #, # ppm);

H. Test Dates: (initiation, date test started, termination, and date of last day of observation);

I. Length of Study: (hours);

J. Results: (LC_{50} value, 95% confidence limits, and no-observed-effect level);

K. Tested Material: (chemical name, formulation, and percent active ingredient);

L. Test Species/Strain: (both common and scientific name);

M. Source of Organisms: (company and address);

N. Age of Organisms at the Initiation of Study: (hours or life stage).

MATERIAL/METHODS

This section is a narrative, which would include the following items.

A. Test Substance

1. Identification (this information may be provided by the laboratory or sponsor):

   a. Chemical name;
   b. Composition (qualitative and quantitative description);
   c. Percent active ingredient;
   d. Molecular structure;
   e. Source, lot number, or code;
   f. Identity and percent composition of impurities in the test substance.

2. Preparation of test solution (control/treatment):

   a. Any vehicle used to dissolve test material;
   b. Amount of vehicle added to control (if used);
   c. Description of method used to get test material into solution;
   d. Water source (reconstituted - if so, how prepared);
   e. Solubility of test material in ppm;
   f. Total amount of test material used;
   g. Temperature of water.

B. Test Organisms

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E (if 1st instar were not used, then give stage of life in which organisms were tested):

   a. Test species name (both scientific and common);
   b. History of test organisms (strain, diseases, and treatment).

2. Description of any pretest conditioning:

   a. Health:

      1. Sickness;
      2. Injuries;
      3. Abnormalities;
      4. Name of medication (if used);
      5. Pretest diet;
      6. Number of days brood organisms were quarantined.

3. Size/age/physical condition:

   a. Age (in hours);
   b. Date of spawn.

4. Source/acclimation:

   a. Complete name and address of test species supplier;
   b. Source of food and dilution water;
   c. Volume of test container [in milliliters (mL)];
   d. Photoperiod and lighting;
   e. Temperature, pH, dissolved oxygen, and name of equipment used to measure water quality;
   f. Chemical treatment (if used);
   g. Feeding schedule, holding and acclimation period;
   h. Test organisms from the same source (yes or no);
   i. Number of days adults were held.

C. Method

1. Test Vessels:

   a. Material type;
   b. Volume of test solution [in milliliters (mL)];
   c. Depth of test solution [in centimeters (cm)];
   d. Size of test containers [in milliliters (mL)].

2. Test system:

   a. Source of dilution water;
   b. If flow-through, description of system and flow rate/day;
   c. Procedures used to prepare toxicant stock solution;
   d. Criteria used to determine effects.

3. Test design:

   a. Method used in assigning test organisms to test and control groups, and the number of replicates used;
   b. Number of organisms per dose level and control group;
   c. Name of protocol followed during this test;
   d. Loading (number of organism per unit volume of water);
   e. Number of treatment levels used (nominal or measured);
   f. Length of exposure period;
   g. Type of control (positive, negative, or solvent control);
   h. Temperature;
   i. pH (when checked - see table 1);
   j. Lighting (time in hours and intensity in footcandles);
   k. Dissolved oxygen (see table 1);
   l. Water hardness (expressed in mg/L as $CaCO_3$ - see table 1);
   m. Alkalinity (expressed in mg/L as $CaCO_3$ - see table 1);
   n. Conductivity (umhos/cm - see table 1);
   o. Range finding test results, concentrations, and mortality (if used);
   p. Aeration (yes or no);
   q. Water physical characteristic at the end of test:
      1. Water depth and volume;
      2. Temperature, pH, and dissolved oxygen;

r. Percent of death/effects at each dose level;
s. Concentration analysis (see table 1);
t. Examination of invertebrate physiology, locomotion, and behavior;
u. Mortalities during the test.

D. Statistical Analysis

1. Cite references (author, title, journal, number, page, etc.).

III. DISCUSSION AND RESULTS

A. Provide LC_{50} value in ppm with 95% confidence limits (graphs, printouts, and other calculations should be attached to report). Provide raw mortality data (see table 2).

B. Discuss the relationship, if any, between the physical factors, toxicant, and observation, and describe any precipitation and solubility problems.

C. Provide statistical method used (cite references, author, etc.).

D. Observation - Provide the following data:

1. Signs of intoxication - temporal onset and duration, concentrations that showed effects;

2. The no-observed-effect level.

IV. CONCLUSIONS

This is a summation of the above section - Discussion/Results.

TABLE 1

WATER QUALITY PARAMETERS

Client :
Test Material:
Test Organism:
Test Water :

WATER QUALITY

pH	Conductivity umhos/cm	Hardness mg/L as $CaCO_3$	Alkalinity mg/L as $CaCO_3$	Temperature °C
X	X	X	X	X

Parameter	Time	Control	Nominal Concentration (mg/L)				
			X	X	X	X	X
			High		Medium		Low
Dissolved Oxygen (mg/L)	Initial	X	X		X		X
	48 hours	X	X		X		X
pH	Initial	X	X		X		X
	48 hours	X	X		X		X

TABLE 2

PERCENT MORTALITIES AND LC_{50} VALUES

Client :
Test Material:
Test Organism:
Test Water :

PERCENT MORTALITY

TEST MATERIAL NOMINAL OR MEASURED CONCENTRATION (mg/L)

	Control	X	X	X	X	X
24 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)
48 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)

		24 hour	48 hour
LC_{50} (mg/L)		X	X
95% Confidence Limits	Low	X	X
	High	X	X

Observed Effects

a floating
b lying on bottom
c swimming near surface
d etc.

1/ How the percent of mortality table works.

1. #(#/#)[a].
2. # = the total number of organisms tested per dose level.
3. #(#/#) = % mortality [No. dead/No. treated, e.g., 10% (1/10)].
4. a = the observed effects.

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Acute Toxicity Test for Shrimp

Subdivision E, Series 72-3

DATA REPORTING

GUIDELINE

The following describes the order and format for a study report item by item.

TITLE/COVER PAGE

Refer to PR Notice 86-5.

TABLE OF CONTENTS

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A. Sponsor: (name of study owner);

B. Name: (person(s) who can provide details of test procedures;

C. Location of study;

D. Location of raw data and final report;

E. Material: (common/trade name);

F. Subject: (final or draft report, 96-hour LC_{50}, and species tested);

G. Test Doses: (control, concurrent vehicle control, #, #, #, #, # ppm);

H. Test Dates: (initiation, date test started, termination, and date of last day of observation);

I. Length of Study: (days);

J. Results: (LC_{50} value, 95% confidence limits, and no-observed-effect level);

K. Tested Material: (chemical name, formulation, and percent active ingredient);

L. Test Species/Strain: (both common and scientific name);

M. Source of Organisms: (company and address);

N. Size/Age of Organisms at the Initiation of Study: (hours).

II. MATERIAL/METHODS

This section is a narrative, which would include the following items.

A. Test Substance

1. Identification (this information may be provided by the laboratory or sponsor):

   a. Chemical name;
   b. Composition (qualitative and quantitative description);
   c. Percent active ingredient;
   d. Molecular structure;
   e. Source, lot number, or code;
   f. Identity and percent composition of impurities in the test substance.

2. Preparation of test solution (control/treatment):

   a. Any vehicle used to dissolve test material;
   b. Amount of vehicle added to control (if used);
   c. Description of method used to get test material into solution;
   d. Water source (reconstituted - if so, how prepared);
   e. Solubility in ppm;
   f. Total amount of test material used;
   g. Salinity (o/oo).

B. Test Shrimp

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E:

   a. Test species name (both scientific and common);
   b. History of test organisms (strain, diseases, and treatment).

2. Description of any pretest conditioning:

   a. Health:

      1. Sickness;
      2. Injuries;
      3. Abnormalities;
      4. Pretest diet.

3. Size/age/physical condition:

   a. Age;
   b. Size;
   c. Date hatched (if known).

4. Source/acclimation;

   a. Complete name and address of test species supplier;
   b. Source of food and dilution water;
   c. Volume of test container [in liters (L)];
   d. Photoperiod and lighting;
   e. Temperature, pH, and dissolved oxygen, name of equipment used to measure water quality;
   f. Chemical treatment (if used);
   g. Percent mortality;
   h. Feeding schedule;
   i. Test organisms from the same source (yes or no);
   j. Acclimation period (in days).

C. Method

1. Test Vessels:

   a. Material type;
   b. Volume [in liters (L)];
   c. Depth of test solution [in centimeters (cm)];
   d. Size [in centimeters (cm)].

2. Test system:

   a. Source of dilution water;
   b. If flow-through, description of system and flow rate/day;
   c. Procedures used to prepare toxicant stock solution;
   d. Criteria used to determine effects.

3. Test design:

   a. Method used in assigning test organisms to test and control groups;
   b. Number of organisms per dose level and control group;
   c. Number of days food was withheld prior to test;
   d. Loading (weight of organism per unit volume of water);
   e. Number of treatment levels used (nominal or measured);
   f. Length of treatment period;
   g. Type of control (positive, negative, or solvent control);
   h. Temperature;
   i. pH (when checked - see table 1);
   j. Lighting (time in hours and intensity in footcandles);
   k. Dissolved oxygen (see table 1);
   l. Range finding test results, concentrations, and mortality (if used);
   m. Aeration (yes or no);
   n. Water physical characteristic at the end of test:
      a. Water depth and volume;
      b. Temperature, pH, and dissolved oxygen;
   o. Percent of death/effects at each dose level;
   p. Name of protocol followed during the test;
   q. Salinity (o/oo).

D. Statistical Analysis

1. Cite references (author, title, journal, number, page, etc.).

III. DISCUSSION AND RESULTS

A. Provide LC_{50} value in ppm with 95% confidence limits (graphs, printouts, and other calculations should be attached to report). Provide raw mortality data (see table 2).

B. Discuss the relationship if any, between the physical factors, toxicant, and observation, and describe any precipitation and solubility problems.

C. Provide statistical method used (cite references, author, etc.).

D. Observation - Provide the following data:

1. Signs of intoxication - temporal onset, duration, and concentrations that showed effects;

2. The no-observed-effect level.

IV. CONCLUSIONS

This is a summation of the above section - Discussion/Results.

TABLE 1

WATER QUALITY PARAMETERS

Client :
Test Material:
Test Organism:
Test Water :

WATER QUALITY

Temperature	6 hours, 12 hours, etc.				
			Nominal Concentration (mg/L)		
Parameter	Time	Control	X	X	X
			High	Medium	Low
Dissolved Oxygen (mg/L)	Initial	X	X	X	X
	48 hours	X	X	X	X
	96 hours	X	X	X	X
pH	Initial	X	X	X	X
	96 hours	X	X	X	X
Salinity o/oo	Initial	X	X		
	96 hours	X	X		

TABLE 2

PERCENT MORTALITIES AND LC_{50} VALUES

Client :
Test Material:
Test Organism:
Test Water :

PERCENT MORTALITY

TEST MATERIAL NOMINAL CONCENTRATION (mg/L)

	Control	X	X	X	X	X
24 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)
48 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)
96 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)

		24 hour	48 hour	96 hour
LC_{50} (mg/L)		X	X	X
95% Confidence Limits	Low	X	X	X
	High	X	X	X

Observed Effects

[a] floating
[b] swimming at surface
[c] lying on bottom
[d] turned dark
[e] etc.

1/ How the percent of mortality table works.

1. #(#/#)[a].
2. # = the total number of organisms tested per dose level.
3. #(#/#) = % mortality [No. dead/No. treated, e.g., 10% (1/10)]
4. a = the observed effects.

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Acute Toxicity Test for Estuarine and Marine Fish

Subdivision E, Series 72-3

DATA REPORTING

GUIDELINE

The following describes the order and format for a study report item by item.

TITLE/COVER PAGE

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TABLE OF CONTENTS

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B. Name: (person(s) who can provide details of test procedures);

C. Location of study;

D. Location of raw data and final report;

E. Material: (common/trade name);

F. Subject: (final or draft report, acute 96-hour LC_{50}, and, species tested);

G. Test Doses: (control, concurrent vehicle control, #, #, #, #, #, and # ppm);

H. Test Dates: (initiation - date test started, termination - date of last day of observation);

I. Length of Study: (hours);

J. Results: (LC_{50} value, 95% confidence limits, and no-observed-effects level);

K. Tested Material: (chemical name, formulation, and percent active ingredient);

L. Test Species/Strain: (both common and scientific name);

M. Source of Organisms: (company and address);

N. Weight/Length and Physical Condition at the Initiation of Study.

II. MATERIAL/METHODS

This section is a narrative, which would include the following items.

A. Test Substance

1. Identification (this information may be provided by the laboratory or sponsor):

   a. Chemical name;
   b. Composition (qualitative and quantitative description);
   c. Percent active ingredient;
   d. Molecular structure;
   e. Source, lot number, or code;
   f. Identity and percent composition of impurities in the test substance.

2. Preparation of test solution (control/treatment):

   a. Any vehicle used to dissolve test material;
   b. Amount of vehicle added to control (if used);
   c. Description of method used to get test material into solution;
   d. Water source (reconstituted - if so, how prepared);
   e. Solubility of test material in ppm;
   f. Total amount of test material used;
   g. Temperature of water;
   h. Salinity (o/oo).

B. Test Fish

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E:

   a. Test species name (both scientific and common);
   b. History of test organisms (strain, diseases, and treatment).

2. Description of any pretest conditioning:

   a. Health:

      1. Sickness;
      2. Injuries;
      3. Abnormalities;
      4. Name of medication (if used);
      5. Pretest diet;
      6. Number of days brood fish were quarantined;

3. Size/age/physical condition:

   a. Age (if known);
   b. Size (weight in grams and length in mm).

4. Source/acclimation:

   a. Complete name and address of test fish supplier;
   b. Source of food and dilution water;
   c. Size of test container [in milliliters (mL)];
   d. Temperature, pH, dissolved oxygen, and name of equipment used to measure water quality;
   e. Feeding schedule, holding, and acclimation period;
   f. Test organisms from the same source (yes or no);
   g. Number of days held;
   h. Salinity (o/oo).

C. Method

1. Test Vessels:

   a. Material type;
   b. Volume [in liters (L)];
   c. Depth of test solution [in centimeters (cm)];
   d. Size [in liters (L)].

2. Test system:

   a. Source of dilution water;
   b. If flow-through, description of system and flow rate/day;
   c. Procedures used to prepare toxicant stock solution;
   d. Criteria used to determine effects.

3. Test design:

   a. Method used in assigning test organisms to test and control groups, and the number of replicates used;
   b. Number of fish per dose level and control group;
   c. Name of protocol followed during this test;
   d. Loading (weight per unit volume of water);
   e. Number of treatment levels used (nominal or measured);
   f. Length of exposure period;
   g. Type of control (positive, negative, or solvent control);
   h. Temperature;
   i. pH (when checked - see table 1);
   j. Lighting (time in hours and intensity in footcandles);
   k. Dissolved oxygen (see table 1);
   l. Range finding test results, concentrations, and mortality (if used);
   m. Aeration (yes or no);
   n. Water physical characteristic at the end of test:
      1. Water depth and volume;
      2. Temperature, pH, and dissolved oxygen;

o. Concentration analysis;
p. Examination of fish physiology, locomotion, behavior and pathology;
q. Mortalities during the test.

D. Statistical Analysis

1. Cite references (author, title, journal, number, page, etc.).

III. DISCUSSION AND RESULTS

A. Provide LC_{50} value in ppm with 95% confidence limits (graphs, printouts, and other calculations should be attached to report). Provide raw mortality data (see table 2).

B. Discuss the relationship, if any, between the physical factors, toxicant, and observation, and describe any precipitation and solubility problems.

C. Provide statistical method used (cite references, author, etc.).

D. Observation - Provide data on the following:

1. Signs of intoxication - temporal onset, duration, and concentrations that showed effects;

2. The no-observed-effect level.

IV. CONCLUSIONS

This is a summation of the above section - Discussion/Results.

TABLE 1

WATER QUALITY PARAMETERS

Client:
Test Material:
Test Organism:
Test Water:

WATER QUALITY

Temperature (°C)	6 hrs, 12 hrs, etc				
		Control	High	Medium	Low
Dissolved Oxygen (mg/L)	Initial	X	X	X	X
	48 hours	X	X	X	X
	96 hours	X	X	X	X
pH	Initial	X	X	X	X
	96 hours	X	X	X	X

TABLE 2

PERCENT MORTALITIES AND LC_{50} VALUES

Client :
Test Material:
Test Organism:
Test Water :

PERCENT MORTALITY

TEST MATERIAL NOMINAL CONCENTRATION (mg/L)

	Control	X	X	X	X	X
24 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)
48 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)
96 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)

		24 hour	48 hour	96 hour
LC_{50} (mg/L)		X	X	X
95% Confidence Limits	Low	X	X	X
	High	X	X	X

Observed Effects

[a] floating
[b] swimming at surface
[c] lying on bottom
[d] turned dark
[e] loss of equilibrium
[f] gulping of air
[g] etc.

1/ How the percent of mortality table works.

1. #(#/#)[a].
2. # = the total number of organisms tested per dose level.
3. #(#/#) = % mortality [No. dead/No. treated, e.g., 10% (1/10)]
4. a = the observed effects.

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Oyster Embryo Test

Subdivision E, Series 72-3

DATA REPORTING

GUIDELINE

The following describes the order and format for a study item by item.

TITLE/COVER PAGE

Refer to PR Notice 86-5.

TABLE OF CONTENTS

The Table of Contents should indicate the overall organization of the study, including tables and figures. It must follow the title, data confidentiality, and GLP (if appropriate) pages as described in PR Notice 86-5.

I. SUMMARY/INTRODUCTION

A. Sponsor: (name of study owner);

B. Name: (person(s) who can provide details on test procedures);

C. Location of study;

D. Location of raw data and final report;

E. Material: (common/trade name);

F. Subject: (final or draft report, 48-hour EC_{50} and species tested);

G. Test Doses: (control, concurrent vehicle control, #, #, #, #, and # ppm);

H. Test Dates: (initiation, date test started, termination and date of last day of observation);

I. Length of Study: (days);

J. Results: (EC_{50} value, 95% confidence limits, and no-observed-effect level);

K. Tested Material: (chemical name, formulation, and percent active ingredient);

L. Test Species/Strain: (both common and scientific name);

M. Source of Organisms: (company and address);

N. Stage of Organisms at the Initiation of Study: (hours).

II. MATERIAL/METHODS

This section is a narrative, which would include the following items.

A. Test Substance

1. Identification (this information may be provided by the laboratory or sponsor):

   a. Chemical name;
   b. Composition (qualitative and quantitative description);
   c. Percent active ingredient;
   d. Molecular structure;
   e. Source, lot number, or code;
   f. Identity and percent composition of impurities in the test substance.

2. Preparation of test solution (control/treatment):

   a. Any vehicle used to dissolve test material;
   b. Amount of vehicle added to control (if used);
   c. Description of method used to get test material into solution;
   d. Water source (reconstituted - if so, how prepared);
   e. Solubility in ppm;
   f. Total amount of test material used;
   g. Salinity (o/oo).

B. Test Oyster

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E:

   a. Test species name (both scientific and common);
   b. History of test organisms (strain, diseases, and treatment).

2. Description of fertilization process and method used to collect embryos.

3. Size/age:

   a. Stage of life at initiation of test;
   b. Date of spawn;
   c. List of abnormalities (if any occurred).

4. Source/acclimation:

   a. Complete name and address of test species supplier;
   b. Source of food and water;
   c. Volume of test container [in liters (L)];
   d. Photoperiod and lighting;
   e. Temperature, pH, dissolved oxygen, and name of equipment used to measure water quality;

f. Chemical treatment (if used);
g. Percent mortality;
h. Feeding schedule;
i. Test organisms from the same source (yes or no);
j. Salinity (o/oo).

C. Method

1. Test vessels:

a. Material type;
b. Volume [in liters (L)];
c. Volume of test solution [in centimeters (cm)];
d. Size [in centimeters (cm)].

2. Test system:

a. Source of dilution water;
b. If flow-through, description of system and flow rate/day;
c. Procedures used to prepare toxicant stock solution;
d. Criteria used to determine effects;
e. Duplication of treatment levels (yes or no).

3. Test design:

a. Method used in assigning test organisms to test and control groups;
b. Number of fertilized eggs per liter of test solution;
c. Loading (weight of organism per unit volume of water);
d. Number of treatment levels used (nominal or measured);
e. Length of treatment period;
f. Type of control (positive, negative, or solvent control);
g. Temperature (prior to and during spawning);
h. pH (when checked - see table 1);
i. Lighting (time in hours, intensity in footcandles, and transition period between light and dark);
j. Dissolved oxygen (see table 1);
k. Range finding test results (if used);
l. Aeration (yes or no);
m. Water physical characteristics at the end of test:
   1. Water depth and volume;
   2. Temperature, pH, dissolved oxygen, and salinity;
n. Name of protocol used during the test;
o. How embryos collected and preserved;
p. Number of normal developed larvae at the end of observation;
q. Number of deformed larvae at the end of observation period.

D. Statistical Analysis

A. Cite references (author, title, journal, number, page, etc.).

III. DISCUSSION AND RESULTS

A. Provide EC_{50} value in ppm with 95% confidence limits (graphs, printouts, and other calculations should be attached to report). Provide raw mortality data (see table 2).

B. Discuss the relationship, if any, between the physical factors, toxicant, and observation, and describe any precipitation and solubility problems.

C. Provide statistical method used (cite references, author, etc.).

D. Observation - Provide the following data:

1. Signs of intoxication - temporal onset and duration, and concentrations that showed effects;

2. The no-observed-effect level.

IV. CONCLUSIONS

This is a summation of the above section - Discussion/Results.

TABLE 1

WATER QUALITY PARAMETERS

Client :
Test Material:
Test Organism:
Test Water :

WATER QUALITY

Temperature (°C)	6 hrs, 12 hrs, etc.				
			High	Medium	Low
Dissolved Oxygen (mg/L)	Initial	X	X	X	X
	48 hours	X	X	X	X
pH	Initial	X	X	X	X
	48 hours	X	X	X	X

TABLE 2

PERCENT REDUCTION OF NORMAL LARVAE AND EC_{50} VALUES

Client :
Test Material:
Test Organism:
No. Per Conc.:
Test Water :

PERCENT REDUCTION OF NORMAL LARVAE

TEST MATERIAL NOMINAL OR MEASURED CONCENTRATION (mg/L)

	Control	X	X	X	X	X
24 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)
48 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)

		24 hour	48 hour
EC_{50} (mg/L)		X	X
95% Confidence Limits	Low	X	X
	High	X	X

Observed Effects

[a] floating
[b] dead
[c] lying on bottom
[d] etc.

1/ How the percent of mortality table works.

1. #(#/#)[a].
2. # = the total number of organisms tested per dose level.
3. #(#/#) = % mortality [No. dead/No. treated, e.g., 10% (1/10)].
4. a = the observed effects.

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Shell Deposition Study for Oyster

Subdivision E, Series 72-3

DATA REPORTING

GUIDELINE

The following describes the order and format for a study report item by item.

TITLE/COVER PAGE

Refer to PR Notice 86-5.

TABLE OF CONTENTS

The Table of Contents should indicate the overall organization of the study, including tables and figures. It must follow the title, data confidentiality, and GLP (if appropriate) pages as described in PR Notice 86-5.

I. SUMMARY/INTRODUCTION

A. Sponsor: (name of study owner);

B. Name(s) of principal investigator(s);

C. Location of study;

D. Location of raw data and final report;

E. Material: (common/trade name);

F. Subject: (final or draft report, 96-hour EC_{50}, and species tested);

G. Test Doses: (control, concurrent vehicle control, #, #, #, #, # ppm);

H. Test Dates: (initiation, date test started, termination, and date of last day of observation);

I. Length of Study: (days);

J. Results: (EC_{50} value, 95% confidence limits, and no-observed-effect level);

K. Tested Material: (chemical name, formulation, and percent active ingredient);

L. Test Species/Strain: (both common and scientific name);

M. Source of Organisms: (company and address);

N. Size of Organisms at the Initiation of Study: (mm).

II. MATERIAL/METHODS

This section is a narrative, which would include the following items.

A. Test Substance

1. Identification (this information may be provided by the laboratory or sponsor):

   a. Chemical name;
   b. Composition (qualitative and quantitative description);
   c. Percent active ingredient;
   d. Molecular structure;
   e. Source, lot number, or code;
   f. Identity and percent composition of impurities in the test substance.

2. Preparation of test solution (control/treatment):

   a. Any vehicle used to dissolve test material;
   b. Amount of vehicle added to control (if used);
   c. Description of method used to get test material into solution;
   d. Water source (reconstituted - if so, how prepared);
   e. Solubility of test material in ppm;
   f. Total amount of test material used;
   g. Salinity (o/oo).

B. Test Oyster

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E:

   a. Test species name (both scientific and common);
   b. History of test organisms (strain, diseases, and treatment).

2. Description of any pretest conditioning:

   a. Health:

      1. Sickness;
      2. Injuries;
      3. Abnormalities;
      4. Pretest diet;

3. Size/age/physical condition:

   a. Height (in mm);
   b. Abnormalities (if any occurred).

4. Source/acclimation:

   a. Complete name and address of test species supplier;
   b. Source of food and water;
   c. Size of test container [in liters (L)];
   d. Photoperiod and lighting;
   e. Temperature, pH, dissolved oxygen, and name of equipment used to measure water quality;
   f. Chemical treatment (if used);
   g. Percent mortality;
   h. Feeding schedule;
   i. Test organisms from the same source (yes or no);
   j. Salinity and acclimation period in days.

C. Method

1. Test Vessels:

   a. Material type;
   b. Volume [in liters (L)];
   c. Depth of test solution [in centimeters (cm)];
   d. Size [in liters (L)].

2. Test system:

   a. Source of dilution water;
   b. If flow-through, description of system and flow rate/day;
   c. Procedures used to prepare toxicant stock solution;
   d. Criteria used to determine effects.

3. Test design:

   a. Method used in assigning test organisms to test and control groups;
   b. Number of organisms per dose level;
   c. Loading (weight of organism per unit volume of water);
   d. Number of treatment levels used (nominal or measured);
   e. Length of treatment period;
   f. Type of control (positive, negative, or solvent control);
   g. Temperature (how often measured);
   h. pH (when checked - see table 1);
   i. Lighting (time in hours, intensity in footcandles, and transition period between light and dark);
   j. Dissolved oxygen (see table 1);
   k. Range finding test results (if used - include sample size, test levels, and mortality data);
   l. Aeration (yes or no);
   m. Water physical characteristic at the end of test:
      1. Water depth and volume;
      2. Temperature, pH, and dissolved oxygen;
   n. Percent of death/effects at each dose level;
   o. Name of protocol followed during the test;
   p. How peripheral valve edge removed;
   q. Method used to measure oyster size.

D. Statistical Analysis

1. Cite references (author, title, journal, number, page, etc.).

III. DISCUSSION AND RESULTS

A. Provide EC_{50} value in ppm with 95% confidence limits (graphs, printouts, and other calculations should be attached to report). Provide raw mortality data (see table 2).

B. Discuss the relationship, if any, between the physical factors, toxicant, and observation, and describe any precipitation and solubility problems.

C. Provide statistical method used (cite references, author, etc.).

D. Observation - Provide the following data:

1. Signs of intoxication - temporal onset and duration, and concentrations that showed effects;

2. The no-observed-effect level.

IV. CONCLUSIONS

This is a summation of the above section - Discussion/Results.

TABLE 1

WATER QUALITY PARAMETERS

Client :
Test Material:
Test Organism:
Test Water :

WATER QUALITY

Temperature 6 hours, 12 hours, etc.

Parameter	Time	Control	Nominal Concentration (mg/L) X	X	X	X	X
			High		Medium		Low
Dissolved Oxygen (mg/L)	Initial	X	X		X		X
	48 hours	X	X		X		X
	96 hours	X	X		X		X
pH	Initial	X	X		X		X
	96 hours	X	X		X		X
Salinity o/oo	Initial	X	X				
	96 hours	X	X				

TABLE 2

PERCENT CHANGE IN SHELL GROWTH AND EC_{50} VALUES

Client :
Test Material:
Test Organism:
Test Water :

PERCENT CHANGE IN SHELL GROWTH

TEST MATERIAL NOMINAL OR MEASURED CONCENTRATION
(mg/L)

	Control	X	X	X	X	X
24 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)
48 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)
96 Hour	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)

		24 hour	48 hour	96 hour
EC_{50} (mg/L)		X	X	X
95% Confidence Limits	Low	X	X	X
	High	X	X	X

Observed Effects

[a] floating
[b] dead
[c] no growth
[d] etc.

1/ How the percent of change in shell growth table works.

1. #(#/#)[a].
2. # = the total number of organisms tested per dose level.
3. #(#/#) = % mortality [No. dead/No. treated, e.g., 10% (1/10)]
4. a = the observed effects.

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Fish Early Life-Stage

Subdivision E, Series 72-4

DATA REPORTING

GUIDELINE

The following describes the order and format for a study report item by item.

TITLE/COVER PAGE

Refer to PR Notice 86-5.

TABLE OF CONTENTS

The Table of Contents should indicate the overall organization of the study, including tables and figures. It must follow the title, data confidentiality, and GLP (if appropriate) pages as described in PR Notice 86-5.

I. SUMMARY/INTRODUCTION

A. Sponsor: (name of study owner);

B. Name(s) of principal investigator(s)

C. Location of study;

D. Location of raw data and final report;

E. Material: (common/trade name);

F. Subject: (final or draft report, fish early life-stage, and species tested);

G. Test Doses: (control, concurrent vehicle control, #, #, #, #, # ppm);

H. Test Dates: (initiation, date test started, termination and date of last day of observation);

I. Length of Study: (days);

J. Results: (MATC value ppm and no-observed-effects level);

K. Tested Material: (chemical name, formulation, and percent active ingredient);

L. Test Species/Strain: (both common and scientific name);

M. Source of Eggs: (company and address);

N. Stage of Eggs development at the Initiation of Study.

I. MATERIAL/METHODS

This section is a narrative, which would include the following items.

A. Test Substance

1. Identification (this information may be provided by the laboratory or sponsor):

   a. Chemical name;
   b. Composition (qualitative and quantitative description);
   c. Percent active ingredient;
   d. Molecular structure;
   e. Source, lot number, or code;
   f. Identity and percent composition of impurities in the test substance.

2. Preparation of test solution (control/treatment):

   a. Any vehicle used to dissolve test material;
   b. Amount of vehicle added to control (if used);
   c. Description of method used to get test material into solution;
   d. Water source (reconstituted - if so, how prepared);
   e. Solubility in ppm;
   f. Total amount of test material used.

B. Test Fish

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E (if eggs were not used, give stage of life cycle in which organisms were tested):

   a. Test species name (both scientific and common);
   b. History of test organisms (strain, diseases, and treatment).

2. Description of any pretest conditioning:

   a. Health:

      1. Sickness;
      2. Injuries;
      3. Abnormalities;
      4. Name of medication (if used);
      5. Pretest diet;
      6. Number of days brood organisms were quarantined.

3. Size/age/physical condition:

   a. Age of unfertilized eggs and milt at the beginning of test;
   b. Description of procedures used to strip eggs and milt;
   c. Description of the procedures used in storage and handling of eggs;
   d. Description of the fertilization process.

4. Source/acclimation:

   a. Complete name and address of test species supplier;
   b. Source of food and dilution water;
   c. Size of test container [in liters (L)];
   d. Number of females used per batch;
   e. Temperature, pH, and dissolved oxygen;
   f. Description of methods used initially to determine different stages of development;
   g. Percent mortality (brood fish);
   h. Feeding schedule, holding and acclimation period;
   i. Test organisms from the same source (yes or no);
   j. Number of males used per batch of eggs.

C. Method

1. Test Vessels:

   a. Material type;
   b. Volume [in milliliters (mL)];
   c. Depth of test solution [in centimeters (cm)];
   d. Size of vessels [in centimeters (cm)].

2. Test system:

   a. Source of dilution water;
   b. If flow-through, description of system construction material, system, and flow rate/day;
   c. Procedures used to prepare toxicant stock solution;
   d. Criteria used to determine effects.

3. Test design:

   a. Method used in assigning test organisms to test and control groups;
   b. Number of eggs per dose level;
   c. Name of protocol followed during this test;
   d. Loading (number of organism per unit volume of water);
   e. Number of treatment levels used (nominal or measured);
   f. Length of exposure period;
   g. Type of control (positive, negative, or solvent control);
   h. Temperature;
   i. pH (when checked - see table 2);
   j. Lighting (time in hours and intensity in footcandles);
   k. Dissolved oxygen (see table 2);
   l. Water hardness (expressed in mg/L as $CaCO_3$ - see table 2);
   m. Alkalinity (expressed in mg/L as $CaCO_3$ - see table 2);
   n. Conductivity (umhos/cm - see table 2);
   o. Range finding test results, concentrations, and mortality (if used);
   p. Aeration (yes or no);

q. Water physical characteristic at the end of test:
   1. Water depth and volume;
   2. Temperature, pH, dissolved oxygen, and name of equipment used to measure the water quality;
r. Period food was withheld prior to termination;
s. Number of normal fry at the end of test (see table 3);
t. Concentration analysis (see table 1);
u. Examination of fish physiology, locomotion, and behavior;
v. Length and weight (see table 5);
w. Mortalities during the test;
x. Fry survival and growth;
y. The larvae fish released from the retaining cup when batch was ( ) % complete or ( ) hours has elapsed;
z. Description of method used to keep eggs clean.

D. Statistical Analysis

1. Cite references (author, title, journal, number, page, etc.).

III. DISCUSSION AND RESULTS

A. Provide MATC value in ppm (graphs, printouts, and other calculations should be attached to report). Provide raw mortality data (see table 3).

B. Discuss the relationship, if any, between the physical factors, toxicant, and observation.

C. Provide statistical method used (cite references, author, etc.).

D. Observation - Provide the following data:

1. Signs of intoxication - temporal onset and duration;

2. The no-observed-effect level.

IV. CONCLUSIONS

This is a summation of the above section - Discussion/Results.

TABLE 1

MEASURED CONCENTRATION OF TEST COMPOUND IN
WATER TO WHICH (Test fish name) (Scientific Name)
WERE EXPOSED FOR # DAYS

Nominal Concentration (mg/L)	Mean ± SD	Range	Number of Samples
"	" ± "	"___"	"
"	" ± "	"___"	"
"	" ± "	"___"	"
"	" ± "	"___"	"
"	" ± "	"___"	"
Water Control	" --- " (Detected)	"---"	"
Solvent Control	Detected or not		"

TABLE 2

WATER QUALITY CHARACTERISTICS MEASURED
DURING EXPOSURE OF (TEST FISH)
EMBRYOS AND LARVAE TO (TEST COMPOUND)

Water Quality Characteristic	Mean ± SD	Range	Number of Measurements
Temperature (°C)	" ± "	"—"	"
Dissolved Oxygen (mg/L)	" ± "	"—"	"
pH	"— —"	"—"	"
Total Hardness (mg/L as $CaCO_3$)	" ± "	"—"	"
Total Alkalinity (mg/L as $CaCO_3$)	" ± "	"—"	"
Specific Conductance (umhos/cm)	" ± "	"—"	"

TABLE 3

SURVIVAL OF (TEST FISH NAME) AT HATCH (DAY #), AT # DAYS POST-HATCH, AFTER EXPOSURE TO SEVERAL CONCENTRATIONS OF (TEST COMPOUND)

Mean Measured Concentration (mg/L)	Tank	Percent Larvae Survival At Hatch on Day # (Mean)		Percent Survival At # Days Post-Hatch (Mean)		Percent Survival At # Days Post-Hatch (Mean)	
#	#	#	#	#	#	#	#
	#	#		#		#	
#	#	#	#	#	#	#	#
	#	#		#		#	
#	#	#	#	#	#	#	#
	#	#		#		#	
#	#	#	#	#	#	#	#
	#	#		#		#	
#	#	#	#	#	#	#	#
	#	#		#		#	
Water Control	#	#	#	#	#	#	#
	#	#		#		#	
Solvent Control	#	#	#	#	#	#	#
	#	#		#		#	

TABLE 4

LENGTH AND WEIGHT OF [TEST FISH NAME] AT # DAYS POST-HATCH AFTER EXPOSURE TO SEVERAL CONCENTRATIONS OF (TEST COMPOUND)

Mean Measured Concentration (mg/L)	Tank	Total Length (mm) Mean ± SD (N) (Treatment Mean ± SD)	Weight (g) Replicate (N) Average (Treatment Average)
#	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)
#	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)
#	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)
#	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)
#	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)
Water Control	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)
Solvent Control	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)

[a] The mean length of these fish was/was not significantly less than the mean length of fish in solvent control group according to a (statistic name) test ($P \leq 0.05$).

TABLE 5

LENGTH AND WEIGHT OF [TEST FISH NAME] AT # DAYS POST-HATCH AFTER EXPOSURE TO SEVERAL CONCENTRATIONS OF (TEST COMPOUND)

Mean Measured Concentration (mg/L)	Tank	Total Length (mm) Mean ± SD (N) (Treatment Mean ± SD)	Weight (g) Replicate (N) Average (Treatment Average)
#	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)
#	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)
#	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)
#	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)
#	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)
Water Control	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)
Solvent Control	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)

[a] Mean lengths and mean weights of the groups of fish exposed to (test compound) were/were not significantly different than the mean length of fish in water and solvent control groups according to a (statistic name) test (   ).

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Aquatic Invertebrate Life-Cycle

Subdivision E, Series 72-4

DATA REPORTING

GUIDELINE

The following describes the order and format for a study report item by item.

TITLE/COVER PAGE

Refer to PR Notice 86-5.

TABLE OF CONTENTS

The Table of Contents should indicate the overall organization of the study, including tables and figures. It must follow the title, data confidentiality, and GLP (if appropriate) pages as described in PR Notice 86-5.

I. SUMMARY/INTRODUCTION

A. Sponsor: (name of study owner);

B. Name: (person(s) who can provide details of test procedures);

C. Location of study;

D. Location of raw data and final report;

E. Material: (common/trade name);

F. Subject: (final or draft report, invertebrate life-cycle, and species tested);

G. Test Doses: (control, concurrent vehicle control, #, #, #, #, # ppm);

H. Test Dates: (initiation, date test started, termination, and date of last day of observation);

I. Length of Study: (days);

J. Results: (MATC value in ppm and no-observed-effects level);

K. Tested Material: (chemical name, formulation, and percent active ingredient);

L. Test Species/Strain: (both common and scientific name);

M. Source of Organisms: (company and address);

N. Age of Organisms at the Initiation of Study: (hours).

II. MATERIAL/METHODS

This section is a narrative which would include the following items.

A. Test Substance

1. Identification (this information may be provided by the laboratory or sponsor):

   a. Chemical name;
   b. Composition (qualitative and quantitative description);
   c. Percent active ingredient;
   d. Molecular structure;
   e. Source, lot number, or code;
   f. Identity and percent composition of impurities in the test substance.

2. Preparation of test solution (control/treatment):

   a. Any vehicle used to dissolve test material;
   b. Amount of vehicle added to control (if used);
   c. Description of method used to get test material into solution;
   d. Water source (reconstituted - if so, how prepared);
   e. Solubility in ppm;
   f. Total amount of test material used;

B. Test Invertebrate

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E:

   a. Test species name (both scientific and common);
   b. History of test organisms (strain, diseases, and treatment).

2. Description of any pretest conditioning:

   a. Health:

      1. Sickness;
      2. Injuries;
      3. Abnormalities;
      4. Mortalities (%);
      5. Pretest diet.

3. Size/age/physical condition:

   a. Age at initiation of test (in hours);
   b. Date spawned;
   c. List of any abnormalities.

4. Source/acclimation:

   a. Complete name and address of test species supplier;
   b. Source of food and dilution water;
   c. Size of test container [in milliliters (mL)];
   d. Temperature, pH, and dissolved oxygen;
   e. Percent mortality;
   f. Feeding schedule;
   g. Test organisms from the same source (yes or no);
   h. Number of days adults were held in laboratory prior to use of offspring.

C. Method

1. Test Vessels:

   a. Material type;
   b. Volume [in milliliters (mL)];
   c. Volume of test solution [in centimeters (cm)];
   d. Size of vessel [in milliliters (mL)].

2. Test system:

   a. Source of dilution water;
   b. If flow-through, description of system and flow rate/day;
   c. Procedures used to prepare toxicant stock solution;
   d. Criteria used to determine effects.

3. Test design:

   a. Method used in assigning test organisms to test and control groups;
   b. Number of organisms per dose level;
   c. Name of protocol followed during this test;
   d. Loading (number of organisms per unit volume of water);
   e. Number of treatment levels used (nominal or measured);
   f. Length of exposure period;
   g. Type of control (positive, negative, or solvent control);
   h. Temperature;
   i. pH (when checked - see table 1);
   j. Lighting (time in hours and intensity in footcandles);
   k. Dissolved oxygen (see table 1);
   l. Water hardness (expressed in mg/L as $CaCO_3$ - see table 1);
   m. Alkalinity (expressed in mg/L as $CaCO_3$ - see table 1);
   n. Conductivity (umhos/cm - see table 1);
   o. Range finding test results, concentrations, and mortality (if used);
   p. Aeration (yes or no);
   q. Water physical characteristic at the end of test:
      1. Water depth and volume;
      2. Temperature, pH, and dissolved oxygen;
   r. Number of normal organisms at the end of test (see table 3);
   s. Concentration analysis (see table 1);

t. Examination of invertebrate physiology, locomotion, behavior, and pathology;
u. Organisms count/measurement (see table 3);
v. Mortalities during the test;
x. If static system was used, how often test solution renewed.

D. Statistical Analysis

1. Cite references (author, title, journal, number, page, etc.).

III. DISCUSSION AND RESULTS

A. Provide MATC value in ppm (graphs, printouts, and other calculations should be attached to report). Provide raw mortality data (see table 3).

B. Discuss the relationship, if any, between the physical factors, toxicant, and observations.

C. Provide statistical method used (cite references, author, etc.).

D. Observation - Provide the following data:

1. Detailed record of spawning, fertility, and fecundity;

2. Signs of intoxication - temporal onset and duration;

3. The no-observed-effect level and reproductive effects.

IV. CONCLUSIONS

This is a summation of the above section - Discussion/Results.

TABLE 1

WATER QUALITY CHARACTERISTICS MEASURED
DURING EXPOSURE OF (TEST ORGANISMS)
TO (TEST COMPOUND)

Water Quality Characteristic	Mean ± SD	Range	Number of Measurements
Temperature (°C)	" ± "	"—"	"
Dissolved Oxygen (mg/L)	" ± "	"—"	"
pH	"— —"	"—"	"
Total Hardness (mg/L as $CaCO_3$)	" ± "	"—"	"
Total Alkalinity (mg/L as $CaCO_3$)	" ± "	"—"	"
Specific Conductance (umhos/cm)	" ± "	"—"	"

TABLE 2

PERCENT MORTALITIES AND MATC VALUES

Client :
Test Material:
Test Organism:
Test Water :

PERCENT MORTALITY

TEST MATERIAL NOMINAL CONCENTRATION
(mg/L)

	Control					
Day #	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)
Day #	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)	#(#/#)

		Day #	Day #
Chronic Results			
MATC (mg/L)		"	"
No effect level		"	"
95% Confidence Limits	Low	"	"
	High	"	"

Observed Effects

[a] floating
[b] swimming near surface
[c] lying on bottom
[d] turned dark
[e] etc.

1/ How the percent of mortality table works.

1. #(#/#)[a].
2. # = the total number of organisms tested per dose level.
3. #(#/#) = % mortality [No. dead/No. treated, e.g., 10% (1/10)]
4. a = the observed effects.

TABLE 3

INVERTEBRATE MEASUREMENT/COUNT

CONCENTRATIONS	# OF ADULTS	# OF YOUNG/ALIVE/DEAD			LENGTH
#	#	#	#	#	#
#	#	#	#	#	#
#	#	#	#	#	#
CONTROLS					
#	#	#	#	#	#
#	#	#	#	#	#

PESTICIDE ASSESSMENT GUIDELINES

WILDLIFE AND AQUATIC ORGANISMS

Life-Cycle Tests for Fish

Subdivision E, Series 72-5

DATA REPORTING

GUIDELINE

The following describes the order and format for a study report item by item.

TITLE/COVER PAGE

Refer to PR Notice 86-5.

TABLE OF CONTENTS

The Table of Contents should indicate the overall organization of the study, including tables and figures. It must follow the title, data confidentiality, and GLP (if appropriate) pages as described in PR Notice 86-5.

I. SUMMARY/INTRODUCTION

A. Sponsor: (name of study owner);

B. Name: (person(s) who can provide details of test procedures);

C. Location of study;

D. Location of raw data and final report;

E. Material: (common/trade name);

F. Subject: (final or draft report, fish life-cycle, and species tested);

G. Test Doses: (control, concurrent vehicle control, #, #, #, #, # ppm);

H. Test Dates: (initiation, date test started, termination, and date of last day of observation);

I. Length of Study: (days);

J. Results: (MATC value and no-observed-effects level);

K. Tested Material: (chemical name, formulation, and percent active ingredient);

L. Test Species/Strain: (both common and scientific name);

M. Source of Organisms: (company and address);

N. Stage of Organisms at the Initiation of Study: (hours).

II. MATERIAL/METHODS

This section is a narrative, which would include the following items.

A. Test Substance

1. Identification (this information may be provided by the laboratory or sponsor):

   a. Chemical name;
   b. Composition (qualitative and quantitative description);
   c. Percent active ingredient;
   d. Molecular structure;
   e. Source, lot number, or code;
   f. Identity and percent composition of impurities in the test substance.

2. Preparation of test solution (control/treatment):

   a. Any vehicle used to dissolve test material;
   b. Amount of vehicle added to control (if used);
   c. Description of method used to get test material into solution;
   d. Water source (reconstituted - if so, how prepared);
   e. Solubility of test material in ppm;
   f. Total amount of test material used.

B. Test Fish

1. Rationale for selection of species if the species used is different than that preferred in Subdivision E (if eggs were not used, give stage of life in which organisms were tested):

   a. Test species name (both scientific and common);
   b. History of test organisms (strain, diseases, and treatment).

2. Description of any pretest conditioning:

   a. Health:

      1. Sickness;
      2. Injuries;
      3. Abnormalities;
      4. Pretest diet;
      5. Number of days brood fish were quarantined.

3. Size/age/physical condition:

   a. Age of unfertilized eggs and milt (in hours);
   b. Description of the procedures used to strip the eggs and milt;
   c. Description of the procedures used in handling and storage of eggs;
   d. Description of the fertilization procedures.

4. Source/acclimation:

   a. Complete name and address of test species supplier;
   b. Source of food and dilution water;
   c. Size of test container [in liters (L)];
   d. Number of females used per batch;
   e. Temperature, pH, dissolved oxygen, and name of equipment used to measure water quality;
   f. Description of methods used initially to determine different stages of development;
   g. Percent mortality (brood fish);
   h. Feeding schedule, holding, and acclimation period;
   i. Test organisms from the same source (yes or no);
   j. Number of males used per batch of eggs.

C. Method

1. Test vessels:

   a. Material type;
   b. Volume [in milliliters (mL)];
   c. Volume of test solution [in milliliters (mL)];
   d. Size [in centimeters (cm)].

2. Test system:

   a. Source of dilution water;
   b. If flow-through, description of system construction material, system, and flow rate/day;
   c. Procedures used to prepare toxicant stock solution;
   d. Criteria used to determine effects.

3. Test design:

   a. Method used in assigning test organisms to test and control groups and number of replicates used;
   b. Number of eggs per dose level;
   c. Name of protocol followed during this test;
   d. Loading (number of eggs per unit volume of water - if known);
   e. Number of treatment levels used (nominal or measured);
   f. Length of exposure period;
   g. Type of control (positive, negative, or solvent control);
   h. Temperature;
   i. pH (when checked - see table 2);
   j. Lighting (time in hours and intensity in footcandles);
   k. Dissolved oxygen (see table 2);
   l. Water hardness (expressed in mg/L as $CaCO_3$ - see table 2);
   m. Alkalinity (expressed in mg/L as $CaCO_3$);
   n. Conductivity (umhos/cm - see table 2);
   o. Range finding test results, concentrations, and mortality (if used);
   p. Aeration (yes or no);

q. Water physical characteristic at the end of test:
   1. Water depth and volume;
   2. Temperature, pH, and dissolved oxygen;
r. Period food was withheld prior to termination;
s. Number of normal fry at the end of test (see tables 3a and 3b);
t. Concentration analysis (see table 1);
u. Examination of fish physiology, locomotion, and behavior;
v. Organisms length and weight (see table 5);
w. Mortalities and effects during the test;
x. If static system was used, how often test solution renewed;
y. Fry survival, growth, and deformities.

D. Statistical Analysis

1. Cite references (author, title, journal, number, page, etc.).

III. DISCUSSION AND RESULTS

A. Provide maximum acceptable toxic concentration (MATC) expressed as the range between the lowest observed effect concentration and the highest observed no effect concentration in ppm.

B. Discuss the relationship, if any, between the physical factors, toxicant, and observation.

C. Provide statistical method used (cite references, author, etc.)

IV. CONCLUSIONS

This is a summation of the above section - Discussion/Results.

TABLE 1

MEASURED CONCENTRATION OF TEST COMPOUND IN
WATER TO WHICH (Test fish name) (Scientific Name)
WERE EXPOSED FOR # DAYS

Nominal Concentration (mg/L)	Mean ± SD	Range	Number of Samples
"	" ± "	"___"	"
"	" ± "	"___"	"
"	" ± "	"___"	"
"	" ± "	"___"	"
"	" ± "	"___"	"
Water Control	" --- " (Detected)	"___"	"
Solvent Control	Detected or not		"

TABLE 2

WATER QUALITY CHARACTERISTICS MEASURED
DURING EXPOSURE OF (TEST FISH)
EMBRYOS AND LARVAE TO (TEST COMPOUND)

Water Quality Characteristic	Mean ± SD	Range	Number of Measurements
Temperature (°C)	" ± "	"—"	"
Dissolved Oxygen (mg/L)	" ± "	"—"	"
pH	"— —"	"—"	"
Total Hardness (mg/L as $CaCO_3$)	" ± "	"—"	"
Total Alkalinity (mg/L as $CaCO_3$)	" ± "	"—"	"
Specific Conductance (umhos/cm)	" ± "	"—"	"

TABLE 3a (F_1)

SURVIVAL OF (TEST FISH NAME) AT HATCH (DAY #), AT # DAYS POST-HATCH, AT # DAYS POST-HATCH AND SWIM-UP AFTER EXPOSURE TO SEVERAL CONCENTRATIONS OF (TEST COMPOUND)

Mean Measured Concentration (mg/L)	Tank	Percent Larvae Survival At Hatch on Day # (Mean)		Percent Survival At # Days Post-Hatch (Mean)		Percent Survival At # Days Post-Hatch (Mean)		Percent At Swim-up At # Days Post-Survival (Mean)	
#	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
#	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
#	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
#	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
#	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
Water Control	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
Solvent Control	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	

TABLE 3b (F_2)

SURVIVAL OF (TEST FISH NAME) AT HATCH (DAY #), AT # DAYS POST-HATCH, AT + DAYS POST-HATCH AND SWIM-UP AFTER EXPOSURE TO SEVERAL CONCENTRATIONS OF (TEST COMPOUND)

Mean Measured Concentration (mg/L)	Tank	Percent Larvae Survival At Hatch on Day #	(Mean)	Percent Survival At # Days Post-Hatch	(Mean)	Percent Survival At # Days Post-Hatch	(Mean)	Percent At Swim-up At # Days Post-Survival	(Mean)
#	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
#	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
#	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
#	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
#	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
Water Control	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	
Solvent Control	#	#	#	#	#	#	#	#	#
	#	#		#		#		#	

TABLE 4

LENGTH AND WEIGHT OF [TEST FISH NAME] AT # DAYS POST-HATCH AFTER EXPOSURE TO SEVERAL CONCENTRATIONS OF (TEST COMPOUND)

Mean Measured Concentration (mg/L)	Tank	Total Length (mm) Mean ± SD (N) (Treatment Mean ± SD)	Weight (g) Replicate (N) Average (Treatment Average)
#	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	# ± #
#	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)
#	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)
#	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)
#	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)
Water Control	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)
Solvent Control	#	# ± #	# ± #
	#	# ± #	# ± #
		(# ± #)	(# ± #)

[a] The mean length of these fish was/was not significantly less than the mean length of fish in solvent control group according to a (statistic name) test ($P \leq 0.05$).

TABLE 5

LENGTH AND WEIGHT OF [TEST FISH NAME] AT # DAYS POST-HATCH AFTER EXPOSURE TO SEVERAL CONCENTRATIONS OF (TEST COMPOUND)

Mean Measured Concentration (mg/L)	Tank	Total Length (mm) Mean ± SD (N) (Treatment Mean ± SD)	Weight (g) Replicate (N) Average (Treatment Average)
#	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)
#	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #
#	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)
#	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± (#)
		(# ± #)	(# ± #)
#	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)
Water Control	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)
Solvent Control	#	# ± # (#)	# ± # (#)
	#	# ± # (#)	# ± # (#)
		(# ± #)	(# ± #)

[a] The mean length of these fish was/was not significantly less than the mean length of fish in solvent control group according to a (statistic name) test ($P \leq 0.05$).