

Data Evaluation Record on the Fish Short-Term Reproduction Assay with **[TGAI or EP]**

EPA MRID Number [.....]

**Data Requirement:** EPA DP Barcode [.....] *if applicable*  
OECD Data Point [.....] *if applicable*  
EPA MRID [.....] *if applicable*  
EPA Guideline 890.1350  
Fish Short-Term Reproduction Assay

**Test Material:** [.....] **Purity (%):** [.....]  
Common Name [.....]  
Chemical Name IUPAC [.....]  
CAS Name [.....]  
CAS No. [.....]  
Synonyms [.....]  
EPA PC Code [.....]

**Primary Reviewer:** [.....] **Date:** [.....] **[EPA/OECD/PMRA]**

**Secondary Reviewer(s):** [.....] **Date:** [.....] **[EPA/OECD/PMRA]**

**Date Evaluation Completed:** [dd-mmm-yyyy]

**CITATION:** [Indicate: Author(s), Year, Study Title, Laboratory Name and Location, Laboratory Report Number, Sponsor, Full Study Date. If published, list the name of the journal, vol., pages, year.]

**[Instructions, prompts, and example values for the individual(s) completing the DER are shown in the DER template in bracketed red text; these instructions and examples do not need to remain visible in the completed DER.]**

***Guideline recommendations are provided in italics; these recommendations should remain visible in the completed DER.***

***Disclaimer: The guideline recommendations in this DER template are offered as a general reference to aid in preparation of the DER. The purpose of these recommendations is not to serve as substitute for the Test Guidelines, nor to provide any guidance on how the study should be conducted.***

**EXECUTIVE SUMMARY**

The 21-day short-term reproduction assay of [test chemical] with [common name and scientific name] was studied under [flow-through, specify if other] conditions. Adult fish [enter number of fish and age] were exposed to [control, solvent control (if applicable), and test chemical nominal/measured concentrations] of [X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, .... X<sub>n</sub>] mg a.i./L. The test system was maintained at [...] to [...]°C and a pH of [...] to [...].

[Modify as appropriate.] Spawning frequency and fecundity in controls, respectively, were every [...] days and [...] eggs/female/day/replicate; fertilization success in controls was [...]%. [Test chemical] significantly [increased or decreased] fecundity at [list all relevant concentrations] mg a.i./L and [increased or decreased] fertilization success at [list all relevant concentrations] mg a.i./L. Plasma [vitellogenin, testosterone, and 17β-estradiol, if measured], respectively, were significantly [increased or decreased] in male fish at [list all relevant concentrations] mg a.i./L and were significantly [increased or decreased] in female fish at [list all relevant concentrations] mg a.i./L. Effects on gonadal histopathology were observed in male fish at [list all relevant concentrations] mg a.i./L and in female fish at [list all relevant concentrations] mg a.i./L. Histopathological effects included ..... [provide details of treatment-related effects, i.e., presence of testis-ova in males, increased oocyte atresia in females]. A significant [increase or decrease] in gonado-somatic index (GSI) was observed in male fish at [list all relevant concentrations] mg a.i./L and in female fish at [list all relevant concentrations] mg a.i./L. Nuptial tubercle score was significantly [increased or decreased] in [female or males] at [list all relevant concentrations] mg a.i./L. [Test chemical] exposure was associated with an [increase or decrease] in [body weight and/or length] in [male or female] fish at [list all relevant concentrations] mg a.i./L. Other effects on secondary sex characteristics and clinical signs (i.e., behavioral and other sublethal effects) including [discoloration, changes in dorsal nape pad, lethargy, loss of equilibrium] were observed in [number of fish] at [each relevant concentration] mg a.i./L. Unless otherwise indicated, all effects are reported based on comparison to the negative (clean water) control.

This assay [does or does not] satisfy the Test Order requirement for a Fish Short-Term Reproduction Assay (OCSPG Guideline 890.1350). [If it does not satisfy the requirement, concisely list the major deficiencies.]

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Results Synopsis:

Test organism age at test initiation: [...] months  
 Mean body weight at test initiation (if measured): [...] g for males, [...] g for females  
 Mean length at test initiation (if measured): [...] mm for males, [...] mm for females  
 Test type: [flow-through, other]

Table 1: Summary of Reproductive and HPG Effects<sup>1,2</sup> in the Fish Short-Term Reproduction Assay (FSTRA) with [test chemical].

Treatment (mg a.i./L) [measured]	Fecundity	Fert. Success	Tubercle Score		GSI		Gonadal Histo.		Plasma VTG		Plasma T		Plasma E2	
			M	F	M	F	M	F	M	F	M	F	M	F
Test conc. 1	No	No	Yes	No	Yes	No	Yes	No	No	No	No	No	No	No
Test conc. 2														
Test conc. 3														
Test conc. n														
Positive control, if used														

Abbreviations: <sup>Conc.</sup> Concentration. <sup>Diff.</sup> Difference. <sup>E2</sup> 17β-estradiol. <sup>F</sup> Female. <sup>Fert</sup> Fertilization. <sup>GSI</sup> Gonado-Somatic Index. <sup>Histo.</sup> Histopathology.

<sup>M</sup> Male. <sup>NA</sup> Not applicable. <sup>T</sup> Testosterone. <sup>VTG</sup> Vitellogenin.

<sup>1</sup> A “yes” indicates a significant difference based on comparison to the negative (clean water) control, unless otherwise specified.

<sup>2</sup> The criteria for significance are described in the Reviewer’s Analysis and Statistical Verification sections of the DER. Conclusions regarding histopathology may be heavily weighted by the expert opinion of a board-certified pathologist.

**I. MATERIALS AND METHODS**

**Guideline Followed:** [Specify the guideline(s) that were followed and any deviations from the guideline(s). State if the deviations affect the validity of the study.]

**Compliance:** [Indicate if signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.]

**A. Test Material** [Complete this subsection using the information provided in the methodology section of the study report. Include the name of the test material and CAS number as cited in the study report.]

**Description:** [eg. Chemical state of the test material]

*OECD recommends describing water solubility, melting/boiling point stability in water and light, pKa, Pow or Kow, vapor pressure of test compound, expiration date.*

**Lot No./Batch No. :** [.....]

**Purity:** [Indicate the % of active ingredient or purity of the test substance. If radiolabeled material was used, indicate the radiopurity and the location(s) of the label.]

**Impurities:** [Identify any impurities reported.]

**Stability of Compound:** [Briefly describe the stability of the test item and identify the source of information.]

**Storage Conditions of Test Chemicals:** [Indicate if the test material was frozen, refrigerated, maintained in the dark, and duration of storage.]

**B. Test Organism**

**Table 2: General Information About the Test Species and Acclimation.**

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Species common name:	[.....]		<i>EPA recommends fathead minnow (Pimephales promelas).</i>
Species scientific name:	[.....]		
Species strain (if stated):	[.....]		
Were fish obtained from a single laboratory stock?	[Yes/No]	[Provide additional information about the source of animals, if available.]	<i>EPA recommends that fish be from a single laboratory stock.</i>
Were acclimation conditions same as definitive test?	[Yes/No]		<i>EPA recommends that fish be acclimated under water quality and illumination conditions that are similar to the definitive test.</i>
Acclimation period:	[...] days		<i>EPA recommends a minimum two-week acclimation period. Note that the acclimation period is different from the subsequent, in situ pre-exposure phase.</i>
Details on health:		[Describe the health of the stock: Were any behavioral abnormalities, deformities, other clinical signs, or mortality observed? ]	<i>EPA recommends that mortality during the 7 days prior to the pre-exposure phase be less than 5% of the culture population. If mortality during these 7 days is greater than 10%, EPA recommends that the fish be rejected. If mortality is between 5-10%, EPA recommends that fish be held another 7 days. If mortalities greater than 5% occur during this extended acclimation period, EPA recommends that the fish not be used.</i>
Type of food:	[frozen brine shrimp, other]		<i>EPA recommends that fish be fed frozen brine shrimp twice per day to promote active reproduction and maintain body condition.</i>
Source of food:	[.....]		
Frequency of feeding:	[.....] times/day		
Details on feeding:		[Describe any other relevant information	

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Parameter	Value(s)	Details or Remarks	Guideline Recommendations
		about the feeding regime, including the amount given. Provide additional information if feeding conditions differed between the acclimation, pre-exposure, and definitive test periods.]	

**Table 3: Fish Selection and Pre-Exposure Performance.**

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Age at test initiation:	[...] months		<i>EPA recommends reproductively mature (sexually dimorphic) fish, 4.5 - 6 months old.</i>
Mean weight of males at test initiation (if determined):	[...] g	[Indicate whether the mean is based on a subsample of male fish or based on all male fish selected for the test.]	<i>EPA recommends that a subsample of fish be weighed before the test to estimate the mean weight for each sex. It is recommended that the individual weight of each fish selected for the test be within ±20% of the estimated mean for each sex.</i>
Range of individual weights (males) at test initiation (if determined):	[...] g : [...] g		
Mean weight of females at test initiation (if determined):	[...] g	[Indicate whether the mean is based on a subsample of female fish or based on all female fish selected for the test.]	
Range of individual weights (females) at test initiation (if determined):	[...] g : [...] g		
Mean length of males at test initiation (if determined):	[...] mm		
Mean length of females at test initiation (if determined):	[...] mm		
Duration of pre-exposure phase:	[...] days		<i>EPA recommends a minimum of 14 days.</i>
Were pre-exposure conditions identical to the definitive test?	[Yes/No]		<i>EPA recommends that pre-exposure conditions, including temperature, photoperiod, feeding, etc., be identical to definitive test conditions.</i>
Number of pre-exposure tanks:	[...]	[Were extra replicates established?]	<i>EPA recommends that additional tanks set up at</i>

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Parameter	Value(s)	Details or Remarks	Guideline Recommendations
			<i>the beginning of pre-exposure will ensure that sufficient replicates with the correct sex ratio are available for the definitive test.</i>
Number of males per tank:	[...]		
Number of females per tank:	[...]		
Pre-exposure fecundity:	≥ [...] eggs/female/ reproductive day/ replicate		<i>EPA recommends that pre-exposure fecundity in each replicate (tank) selected for use in the definitive test be at least 15 eggs/female/reproductive day/replicate during the 7 days prior to the definitive test.</i>
Number of spawns during pre-exposure:	≥ [...] times in 7 days		<i>EPA recommends that spawning occur at least twice in the 7 days prior to the definitive test.</i>
Details on pre-exposure:		[Were any pre-exposure replicates excluded due to mortality, incorrect sex ratio, or poor reproductive performance?]	

C. Exposure System

Table 4: Summary of Information on the Exposure System and Test Vessel Characteristics.

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Type of exposure:	[flow-through, other]	[Provide details if a different exposure system was used.]	<i>EPA recommends the use of a flow-through system. As noted in the Corrections and Clarifications document<sup>1</sup>, the use of a static renewal system is not recommended for this assay.</i>

<sup>1</sup> U.S. Environmental Protection Agency (EPA). (2011). Corrections and Clarifications on Technical Aspects of the Test Guidelines for the Endocrine Disruptor Screening Program Tier 1 Assays (OCSPP Test Guideline Series 890). March 3, 2011. Office of Chemical Safety and Pollution Prevention (OCSPP), Washington, D.C. (<http://www.epa.gov/endo/pubs/assayvalidation/clarificationdoc.pdf>).

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Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Type of flow-through dilution system:	[intermittent flow proportional diluters, continuous flow serial diluters, other]		<i>Intermittent flow proportional diluters or continuous flow serial diluters are recommended.<sup>2</sup></i>
Flow-through rate:	[...] mL/min		<i>Recommended flow-through rate is 45 mL/min (2.7 L/hr), or at least 6 total volume exchanges per day.</i>
Details on toxicant mixing for flow-through systems:		[Briefly summarize any relevant information about toxicant mixing, flow-splitting accuracy, and the performance of the flow-through system.]	<i>Recommended toxicant mixing for flow-through systems: 1) Mixing chamber is recommended but not required; 2) Aeration is not recommended for mixing; 3) A demonstration that the test solution is completely mixed before introduced into the test system is recommended; 4) The recommended flow splitting accuracy is within 10%.</i>
Aeration?	[Yes/No]		<i>EPA recommends aeration if dissolved oxygen reaches <math>\leq 4.9</math> mg/L (<math>\leq 60\%</math> saturation).</i>
Source of dilution water:	[natural water, reconstituted water, other]		<i>EPA recommends natural or reconstituted water; it is recommended that natural water be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants. OECD accepts any water in which the test species show control survival at least as good as indicated in the test guideline.</i>
Was dilution water analyzed for pesticides, heavy metals, and other contaminants?	[Yes/No]		
Test vessel type/materials:	[.....]		<i>EPA and OECD recommend that water-contact portions of the system not</i>

<sup>2</sup> Additional guidance for aquatic test design is located in OCSPP Guideline 850.1000, Special Considerations for Conducting Aquatic Laboratory Studies.



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Parameter	Value(s)	Details or Remarks	Guideline Recommendations
			<i>compromise the study (e.g., all glass vessels or glass vessels with stainless steel frames are acceptable examples).</i>
Test vessel size:	[report dimensions and/or total volume, specify units]		<i>EPA recommends the use of 18 L test chambers (e.g., 40 x 20 x 20 cm).</i>
Fill volume:	[...] L		<i>EPA recommends 10 L solution per tank.</i>
Spawning substrate material:	[aged PVC, glass, stainless steel, other]		<i>EPA recommends that each tank contain three semi-circular spawning substrates, e.g., aged PVC pipe, 10 - 20 cm in length, split lengthwise.</i>
Spawning substrate size:	[...] cm		
Additional details on exposure system:		[Briefly summarize other relevant details regarding the test system as they relate to guideline recommendations.]	

Table 5: Summary of Water Quality Characteristics in the Test System.

Parameter	Minimum	Maximum	Mean	Measurement Interval	Guideline Recommendations
Temperature (°C)					<i>EPA recommends temperature 25±1°C; inter-replicate and inter-treatment differentials should not exceed 1°C.</i>
pH					<i>EPA recommends pH 6.5 to 9.0.</i>
Dissolved oxygen (mg/L)					<i>EPA recommends dissolved oxygen (DO) ≥4.9 mg/L (&gt;60% air saturation)</i>
Total alkalinity (mg/L as CaCO <sub>3</sub> )					<i>EPA recommends total alkalinity &gt; 20 mg/L as CaCO<sub>3</sub>.</i>
Hardness [specify units]					
Total organic carbon (mg/L)					<i>EPA recommends that total organic carbon in dilution water be ≤ 2 mg/L.</i>
Unionized ammonia (µg/L)					<i>EPA recommends that unionized ammonia in the dilution water be ≤ 1 µg/L.</i>

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Parameter	Minimum	Maximum	Mean	Measurement Interval	Guideline Recommendations
Residual chlorine (µg/L)					<i>EPA recommends that residual chlorine in dilution water be &lt; 10 µg/L.</i>
Other [specify]					<i>General recommendations for frequency of measurements: EPA recommends that temperature, pH, and dissolved oxygen be measured in all test tanks at least weekly and that hardness and alkalinity be measured in controls and in one tank at the highest test concentration at least weekly. In addition, continuous temperature monitoring of at least one tank is encouraged.</i>

Abbreviations: <sup>NA</sup> Not applicable.

**D. Study Design and Additional Experimental Conditions**

**Table 6: Range-Finding Study Conditions (if Applicable).**

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Was a range-finder conducted?	[Yes/No]		<i>EPA recommends conducting a range-finder if 96-hour LC<sub>50</sub> data for the fathead minnow are unavailable.</i>
If yes, what was the method for determining the highest test concentration in the range-finder?	[other reference study, solubility limit, 100 mg/L]		<i>EPA recommends that the highest test concentration be selected based on toxicity data for other fish studies or species, if available. Otherwise, either the solubility limit of the test compound or 100 mg/L (whichever is lower) is appropriate.</i>
Species:	[scientific name]		
Life stage:	[...]		<i>EPA recommends that range-finding tests be performed with fish of similar age and size to those that would be utilized in the test.</i>
Test duration:	[...] days		<i>EPA recommends a 96-hour exposure.</i>

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Additional details:		[Briefly outline the range-finding test concentrations and other relevant conditions. Indicate the results, e.g., NOEC, LOEC, LC <sub>50</sub> values if obtained, and note any relevant clinical observations.]	<i>EPA recommends conducting a range-finder with five test concentrations plus a control (six total treatment levels), with four females and two males per exposure tank (36 fish total). The number of mortalities that occur may be used to develop a concentration-response curve. Based upon the results, the highest concentration that does not result in increased mortality or signs of overt morbidity compared to controls, or 1/3 the derived 96-hr LC<sub>50</sub>, may be selected as the highest exposure concentration in the 21-day test.</i>
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**Table 7: Definitive Study Conditions.**

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Test duration:	[...] days		<i>EPA recommends that the duration of the definitive test be 21 days.</i>
Method for selecting the highest test concentration in the definitive test:	[range-finder, other reference study, solubility limit, 100 mg/L, other]		<i>EPA recommends that the highest test concentration is either the solubility limit of the test compound, 100 mg/L, or demonstrates adequate evidence of toxicity (e.g., 1/3 the 96-hour LC<sub>50</sub>), whichever concentration is lowest.</i>
Reference study citation (if applicable):	[MRID, if available, and additional citation information]		
Separation of test concentrations:	[...]		<i>EPA suggests that a concentration separation of between 0.33 (or three-fold) and 0.1 (or ten-fold) is scientifically acceptable<sup>1</sup>.</i>
Number of test concentrations:	[...]		<i>EPA recommends a minimum of 3 concentrations and a control, plus solvent control if appropriate.</i>
Are nominal concentrations adjusted for purity?	[Yes/No]		

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Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Indicate the type of values presented for measured concentrations:	[geometric mean, time-weighted average, other]		
Limit of quantification (LOQ):	[...] mg a.i./L		<i>EPA recommends that for chemical test concentrations below the LOQ, analyses be conducted on the stock solutions.</i>
Level of detection (LOD):	[...] mg a.i./L		
Frequency of measurement:	[...] days		<i>It is recommended that test item concentration be measured prior to the addition of fish in all tanks and at least weekly thereafter in two replicates per treatment level.</i>
Was the randomized complete block design used?	[Yes/No]	[Provide details if the test design differed from guideline recommendations.]	<i>EPA recommends that all fish be randomly assigned to tanks during pre-exposure. Tanks are then ranked according to pre-exposure fecundity, and the tanks with the highest fecundity are randomly assigned to a definitive test treatment and block first. Each block contains one replicate of each treatment, including controls.</i>
Number of replicates in control:	[...]		<i>EPA recommends 4 replicates.</i>
Number of replicates in solvent control (if applicable):	[...]		<i>EPA recommends the use of a concurrent solvent control when a solubilizing agent is used. EPA recommends 4 replicates.</i>
Number of replicates per test item treatment level:	[...]		<i>EPA recommends 4 replicates.</i>
Number of male fish per replicate at test initiation:	[...]		<i>EPA recommends 2 males per replicate.</i>
Number of female fish per replicate at test initiation:	[...]		<i>EPA recommends 4 females per replicate.</i>
Was a solvent used?	[Yes/No]		
Solvent type (if applicable):	[e.g., DMF, acetone,		

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Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Maximum solvent concentration (if applicable):	[...] mL/L		<i>EPA recommends that the solvent not exceed 0.02 ml/L<sup>3</sup>. OECD recommends that solvent have no effect on survival nor produce any other adverse effects and that concentration not be greater than 0.1 ml/L<sup>4</sup>.</i>
Was a positive control used?	[Yes/No]		
Positive control (if applicable):	[name of chemical]		
Positive control concentration(s) (if applicable):	[.....] mg a.i./L		
Photoperiod:	[...] hrs light : [...] hrs dark		<i>EPA recommends photoperiod 16:8 (light:dark).</i>
Light intensity at water's surface:	[...] lux		<i>EPA recommends light intensity 540 – 1080 lux (at water's surface).</i>
Additional details:		[Briefly summarize other relevant details regarding the study conditions for the definitive test, as they relate to guideline recommendations.]	

<sup>3</sup> Hutchinson TH, Shillabeer N, Winter MJ, Pickford DB (2006). Acute and chronic effects of carrier solvents in aquatic organisms: A critical review. Review. Aquatic Toxicology, 76, pp.69–92.

<sup>4</sup> OECD (2000). Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. Environmental Health and Safety Publications. Series on Testing and Assessment. No. 23. Paris, France.

**Table 8: Summary of Treatment Concentrations in the Fish Short-Term Reproduction Assay with [test chemical].**

Treatment ID	Nominal Concentration (mg a.i./L)	Measured Concentration (mg a.i./L)	Mean CV (%)	Details or Remarks	Guideline Recommendations
Control (dilution water only)	0.00	<LOQ	N/A		<i>EPA recommends that test item concentrations be maintained at a coefficient of variation (CV) ≤20%.</i>
Solvent control (if applicable)	0.00	<LOQ	N/A		
Treatment 1					
Treatment 2					
Treatment 3					
Treatment n					

Abbreviations: <sup>CV</sup> Coefficient of variation.

**E. Observations**

**Biological Endpoints:** [List the parameters measured, including the specific clinical signs/sublethal effects that were considered. Include the measurement interval for each observation (e.g., daily, day 21, other).]

**Were raw (individual) data provided?** [Yes/No]

*EPA recommends that observations of survival, fecundity, fertilization success, secondary sex characteristics, and other clinical signs occur at least daily. At test termination (day 21), additional observations include body weight and length, nuptial tubercle score, gonadal staging and histopathology, plasma vitellogenin, and plasma sex steroids (testosterone and 17β-estradiol, if measured). Gonado-somatic index (GSI) is calculated using a ratio of gonad weight to body weight (gonad weight to the nearest 0.1 mg / body weight in mg x 100) at test termination.*

*Clinical signs of overt toxicity may include (but are not limited to) hemorrhage, cessation of feeding, and other abnormal behavior.*

**II. RESULTS AND DISCUSSION**

**A. Results**

[Briefly summarize the results and complete the associated tables, modifying if needed to add test item concentrations. Example values are provided in red text in the tables.]

[Describe any effects of the test substance on fish survival. Compare results of the test item treatments with the available reference toxicity endpoints. The guideline recommends that survival in controls be  $\geq 90\%$ .]

**Table 9: Adult Fish Survival in [test organism].**

Treatment (mg a.i./L) [measured]	Males			Females		
	n	# Surviving	% Survival	n	# Surviving	% Survival
Control (dilution water only), if used	8	8	100	16	16	100
Solvent control, if used						
Test concentration 1						
Test concentration 2						
Test concentration 3						
Test concentration n						
Positive control, if used						

Abbreviations: <sup>NA</sup> Not applicable.

[Briefly summarize any effects on growth, as indicated by body weight and length at test termination, respectively. Report all concentrations at which effects were observed and the direction of the effect, i.e., apparent increase or decrease when compared to control.]

**Table 10: Size at Test Termination in [test organism].**

Treatment (mg a.i./L) [measured]	Body Weight						Length					
	Males			Females			Males			Females		
	n	Mean (g)	±SD	n	Mean (g)	±SD	n	Mean (mm)	±SD	n	Mean (mm)	±SD
Control (dilution water only), if used	8	3.02	0.36	16	1.84	0.28	8	50.6	1.6	16	46.2	2.6
Solvent control, if used												
Test concentration 1												
Test concentration 2												
Test concentration 3												
Test concentration n												
Positive control, if used												

Abbreviations: <sup>NA</sup> Not applicable. <sup>ND</sup> Not determined. <sup>SD</sup> Standard deviation.



[Briefly summarize any effects on fecundity and fertilization success. Report all concentrations at which effects were observed and the direction of the effect, *i.e.*, apparent increase or decrease when compared to control.]

**Table 11: Fecundity and Fertilization Success in [test organism].**

Treatment (mg a.i./L) [measured]	Fecundity <sup>1</sup>	Fertilization Success (%) <sup>2</sup>
Control (dilution water only), if used	15	95
Solvent control, if used		
Test concentration 1		
Test concentration 2		
Test concentration 3		
Test concentration n		
Positive control, if used		

Abbreviations: <sup>NA</sup> Not applicable. <sup>ND</sup> Not determined.

<sup>1</sup> Fecundity is calculated as the number of eggs per surviving female per reproductive day per replicate.

<sup>2</sup> Fertilization success (%) is calculated as the number of embryos divided by the number of eggs, multiplied by 100.

[Briefly summarize any effects on nuptial tubercle score. Report all concentrations at which effects were observed and the direction of the effect, *i.e.*, apparent increase or decrease when compared to control.]

**Table 12: Nuptial Tubercle Score in [test organism].**

Treatment (mg a.i./L) [measured]	Males		Females	
	n	Median Tubercle Score	n	Median Tubercle Score
Control (dilution water only), if used	8	38	16	0
Solvent control, if used				

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Test concentration 1				
Test concentration 2				
Test concentration 3				
Test concentration n				
Positive control, if used				

Abbreviations: <sup>NA</sup> Not applicable. <sup>ND</sup> Not determined. <sup>SD</sup> Standard deviation.

[Briefly summarize any effects on gonado-somatic index (GSI). Report all concentrations at which effects were observed and the direction of the effect, i.e., apparent increase or decrease when compared to control.]

**Table 13: Gonado-Somatic Index (GSI) in [test organism].**

Treatment (mg a.i./L) [measured]	Males			Females		
	n	Mean GSI <sup>1</sup> (%)	±SD	n	Mean GSI <sup>1</sup> (%)	±SD
Control (dilution water only), if used	8	1.5	0.6	16	12.8	2.2
Solvent control, if used						
Test concentration 1						
Test concentration 2						
Test concentration 3						
Test concentration n						
Positive control, if used						

Abbreviations: <sup>NA</sup> Not applicable.

<sup>1</sup> Gonado-somatic index (%) is calculated as gonad weight (to the nearest 0.1 mg) / body weight (mg) x 100.

[Briefly summarize any effects on gonadal stage. Report all concentrations at which effects were observed and the direction of the effect, i.e., apparent accelerated or inhibited development when compared to control.]

**Table 14: Gonadal Staging in [test organism].**

Treatment (mg a.i./L) [measured]	Males		Females	
	n	Median Stage <sup>1</sup>	n	Median Stage <sup>2</sup>
Control (dilution water only), if used	8	3	16	5
Solvent control, if used				
Test concentration 1				
Test concentration 2				
Test concentration 3				
Test concentration n				
Positive control, if used				

Abbreviations: J Juvenile. <sup>NA</sup> Not applicable. <sup>ND</sup> Not determined. <sup>UTS</sup> Unable to stage.

<sup>1</sup> The guideline recommends the following gonadal staging scale for male fathead minnow: 0=undeveloped, 1=early spermatogenic, 2=mid-spermatogenic, 3=late spermatogenic, 4=spent.

<sup>2</sup> The guideline recommends the following gonadal staging scale for female fathead minnow: 0=undeveloped, 1=early development, 2=mid-development, 3=late development, 4=late development/hydrated, 5=post-ovulatory.

[Discuss the incidence of gonadal histopathology observations in male and female fish, respectively. Summarize qualitative (narrative) observations not included in the tables below, if provided. Identify any apparent treatment-related effects. Report whether the severity grades were assigned based on comparison to the negative control or based on comparison to normal histology as determined by the pathologist's experience.]

[Complete the following tables, specifying values for each severity grade listed. For example, if 8 control fish were examined for testis-ova and no testis-ova were observed, the incidence value for severity grade 0 (not remarkable) in the control would be 8, and the incidence values for severity grades 1-4 in the control would be 0.]

**Table 15: Gonadal Histopathology in Male [test organism].**

Treatment (mg a.i./L) [measured]	Diagnostic Observations <sup>1</sup>								
	Severity	Increased Proportion of Spermatogonia		Presence of Testis-Ova		Increased Testicular Degeneration		Interstitial Cell Hypertrophy/Hyperplasia	
		n	Incidence	n	Incidence	n	Incidence	n	Incidence
Control (dilution)	0	8	8	8	8	8	8	8	8
	1								

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Treatment (mg a.i./L) [measured]	Diagnostic Observations <sup>1</sup>								
	Severity	Increased Proportion of Spermatogonia		Presence of Testis-Ova		Increased Testicular Degeneration		Interstitial Cell Hypertrophy/Hyperplasia	
		n	Incidence	n	Incidence	n	Incidence	n	Incidence
water only), if used	2								
	3								
	4								
Solvent control, if used	0								
	1								
	2								
	3								
	4								
Test concentration 1	0								
	1								
	2								
	3								
	4								
Test concentration 2	0								
	1								
	2								
	3								
	4								
Test concentration 3	0								
	1								
	2								
	3								
	4								
Test concentration n	0								
	1								
	2								
	3								
	4								
Positive control, if used	0								
	1								
	2								
	3								
	4								

<sup>1</sup> Gonadal histopathology diagnostic observations are graded 0 – 4 based on severity: 0=Not remarkable, 1=Minimal, 2=Mild, 3=Moderate, 4=Severe. See Appendix E of the test guideline for reference.

Table 16: Additional Gonadal Histopathology Observations in Male [test organism].

Treatment (mg a.i./L) [measured]	Additional Diagnostic Observations <sup>1</sup>										
	Severity	Decreased Proportion of Spermatogonia		Increased Vascular or Interstitial Proteinaceous Fluid		Asynchronous Gonad Development		Altered Proportions of Spermatocytes or Spermatids		Granulomatous Inflammation	
		n	Incidence	n	Incidence	n	Incidence	n	Incidence	n	Incidence
Control (dilution water only), if used	0	8	8	8	8	8	8	8	8	8	8
	1										
	2										
	3										
	4										
Solvent control, if used	0										
	1										
	2										
	3										
	4										
Test concentration 1	0										
	1										
	2										
	3										
	4										
Test concentration 2	0										
	1										
	2										
	3										
	4										
	0										

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Treatment (mg a.i./L) [measured]	Additional Diagnostic Observations <sup>1</sup>										
	Severity	Decreased Proportion of Spermatogonia		Increased Vascular or Interstitial Proteinaceous Fluid		Asynchronous Gonad Development		Altered Proportions of Spermatocytes or Spermatids		Granulomatous Inflammation	
		n	Incidence	n	Incidence	n	Incidence	n	Incidence	n	Incidence
Test concentration 3	1										
	2										
	3										
	4										
Test concentration n	0										
	1										
	2										
	3										
	4										
Positive control, if used	0										
	1										
	2										
	3										
	4										

<sup>1</sup> Gonadal histopathology diagnostic observations are graded 0 – 4 based on severity: 0=Not remarkable, 1=Minimal, 2=Mild, 3=Moderate, 4=Severe. See Appendix E of the test guideline for reference.

**Table 17: Gonadal Histopathology in Female [test organism].**

Treatment (mg a.i./L) [measured]	Diagnostic Observations <sup>1</sup>						
	Severity	Increased Oocyte Atresia		Perifollicular Cell Hyperplasia/Hypertrophy		Decreased Yolk Formation	
		n	Incidence	n	Incidence	n	Incidence
Control (dilution water only), if used	0	16	16	16	16	16	16
	1						
	2						
	3						
	4						
Solvent control, if used	0						
	1						
	2						
	3						
	4						
Test concentration 1	0						
	1						
	2						
	3						
	4						
Test concentration 2	0						
	1						
	2						
	3						
	4						
Test concentration 3	0						
	1						
	2						
	3						
	4						
Test concentration n	0						
	1						
	2						
	3						
	4						
Positive control, if used	0						
	1						
	2						
	3						
	4						

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<sup>1</sup> Gonadal histopathology diagnostic observations are graded 0 – 4 based on severity: 0=Not remarkable, 1=Minimal, 2=Mild, 3=Moderate, 4=Severe. See Appendix E of the test guideline for reference.

**Table 18: Additional Gonadal Histopathology Observations in Female [test organism].**

Treatment (mg a.i./L) [measured]	Additional Diagnostic Observations <sup>1</sup>								
	Severity	Interstitial Fibrosis		Egg Debris in Oviduct		Granulomatous Inflammation		Decreased Post-Ovulatory Follicles	
		n	Incidence	n	Incidence	n	Incidence	n	Incidence
Control (dilution water only), if used	0	16	16	16	16	16	16	16	16
	1								
	2								
	3								
	4								
Solvent control, if used	0								
	1								
	2								
	3								
	4								
Test concentration 1	0								
	1								
	2								
	3								
	4								
Test concentration 2	0								
	1								
	2								
	3								
	4								
Test concentration 3	0								
	1								
	2								
	3								
	4								
Test concentration n	0								
	1								
	2								
	3								
	4								
Positive control, if used	0								
	1								
	2								



Treatment (mg a.i./L) [measured]	Additional Diagnostic Observations <sup>1</sup>								
	Severity	Interstitial Fibrosis		Egg Debris in Oviduct		Granulomatous Inflammation		Decreased Post-Ovulatory Follicles	
		n	Incidence	n	Incidence	n	Incidence	n	Incidence
3									
4									

<sup>1</sup> Gonadal histopathology diagnostic observations are graded 0 – 4 based on severity: 0=Not remarkable, 1=Minimal, 2=Mild, 3=Moderate, 4=Severe. See Appendix E of the test guideline for reference.

[Briefly summarize any effects on biochemical endpoints, including plasma vitellogenin (VTG), testosterone (T), and 17β-estradiol, if measured. Report all test concentrations at which effects were observed and the direction of the effect, *i.e.*, apparent increase or decrease when compared to control.]

Table 19: Plasma Vitellogenin in [test organism].

Treatment (mg a.i./L) [measured]	Plasma Vitellogenin (VTG)					
	Males			Females		
	n	Mean (ng/mL plasma)	±SD	n	Mean (ng/mL plasma)	±SD
Control (dilution water only), if used	8	528	39	16	1.2x10 <sup>7</sup>	8.1x10 <sup>6</sup>
Solvent control, if used						
Test concentration 1						
Test concentration 2						
Test concentration 3						
Test concentration n						
Positive control, if used						

Abbreviations: <sup>NA</sup> Not applicable. <sup>ND</sup> Not determined. <sup>SD</sup> Standard deviation.

Table 20: Plasma Sex Steroids in [test organism] (if measured).

Treatment (mg a.i./L) [measured]	Plasma Testosterone (T)						Plasma 17β-estradiol (E2)					
	Males			Females			Males			Females		
	n	Mean (ng/mL plasma)	±SD	n	Mean (ng/mL plasma)	±SD	n	Mean (ng/mL plasma)	±SD	n	Mean (ng/mL plasma)	±SD
Control (dilution water only), if used	8	9.8	0.9	16	3.0	0.2	8	0.4	0.1	16	6.1	1.2
Solvent control, if used												
Test concentration 1												
Test concentration 2												
Test concentration 3												
Test concentration n												
Positive control, if used												

Abbreviations: <sup>NA</sup> Not applicable. <sup>ND</sup> Not determined. <sup>SD</sup> Standard deviation.

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[Discuss qualitative observations of secondary sex characteristics and the incidence of behavioral effects or other clinical signs. Identify when during the study the effects were observed and whether the effects are considered treatment-related. Complete the following table, using a separate line for each type of observation. Add rows as necessary.]

**Table 21: Secondary Sex Characteristics and Clinical Signs in [test organism].**

Treatment (mg a.i./L) [measured]	Secondary Sex Characteristics and Clinical Signs <sup>1</sup>					
	Males			Females		
	Type	n	Incidence	Type	n	Incidence
Control (dilution water only), if used	Light body color	8	1	Vertical bands	16	2
Solvent control, if used						
Test concentration 1						
Test concentration 2						
Test concentration 3						
Test concentration n						

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Treatment (mg a.i./L) [measured]	Secondary Sex Characteristics and Clinical Signs <sup>1</sup>					
	Males			Females		
	Type	n	Incidence	Type	n	Incidence
Positive control, if used						

<sup>1</sup> Define any abbreviations used to identify secondary sex characteristics and clinical signs (*i.e.*, sublethal effects, including behavioral effects) not specified elsewhere in the results. Examples include discoloration, changes in dorsal nape pad, abnormal swimming behavior, lethargy, loss of equilibrium, hemorrhage, malformations, lesions, *etc.* Add rows as necessary. Note that nuptial tubercle score is reported previously in **Table 12**.

**B. Study Author's Analysis and Conclusions**

[List the parameters that were analyzed and the statistical tests that were performed by the study author. Indicate whether the statistical analyses were appropriate and whether they were consistent with the methods recommended in the guideline.]

[Briefly summarize the study author's conclusions.]

**C. Reviewer's Analysis and Conclusions**

[It is recommended that the reviewer confirm the accuracy of the statistical analyses by recalculating both summary statistics and pertinent statistical tests for each endpoint, as well as performing the tests including and excluding any outliers, as appropriate. Include percent differences from the negative control for each endpoint and the criteria for determining significance.]

**Statistical Methods:** [Modify as appropriate.] The reviewer analyzed data for survival (mortality) using Fisher's Exact Test [ToxStat 3.5 or CETIS v.xx]. Data for fecundity, normalized to the number of female reproductive days; fertility; gonado-somatic index (GSI), vitellogenin (VTG); sex steroids (if measured); weight; and length (respectively), ...

[Choose the appropriate option below]

- were consistent with a monotonic concentration-response and therefore were analyzed using the Jonckheere-Terpstra test [SAS v.xx or CETIS v.xx].
- were not consistent with a monotonic concentration-response. The data were tested for normality using Shapiro-Wilks test and for homogeneity of variance using Levene's test [ToxStat 3.5 or CETIS]. Data [specify] that met the assumptions of normality and homogeneity of variance were then analyzed using Dunnett's test [ToxStat 3.5 or CETIS]. Data [specify] that failed either of these assumptions [specify] were analyzed using the Mann-Whitney test with the Bonferroni-Holm adjustment [ToxStat 3.5 or CETIS].

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Replicate median values for nuptial tubercle score were analyzed using the Jonckheere-Terpstra test **[SAS v.xx or CETIS v.xx]**. Unless otherwise indicated, effects were considered statistically significant at **[p<0.05]**. **[Describe any summary statistics presented for gonadal staging and histopathology.]**

**Conclusions:**

**[Briefly summarize the study findings and complete the following tables.]**

**Table 22: Reproductive and HPG Endpoints<sup>1,2</sup> for Male **[test organism]** in the FSTRA with **[test chemical]**.**

Treatment (mg a.i./L) [measured]	Tubercle Score		GSI		Gonadal Staging and Histo.	Plasma VTG		Plasma T		Plasma E2	
	Median	p	% Diff.	p	Effect? (Yes/No)	% Diff.	p	% Diff.	p	% Diff.	p
Control (dilution water only), if used	38	NA	0	NA	NA	0	NA	0	NA	0	NA
Solvent control, if used											
Test conc. 1											
Test conc. 2											
Test conc. 3											
Test conc. n											
Positive control, if used											

Abbreviations: <sup>Conc.</sup> Concentration. <sup>Diff.</sup> Difference. <sup>E2</sup> 17β-estradiol. <sup>GSI</sup> Gonado-Somatic Index. <sup>Histo</sup> Histopathology. <sup>NA</sup> Not applicable. <sup>T</sup> Testosterone. <sup>VTG</sup> Vitellogenin.

<sup>1</sup> Unless otherwise indicated, effects and percent (%) differences are reported based on comparison to the negative (clean water) control. Conclusions regarding histopathology may be heavily weighted by the expert opinion of a board-certified pathologist.

<sup>2</sup> Unless otherwise specified, effects are considered statistically significant at **[p<0.05]**.

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**Table 23: Reproductive and HPG Endpoints<sup>1,2</sup> for Female **[test organism]** in the FSTRA with **[test chemical]**.**

Treatment (mg a.i./L) [measured]	Fecundity		Fert. Success		Tubercle Score		GSI		Gonadal Staging and Histo.	Plasma VTG		Plasma T		Plasma E2	
	% Diff.	p	% Diff.	p	Median	p	% Diff.	p	Effect? (Yes/No)	% Diff.	p	% Diff.	p	% Diff.	p
Control (dilution water only), if used	0	NA	0	NA	0	NA	0	NA	NA	0	NA	0	NA	0	NA
Solvent control, if used															
Test conc. 1															
Test conc. 2															
Test conc. 3															
Test conc. n															
Positive control, if used															

Abbreviations: <sup>Conc.</sup> Concentration. <sup>Diff.</sup> Difference. <sup>E2</sup> 17β-estradiol. <sup>Fert.</sup> Fertilization. <sup>GSI</sup> Gonado-Somatic Index. <sup>Histo.</sup> Histopathology.

<sup>NA</sup> Not applicable. <sup>T</sup> Testosterone. <sup>VTG</sup> Vitellogenin.

<sup>1</sup> Unless otherwise indicated, effects and percent (%) differences are reported based on comparison to the negative (clean water) control. Conclusions regarding histopathology may be heavily weighted by the expert opinion of a board-certified pathologist.

<sup>2</sup> Unless otherwise specified, effects are considered statistically significant at **[p<0.05]**.

**Table 24: Growth Endpoints<sup>1,2</sup> in the Fish Short-Term Reproduction Assay (FSTRA) with [test chemical].**

Treatment (mg a.i./L) [measured]	Body Weight				Length			
	Males		Females		Males		Females	
	% Diff.	p	% Diff.	p	% Diff.	p	% Diff.	p
Control (dilution water only), if used	0	NA	0	NA	0	NA	0	NA
Solvent control, if used								
Test concentration 1								
Test concentration 2								
Test concentration 3								
Test concentration n								
Positive control, if used								

Abbreviations: <sup>Diff.</sup> Difference. <sup>NA</sup> Not applicable. <sup>ND</sup> Not determined.

<sup>1</sup> Unless otherwise indicated, percent (%) differences are reported based on comparison to the negative (clean water) control.

<sup>2</sup> Unless otherwise specified, effects are considered statistically significant at [p<0.05].

**E. Study Deficiencies**

[Were validity and performance criteria met? Include a discussion of whether, to what extent, and in what way failure to meet the performance criteria had an impact on the quality or acceptability of the study e.g., major vs. minor deficiencies.]

**F. Reviewer’s Comments**

[Identify whether the reviewer’s interpretation is in agreement with that of the study author; discuss any differences and whether or not the differences substantively impact the analysis. Provide additional comments that do not appear under other sections of the template.]

**III. REFERENCES**

[Provide references that were cited in the study report, studies in the open literature, references to other study reports in the submission or other studies conducted by the sponsor. Do not include references to standard guidelines or methodologies.]