Data Requirement:	EPA DP Barcode	[.....]	*if applicable*
	OECD Data Point	231	
	EPA MRID	[.....]	*if applicable*
	EPA Guideline	890.1100	
		Amphibian Metamorphosis Assay (Frog)	

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 assay of [test chemical] on amphibian metamorphosis of [common name and scientific name] was studied under [flow-through/static-renewal] conditions. Amphibian larvae [enter number of larvae used and age or stage of development] were exposed to [control, solvent control (if applicable), and test chemical nominal/measured concentrations] of [x_1, x_2, x_3, ... x_n] mg a.i./L. The test system was maintained at [...] to [...]°C and a pH of [...] to [...].

[Modify as appropriate.] [Test chemical] significantly [increased or decreased] 7-day hind-limb length (HLL) at [list all relevant concentrations] mg a.i./L and 21-day HLL at [list all relevant concentrations] mg a.i./L. Significant [acceleration or delay] of median Nieuwkoop-Faber (NF) developmental stage was observed at 7 days at [list all relevant concentrations] mg a.i./L and at 21 days at [list all relevant concentrations] mg a.i./L. Asynchronous development was observed in [list number of tadpoles affected and the relevant concentration for each treatment level where asynchrony was observed]. Effects on thyroid gland histopathology were observed at [list all relevant concentrations] mg a.i./L. Histopathological effects included ..... [provide details of treatment-related effects, *e.g.*, increased incidence of follicular cell hyperplasia]. Clinical signs (*i.e.*, behavioral and other sublethal effects) including [discoloration, lethargy, loss of equilibrium, *etc.*] were observed in [number of tadpoles] 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 an Amphibian Metamorphosis Assay (OCSPP Guideline 890.1100). [If it does not satisfy the requirement, concisely list the major deficiencies.]

Results Synopsis:

Test organism NF stage at test initiation: [...]
Test organism total length at test initiation (optional): mean [....] mm, range [...] to [...] mm
Test type: [flow-through, static renewal]

Table 1: Summary of Developmental and Thyroid Pathology/Histopathology Effects[1,2] in the Amphibian Metamorphosis Assay (AMA) with [test chemical].

Treatment (mg a.i../L) [measured]	NF Developmental Stage		Hind Limb Length[3]		Asynchronous Development		Thyroid Gross and Histopathology
	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21	Day 21
Test concentration 1	No	No	No	Yes	No	No	Yes
Test concentration 2							
Test concentration 3							
Test concentration n							
Positive control, if used							

Abbreviations: Diff. Difference. NA Not applicable.

[1] A "yes" indicates a significant difference based on comparison to the negative (clean water) control, unless otherwise specified.

[2] 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.

[3] Hind-limb length is normalized to snout-vent length (SVL).

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 Parental Care.

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Species common name:	[.....]		*EPA recommends African clawed frog (Xenopus laevis). Western [Africa] clawed frog Silurana (Xenopus) tropicalis may be used as an alternate species[1]; however, a list of all of the necessary protocol deviations to accommodate this species is recommended for inclusion in the study report. The guideline recommends that the performance criteria used to support the reliability of the test be identified.*
Species scientific name:	[.....]		
Species strain (if stated):	[.....]		
Were parents maintained as in-house stock?	[Yes/No]	[Provide additional information about the source of animals, if available.]	*EPA recommends that larvae used in the assay be derived from in-house adults.*
Were parental acclimation conditions same as definitive test?	[Yes/No]		
Acclimation period for parental frogs (if applicable):	[...] days		
Details on parental feeding:		[What were the type, source, amount, and frequency of feeding for adult frogs?]	
Details on parental health:		[Describe the health of the parental stock: Were any behavioral	

[1] 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).

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
		abnormalities, deformities, other clinical signs, or mortality observed?]	

Table 3: Larval Selection and Care.

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Best single spawn?	[Yes/No]		*EPA and OECD recommend that the best 2 – 3 individual spawns, with a minimum of 1500 larvae/spawn, be evaluated to identify the best single spawn, and that the larvae selected for testing originate from the best single spawn (i.e., the spawns are not co-mixed)*
Number of spawns evaluated (if applicable):	[...]		
Number of eggs sampled per spawn:	[...]	[On which day(s) were eggs evaluated to check embryonic viability?]	
NF stage at test initiation	[...]		*EPA recommends that the definitive study be initiated with larvae at Nieuwkoop – Faber (NF) developmental stage 51 (≤17 days post-fertilization).*
Age at test initiation:	[...] days post-fertilization (dpf)		
Mean total length at test initiation (if reported):	[...] mm		
Range of total length at test initiation (if reported):	[...] mm : [...] mm		
Was the optional size selection method used?	[Yes/No]		
Details on larval selection:		[If the method used to select larvae for use in the definitive test was different from the guideline recommendations, briefly describe. How were larval number and viability assessed?]	

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Loading rate (rearing density):	[...] larvae/L		*EPA recommends that rearing density (loading rate) not exceed approximately 10 larvae/L culturing system for flow-through systems or 4 tadpoles/L in static-renewal exposure systems.*
Type of food:	[Sera Micron®, other]	[If other, please describe.]	*EPA recommends Sera Micron® throughout pre-exposure (after NF stage 45/46) and during the entire 21-d definitive study. If another diet is used, the study report should provide analysis of iodide content and potential contaminants, and the diet should demonstrate equal performance to Sera Micron®.*
Source of food:	[.....]		
Iodide concentration in diet (if known):	[...]		
Frequency of feeding:	[.....] times/day		*EPA recommends that feeding occur at least twice per day.*
Details on feeding regime:	[Was the feeding regime the same as guideline recommendations? Provide details on any deviations and rationale.]		*It is recommended that food rations during the pre-exposure period be increased along with larval growth to approximately 30 mg/larva/day by test initiation. EPA and OECD recommend that food rations increase from 30 mg/larva/day at test initiation (Study Day 0-4) to 80 mg/larva/day in the last week of the test (Study Day 15-21).*

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, static renewal, other]		*EPA recommends the use of a flow-through system.*
Type of flow-through dilution system (if applicable):	[intermittent flow proportional diluters, continuous flow serial diluters, other]		*Intermittent flow proportional diluters or continuous flow serial diluters are recommended.*[2]
Flow-through rate (if applicable):	[...] mL/min		*Recommended flow-through rate is 25 mL/min (complete volume replacement ca. every 2.7 hrs).*
Details on toxicant mixing for flow-through systems (if applicable):		[Briefly summarize any relevant information about toxicant mixing, flow-splitting accuracy, and the performance of the flow-through system.]	*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%.*
Renewal period for static renewal (if applicable):	[....] hrs		*If static renewal is used, EPA recommends 24-hr renewal; renewal period is recommended not to exceed 72 hours.*
Aeration?	[Yes/No]		*EPA recommends maintaining dissolved oxygen concentrations ≥40% air*

[2] Additional guidance for aquatic test design is located in OCSPP Guideline 850.1000, Special Considerations for Conducting Aquatic Laboratory Studies.

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
			saturation (≥3.5 mg/L). Aeration may be maintained through bubblers. It is recommended to set bubblers at levels that do not cause stress on the tadpoles.
Source of dilution water:	[natural water, reconstituted water, other]		*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, including known substrates of the iodine transporter of the thyroid gland (e.g., fluoride, chlorate, perchlorate). OECD accepts any water in which the test species show control survival at least as good as indicated in the test guideline.*
Was dilution water analyzed for pesticides, heavy metals, and other contaminants?	[Yes/No]		
Iodide supplementation in water?	[Yes/No]		*If reconstituted water is used or if background levels of iodide in natural water are less than 0.5 µg/L, iodide supplementation is recommended. This supplementation is in addition to the recommended dietary source of iodide (e.g.. in Sera Micron).*
Test vessel type/materials:	[.....]		*EPA and OECD recommend that water-contact portions of the system not compromise the study (e.g., all glass vessels or glass vessels with stainless steel frames are acceptable examples).*

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Test vessel size:	[report dimensions and/or total volume, specify units]		
Fill volume:	[...] L		
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
Hardness (mg/L as $CaCO_3$)					*EPA recommends hardness 40 to 48 mg/L as $CaCO_3$.*
pH					*EPA recommends pH 7.5 ± 1, inter-replicate and inter-treatment differentials should not exceed 0.5.*
Dissolved oxygen (mg/L)					*EPA recommends dissolved oxygen (DO) >3.5 mg/L (>40% air saturation). OECD recommends DO concentration >3.5 mg/L (>40% air saturation).*
Temperature					*EPA recommends temperature 22±1°C; inter-replicate and inter-treatment differentials should not exceed 0.5°C.*
Iodide					*EPA recommends aquatic iodide range 0.5 – 10 µg/L (supplemental iodide should not exceed 2 µg/L).*

Ammonia					*General recommendations for frequency of measurements: EPA recommends that water quality parameters be measured in a control and at one test item concentration at least weekly. In static renewal systems, water quality parameters, including ammonia, should be measured just prior to renewal. In addition, EPA recommends that DO be measured at each concentration at least weekly and that temperature be measured continuously. OECD recommends that DO and temperature be measured at least weekly and that pH and hardness be measured at least at the beginning and end of the test.*
Fluoride					
Perchlorate					
Chlorate					
Other [specify]					

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]		
If yes, what was the method for determining the highest test concentration in the range-finder?	[solubility limit, 100 mg/L, ≤10% mortality, other]		*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., ≤10% mortality), whichever concentration is lowest.*
Species:	[scientific name]		
Life stage:	[...]		

Test duration:	[...] days		
Additional details:		[Briefly outline the range-finding test concentrations and other relevant conditions. Indicate the results, *e.g.*, NOEC, LOEC, LC_{50} values if obtained, and note any relevant clinical observations.]	

Table 7: Definitive Study Conditions.

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
Test duration:	[....] days		*EPA recommends that the duration of the definitive test be 21 days.*
Method for selecting the highest test concentration in the definitive test:	[range-finder, other reference study, solubility limit, 100 mg/L, ≤10% mortality, other]		*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., ≤10% mortality), whichever concentration is lowest.*
Reference study citation (if applicable):	[MRID, if available, and additional citation information]		
Separation of test concentrations:	[...]		*EPA recommends that the maximum concentration separation be 0.1 and the minimum be 0.33.*
Number of test concentrations:	[...]		*EPA recommends a minimum of 3 concentrations and a control, plus solvent control if appropriate.*
Are nominal concentrations adjusted for purity?	[Yes/No]		

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		*EPA recommends that for chemical test concentrations below the LOQ, analyses be conducted on the stock solutions.*
Level of detection (LOD):	[...] mg a.i./L		
Frequency of measurement:	[...] days		*It is recommended that test item concentration be measured in one tank at each treatment level at test initiation and every week thereafter.*
Number of replicates in control:	[...]		*EPA recommends 4 replicates.*
Number of replicates in solvent control (if applicable):	[...]		*EPA and OECD recommend the use of a concurrent solvent control when a solubilizing agent is used. EPA recommends 4 replicates.*
Number of replicates per test item treatment level:	[...]		*EPA recommends 4 replicates.*
Number of larvae per treatment at test initiation:	[...]		
Was a solvent used?	[Yes/No]		
Solvent type (if applicable):	[*e.g.*, DMF, acetone, other]		
Maximum solvent concentration (if applicable):	[...] mL/L		*EPA recommends that the solvent not exceed 0.02 ml/L*[3]*. OECD recommends*

[3] Hutchinson TH, Shillabeer N, Winter MJ, Pickford DB (2006). Acute and chronic effects of carrier solvents in aquatic organisms: A critical review.

Parameter	Value(s)	Details or Remarks	Guideline Recommendations
			that solvent have no effect on survival nor produce any other adverse effects and that concentration not be greater than 0.1 ml/L[4]*.*
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		*EPA recommends photoperiod 12:12 (light:dark).*
Light intensity at water's surface:	[...] Klux		*EPA recommends light intensity 0.6 – 2 Klux (at water's surface).*
Additional details:		[Briefly summarize other relevant details regarding the study conditions for the definitive test, as they relate to guideline recommendations.]	

Review. Aquatic Toxicology, 76, pp.69–92.
[4] 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 Amphibian Metamorphosis 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		*EPA and OECD recommend that test item concentrations be maintained at a coefficient of variation (CV) ≤20%.*
Solvent control (if applicable)	0.00	<LOQ	N/A		
Treatment 1					
Treatment 2					
Treatment 3					
Treatment n					

Abbreviations: CV 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 7 and 21, day 21, other).]

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

EPA recommends that observations of mortality and clinical signs occur daily, at a minimum; other observations are recommended as follows: NF developmental stage (days 7 and 21); any asynchronous development, indicated by tadpoles that cannot be assigned an NF stage (days 7 and 21); hind limb length (days 7 and 21); snout-vent length (days 7 and 21); body weight (test initiation, for optional size-based larval selection); and thyroid gland gross pathology and histopathology (day 21). Note the histopathology section of the test guideline also includes thyroid gross pathology observations.

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 larval mortality. Compare results of the test item treatments with the available reference toxicity endpoints. The guideline recommends that mortality in controls be ≤10% and that there be at least two test concentrations without overt toxicity.]

Table 9: Larval Mortality in [test organism].

Treatment (mg a.i../L) [measured]	Larval Mortality					
	Day 7[1]			Day 21		
	n	Mortality #	Mortality %	n	Mortality #	Mortality %
Control (dilution water only), if used	20	0	0	15	2	13
Solvent control, if used						
Test concentration 1						
Test concentration 2						
Test concentration 3						
Test concentration n						
Positive control, if used						

Abbreviations: NA Not applicable.
[1] Sample size and cumulative mortality values at Day 7 prior to interim sacrifice.

[Briefly summarize developmental stage observations. Report whether control performance, based on NF stage at test termination, was consistent with guideline recommendations; the guideline recommends that minimum median developmental stage of controls at test termination be NF Stage 57 and that the 10th and 90th percentiles not differ by more than 4 NF stages. Compare median developmental stage across treatment levels, at Day 7 and Day 21. Report all concentrations at which effects were observed and the direction of the effect, *e.g.*, apparent advance or delay in median developmental stage when compared to control. Report any observations of asynchronous development (tadpoles that cannot be staged), including the number of tadpoles affected and the relevant test concentration(s). Compare any effects observed with

effects in tests with the reference chemical (if reported).]

Table 10: Larval Development in [test organism] – Developmental Stage and Asynchronous Development.

Treatment (mg a.i../L) [measured]	Developmental Stage					
	Day 7			Day 21		
	n	Median Stage	# Asynchronous	n	Median Stage	# Asynchronous
Control (dilution water only), if used	5	53	0	13	58	0
Solvent control, if used						
Test concentration 1						
Test concentration 2						
Test concentration 3						
Test concentration n						
Positive control, if used						

Abbreviations: NA Not applicable.

[Briefly summarize developmental effects based on normalized hind limb length (HLL:SVL). Report all concentrations at which effects were observed and the direction of the effect, *e.g.*, apparent increase or decrease in normalized HLL when compared to control.]

Table 11: Larval Development in [test organism] – Hind Limb Length.

Treatment (mg a.i./L) [measured]	Hind Limb Length (HLL)[1]							
	Day 7				Day 21			
	n	Mean (mm)	±SD	HLL: SVL[2]	n	Mean (mm)	±SD	HLL: SVL[2]
Control (dilution water only), if used	5	1.81	0.32	0.11	13	14.01	3.04	0.62
Solvent control, if used								
Test concentration 1								
Test concentration 2								
Test concentration 3								
Test concentration n								
Positive control, if used								

Abbreviations: NA Not applicable. SD Standard deviation.
[1] Report the treatment mean measurements (mm) for hind limb length (HLL). Then report the normalized value (ratio of HLL:SVL) in the column following standard deviation (SD). The normalized value (HLL: SVL) reported in the table should be the treatment mean of individual ratios of HLL to SVL.
[2] Summary results for snout-vent length (SVL) are presented in the next table (**Table 12**).

[Briefly summarize any effects on growth, as indicated by SVL and body weight at Day 7 and Day 21, respectively. Report all concentrations at which effects were observed and the direction of the effect, *e.g.*, apparent increase or decrease when compared to control.]

Table 12: Larval Growth in [test organism].

Treatment (mg a.i./L) [measured]	Snout-Vent Length (SVL)						Body Weight[1]					
	Day 7			Day 21			Day 7			Day 21		
	n	Mean (mm)	±SD	n	Mean (mm)	±SD	n	Mean (g)	±SD	n	Mean (g)	±SD
Control (dilution water only), if used	5	17.22	1.59	13	26.34	2.79	5	0.51	0.13	13	1.86	0.46
Solvent control, if used												
Test concentration 1												
Test concentration 2												
Test concentration 3												
Test concentration n												
Positive control, if used												

Abbreviations: NA Not applicable. ND Not determined. SD Standard deviation.
[1] Also referred to as "wet weight" in the test guideline.

[Discuss the incidence of gross pathology (thyroid gland hypertrophy and atrophy) and histopathology observations. Summarize qualitative (narrative) observations regarding effects on the thyroid gland, including observations of colloid quality (not included in the tables below), if provided. Identify any apparent treatment-related effects. Complete the tables below, specifying values for each severity grade listed.]

Table 13: Gross Pathology and Histopathology of the Thyroid Gland in [test organism].

Treatment (mg a.i./L) [measured]	Diagnostic Observations[1]								
	Severity	Thyroid Gland Hypertrophy		Thyroid Gland Atrophy		Follicular Cell Hypertrophy		Follicular Cell Hyperplasia	
		n	Incidence	n	Incidence	n	Incidence	n	Incidence
Control (dilution water only), if used	0	5	5	5	5	5	5	5	5
	1								
	2								
	3								
Solvent control, if used	0								
	1								
	2								
	3								
Test concentration1	0								
	1								
	2								
	3								
Test concentration 2	0								
	1								
	2								
	3								
Test concentration 3	0								
	1								
	2								
	3								
Test concentration n	0								
	1								
	2								
	3								
Positive control, if used	0								
	1								
	2								
	3								

[1] Thyroid gland gross pathology and histopathology are graded 0 – 3 based on severity: 0=Not remarkable, 1=Mild, 2=Moderate, 3=Severe. See OECD No. 82 for reference.

Table 14: Additional Thyroid Gland Histopathology Observations in [test organism].

Treatment (mg a.i./L) [measured]	Additional Qualitative Observations[1]										
	Severity	Follicular Lumen Area (Increase)		Follicular Lumen Area (Decrease)		Follicular Cell Height (Increase)		Follicular Cell Height (Decrease)		Follicular Cell Shape	
		n	Incidence	n	Incidence	n	Incidence	n	Incidence	n	Incidence
Control (dilution water only), if used	0	5	5	5	5	5	5	5	5	5	5
	1										
	2										
	3										
Solvent control, if used	0										
	1										
	2										
	3										
Test concentration 1	0										
	1										
	2										
	3										
Test concentration 2	0										
	1										
	2										
	3										
Test concentration 3	0										
	1										
	2										
	3										
Test concentration n	0										
	1										
	2										
	3										
	0										

Treatment (mg a.i./L) [measured]	Additional Qualitative Observations[1]										
	Severity	Follicular Lumen Area (Increase)		Follicular Lumen Area (Decrease)		Follicular Cell Height (Increase)		Follicular Cell Height (Decrease)		Follicular Cell Shape	
		n	Incidence	n	Incidence	n	Incidence	n	Incidence	n	Incidence
Positive control, if used	1										
	2										
	3										

[1] Thyroid histopathology is graded 0 – 3 based on severity: 0=Not remarkable, 1=Mild, 2=Moderate, 3=Severe. See OECD No. 82 for reference.

[Discuss 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 table below, identifying each clinical sign observed on a separate row. Add rows as necessary.]

Table 15: Clinical Signs in [test organism].

Treatment (mg a.i./L) [measured]	Clinical Signs[1]		
	Type	n	Incidence
Control (dilution water only), if used	Spinal curvature	20	1
Solvent control, if used			
Test concentration1			
Test			

Treatment (mg a.i./L) [measured]	Clinical Signs[1]		
	Type	n	Incidence
concentration 2			
Test concentration 3			
Test concentration n			
Positive control, if used			

[1] Define any abbreviations used to identify clinical signs (*i.e.*, sublethal effects, including behavioral effects) not specified elsewhere in the results. Examples include abnormal swimming behavior, lethargy, loss of equilibrium, curvature of the spine (*e.g.*, "bent tail"), other malformations, lesions, *etc.* Add rows as necessary. Note that asynchronous development (unable to stage) is reported previously in **Table 10** and not here.

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 mortality using Fisher's Exact Test [ToxStat 3.5 or CETIS v.xx]. Replicate median values for developmental stage were analyzed using the Jonckheere-Terpstra test [SAS v.xx or CETIS v.xx]. Data for snout-vent-length (SVL), normalized hind-limb-length (HLL:SVL), and weight (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].

... The incidence of asynchronous development [if observed] was analyzed using [specify test]. Unless otherwise indicated, effects were considered statistically significant at [$p<0.05$]. [Describe any summary statistics presented for thyroid gross pathology and histopathology.]

Conclusions:

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

Table 16: Developmental and Thyroid Gross Pathology/Histopathology Endpoints[1,2] in the AMA with [test chemical].

Treatment (mg a.i../L) [measured]	NF Developmental Stage				Hind Limb Length[3]				Asynchronous Development				Thyroid Gross and Histopathology
	Day 7		Day 21		Day 7		Day 21		Day 7		Day 21		Day 21
	Median	p	Median	p	% Diff.	p	% Diff.	p	% Diff.	p	% Diff.	p	Treatment-Related Effects? (Yes/No)
Control (dilution water only), if used	53	NA	58	NA	0	NA	0	NA	0	NA	0	NA	NA
Solvent control, if used													
Test concentration 1													
Test concentration 2													
Test concentration 3													
Test concentration n													
Positive control, if used													

Abbreviations: Diff. Difference. NA Not applicable.
[1] Unless otherwise indicated, effects are reported based on comparison to the clean water control. Conclusions regarding histopathology may be heavily weighted by the expert opinion of a board-certified pathologist.
[2] Unless otherwise specified, effects are considered statistically significant at [$p<0.05$].
[3] Hind-limb length is normalized to snout-vent length (SVL).

Table 17: Growth Endpoints[1,2] in the AMA with [test chemical].

Treatment (mg a.i../L) [measured]	Snout-Vent Length				Body Weight			
	Day 7		Day 21		Day 7		Day 21	
	% 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: Diff. Difference. NA Not applicable.
[1] Unless otherwise indicated, effects are reported based on comparison to the negative (clean water) control.
[2] 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.]