Data Evaluation Report on the Acute Toxicity of Esfenvalerate to the Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number {.....} EPA MRID Number 48831602

Data Requirement:

PMRA Data Code	{.....}
EPA DP Barcode	404032
OECD Data Point	{.....}
EPA MRID	48831602
EPA Guideline	850.1075

Test material: Esfenvalerate Technical **Purity:** 90.9% active isomer
Common name: Esfenvalerate (DPX-YB656)
Chemical name: IUPAC: (*S*)-α-Cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-3-methylbutyrate
CAS name: (*S*)-Cyano(3-phenoxyphenyl)methyl (α*S*)-4-chloro-a-(l-methylethyl)benzeneacetate
CAS No.: 66230-04-4
Synonyms: DPX-YB656

Primary Reviewer: Rebecca L. Bryan **Signature:** Rebecca L. Bryan
Staff Scientist, CSS-Dynamac Corporation **Date:** 5/14/13

Secondary Reviewer: John Marton, Ph.D. **Signature:**
Environmental Scientist, CDM Smith **Date:** 6/07/14

Primary Reviewer: Donna R. Judkins, Ph.D., Biologist **Date:** 3/25/15
EPA/OCSPP/OPP/EFED/ERB2

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Secondary Reviewer(s): {.....} **Date:** {.....}
{EPA/OECD/PMRA}

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	109303	

Date Evaluation Completed: 03/25/2015

CITATION: Brougher, D.S. *et.al.* 2012. Esfenvalerate (DPX-YB656) Technical: A 96-Hour Flow Through Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*). An unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory report number: 112A-402. Study sponsored by E.I. du Pont de Nemours and Company, Wilmington, DE. Study initiated March 7, 2012 and completed May 11, 2012.

Data Evaluation Report on the Acute Toxicity of Esfenvalerate to the Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number {.....} EPA MRID Number 48831602

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Rainbow Trout (*Oncorhynchus mykiss*) were exposed to Esfenvalerate (DPX-YB656) at nominal concentrations of 0 (negative and solvent controls), 0.031, 0.063, 0.13, 0.25, and 0.50 µg ai/L under flow-through conditions. Mean measured concentrations were <0.001 (<LOD, negative and solvent controls), 0.021, 0.046, 0.11, 0.21, and 0.38 µg ai/L. At 96 hours, mortality was 0% for the negative and solvent controls, and 0, 0, 14, 86, and 100% in the 0.021, 0.046, 0.11, 0.21, and 0.38 µg ai/L groups, respectively. Sub-lethal effects observed in the 0.11, 0.21, and 0.38 µg ai/L groups included surfacing, lethargy, erratic swimming, and/or loss of equilibrium. The 96-hour LC_{50} (with 95% CI) was 0.15 (0.11 to 0.21) µg ai/L. Based on the results of this study, Esfenvalerate would be classified as **very highly toxic** to Rainbow Trout (*Oncorhynchus mykiss*) in accordance with the classification system of the U.S. EPA.

This toxicity study is scientifically sound and classified as acceptable. It satisfies the guideline requirement for an acute freshwater fish toxicity study with Rainbow Trout (*Oncorhynchus mykiss*).

Results Synopsis

Test Organism Size/Age (mean weight or length): weight: 1.22 g/length: 5.0 cm/Juveniles
Test Type (Flow-through, Static, Static Renewal): Flow-through

LC_{50}: 0.15 µg ai/L 95% C.I.: 0.11 to 0.21 µg ai/L
Probit Slope: 7.7 95% C.I.: 2.22-13.2

Endpoint(s) affected: Mortality and sublethal effects
Sublethal effects: surfacing, lethargy, erratic swimming, loss of equilibrium

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study followed the protocol "Esfenvalerate (DPX-YB656) Technical: A 96-Hour Flow Through Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)", which was based on the procedures outlined in the OECD Guideline for Testing Chemicals, 203: Fish, Acute Toxicity Test and US Environmental Protection Agency Series 850-Ecological Effects Test Guidelines, OPPTS Number 850.1075, Fish Acute Toxicity Test, Freshwater and Marine. The following deviations from the OPPTS 850.1075 guidelines were noted:

1. Recovery of test substance was below the recommended 70% in the lowest treatment (68%).
2. The total organic carbon and particulate matter contents of the dilution water were not reported.
3. The type of flow-through diluter system and the flow rate were not reported

These deviations do not affect the validity of the study. No evidence of stress was seen in the control fish. The study is classified as acceptable.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study was conducted in accordance with GLP standards with the following exception: the periodic analyses of well water for potential contaminants was not performed according to GLP, but were performed using a certified laboratory and standard US EPA analytical methods.

A. MATERIALS:

1. Test material: Esfenvalerate (DPX-YB656) Technical

Description: Liquid

Lot No./Batch No. : 100605

Purity: 90.9% active isomer

Stability of compound under test conditions: Stable. Mean measured concentrations ranged from 68-85% of nominal. The Day 2 and 4 measured concentrations were 67-98% of Day 0 measured concentrations.

Storage conditions of test chemicals: Ambient conditions.

Physicochemical properties of Esfenvalerate

Parameter	Values	Comments
Water solubility at 20°C	Not reported	According to the Registration Review problem formulation document, two studies screened from the open literature provided useable solubility estimates for esfenvalerate of 0.002-0.006 mg/L.[1]
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism:

Species: Rainbow Trout (*Oncorhynchus mykiss*)
Age at test initiation: Juvenile
Weight at study initiation: Mean: 1.22 g (range: 0.88-1.8 g) at test termination
Length at study initiation: Mean: 5.0 cm (range: 4.6-5.5 cm) at test termination
Source: Thomas Fish Company (Anderson, CA)

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: It was stated that the nominal concentrations for the definitive test were selected in consultation with the Sponsor based on the results of exploratory range-finding toxicity and limit of solubility data. However, no details of the range-finding or solubility data were provided.

b. Definitive study:

[1] DP Barcode: 379293 – June 21, 2010. Registration Review: Preliminary Problem Formulation for Ecological Risk, Environmental Fate, Endangered Species, and Drinking Water Assessment for Esfenvalerate, internal correspondence to the Special Review and Reregistration Division.

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions (same as test or not): Feeding: Health (any mortality observed):	At least 14 days Same as test Fed a commercially-prepared diet (Zeigler Brothers, Inc., Gardners, PA) No signs of disease, stress, or mortality were observed during the 14-day holding period.	Fish were not fed for 48 hours prior to the study, nor during the study. *The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Duration of the test:	96 hours	*The recommended test duration is 96 hours.*
Test conditions Static/flow-through: Type of dilution system - for flow-through method: Renewal rate for static renewal:	Flow-through Not reported N/A	The flow rate was not reported. *A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*

Parameter	Details	Remarks *Criteria*
Aeration, if any:	The dilution water was aerated prior to use.	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*
Test vessel Material (glass/stainless steel): Size: Fill volume:	 Teflon lined stainless steel aquaria 25 L *Ca.* 15 L (18 cm depth)	*Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.* *Fill volume is usually 15-30 L of solution.*
Source of dilution water:	Moderately-hard water was obtained from a 40-m deep on-site well. The water was passed through a sand filter (25 µm), aerated, filtered again (0.45 µm), and UV sterilized prior to use.	*Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf) Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness: pH: Dissolved oxygen: Total Organic Carbon: Particulate Matter: Metals: Pesticides: Chlorine: Temperature: Salinity {for marine or estuarine species}: Intervals of water quality measurement:	 136 mg/L as $CaCO_3$ 8.1-8.2 8.0 to 9.3 mg/L (≥62% saturation) Not reported Not reported (see remarks) (see remarks) (see remarks) Daily: 12.3-12.7°C Continuous: 12.0-12.5°C N/A Dissolved oxygen and pH were measured in each test chamber at the beginning and end of the test and at approximately 24 hour intervals during the test. Temperature was measured in each test chamber at the beginning and end of the test, and continuously in one negative control chamber.	Results from the analysis of the Wildlife International well water from samples taken on December 28, 2011 were provided. No metals or pesticides were detected at levels considered toxic. *Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg/L)* *pH:* *EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static: >60% during first 48 hrs and >40% during second 48 hrs; flow-through: >60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12 °C for coldwater species, 17 or 22 °C for warmwater species, and 22 ± 1 °C for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity:* *EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰.* *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/group: Control: Solvent control: Treated:	 1 1 1	*Recommended numbers of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.*
Number of organisms per replicate /group: Control: Solvent control: Treated ones:	 7 7 7	*Number of organisms per replicate should be ≥ 10/concentration; OECD guideline recommends at least 7 fish/concentration.*

Parameter	Details	Remarks *Criteria*
Biomass loading rate:	0.57 g fish/L	*Recommended static conditions are ≤ 0.8 g/L at ≤ 17°C and ≤ 0.5 g/L at >17°C. Recommended flow-through conditions are ≤ 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
Test concentrations: Nominal: Mean measured:	 0 (negative and solvent controls), 0.031, 0.063, 0.13, 0.25, and 0.50 µg ai/L <0.001 (<LOD, negative and solvent controls), 0.021, 0.046, 0.11, 0.21, and 0.38 µg ai/L	
Solvent (type, percentage, if used):	Triethylene Glycol (TEG) at 0.1 mL/L	*The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.*
Lighting:	16 hour light/8 hour dark photoperiod with 30 minute transition periods. Light intensity at test initiation was 251 lux.	*The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*
Feeding:	Not fed during the test, nor 48 hours prior	*Fish should not feed during the study.*
Recovery of chemical: Frequency of determination: Limit of quantitation: Limit of detection:	68-85% of nominal 0, 48, and 96 hours 0.0200 µg ai/L 0.00101 µg ai/L	GC/ECD method
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any:	At test initiation, the specific conductance was 337 µS/cm, hardness was 136 mg/L as $CaCO_3$, and alkalinity was 174 mg/L as $CaCO_3$ in the well water.	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	Mortality Sublethal effects	
Observation intervals:	3, 24, 48, 72, and 96 hours	
		Observation intervals should be a minimum of every 24 hours.
Were raw data included?	Yes	
Other observations, if any:	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

At 96 hours, mortality was 0, 0, 0, 0, 14, 86, and 100% in the negative control, solvent control, 0.021, 0.046, 0.11, 0.21, and 0.38 µg ai/L groups, respectively. The LC_{50} (with 95% CI) and NOAEC values were 0.15 (0.11-0.21) µg ai/L and 0.046 µg ai/L, respectively, based on mean measured concentrations.

Table 3: Effect of Esfenvalerate on Mortality in Rainbow Trout (*Oncorhynchus mykiss*).[a]

Mean measured (and nominal) Concentrations (µg ai/L)	No. of fish at start of study	Observation period							
		24 Hours		48 Hours		72 Hours		96 Hours	
		No. Dead	% Mortality	No. Dead	% Mortality	No. Dead	% Mortality	No. Dead	% Mortality
<LOD [b] (Negative Control)	7	0	0	0	0	0	0	0	0
<LOD [b] (Solvent Control)	7	0	0	0	0	0	0	0	0
0.021 (0.031)	7	0	0	0	0	0	0	0	0
0.046 (0.063)	7	0	0	0	0	0	0	0	0
0.11 (0.13)	7	0	0	0	0	0	0	1	14
0.21 (0.25)	7	0	0	1	14	4	57	6	86
0.38 (0.50)	7	0	0	6	86	7	100	7	100
NOAEC	0.046 µg ai/L								
LC_{50} (with 95% CI)	0.15 (0.11-0.21) µg ai/L								

a Data were obtained from Tables 6 and 7 on pages 27-29 of the study report.
b LOD = 0.001 µg ai/L

B. NON-LETHAL TOXICITY ENDPOINTS:

All fish in the control and ≤0.046 µg ai/L test levels appeared normal throughout the test. Sub-lethal effects observed in the 0.11, 0.21, and 0.38 µg ai/L groups included surfacing, lethargy, erratic swimming, and/or loss of equilibrium. The NOAEC was 0.046 µg ai/L.

Table 4: Sub-lethal Effects of Esfenvalerate on Rainbow Trout (*Oncorhynchus mykiss*).[a]

Mean measured (and nominal) Concentrations (µg ai/L)	Observation period			
	24 Hours	48 Hours	72 Hours	96 Hours
	% affected	% affected	% affected	% affected
<LOD [b] (Negative Control)	AN	AN	AN	AN
<LOD [b] (Solvent Control)	AN	AN	AN	AN
0.021 (0.031)	AN	AN	AN	AN
0.046 (0.063)	AN	AN	AN	AN
0.11 (0.13)	AN	14% C	29% C; 14% N	33% C; 17% N
0.21 (0.25)	14% N	17% C; 17% E; 17% N	33% C; 67% N	100% N
0.38 (0.50)	57% A; 14% N	100% N	All dead	All dead
NOAEC	0.046 µg ai/L			
LOAEC	0.11 µg ai/L			
EC_{50}	Not reported			

a Data were obtained from Table 6 on pages 27 and 28 of the study report.
b LOD = 0.001 µg ai/L
AN = all surviving fish appeared normal; A = surfacing; C = lethargy; E = erratic swimming; N = loss of equilibrium

C. REPORTED STATISTICS:

The probit method was used to calculate the 96-hour LC_{50} and the associated 95% confidence interval. The no-mortality concentration and the NOAEC were determined by visual interpretation of the mortality and observation data.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The 96-hour LC_{50} value was calculated using the probit method via CETIS version 1.8.7.7 statistical software with backend database settings implemented by EFED on 31 May 2013. There was no mortality in either control group and all treatment level mortality was compared to the negative control only. The results are based on mean-measured concentrations.

LC_{50}: 0.15 µg ai/L 95% C.I.: 0.11 to 0.21 µg ai/L
Probit Slope: 7.7 95% C.I.: 2.22-13.2

E. STUDY DEFICIENCIES: None

F. REVIEWER'S COMMENTS:

The reviewer's results were comparable to those reported by the study author with the exception that the reviewer reported the 95% confidence interval associated with the probit slope. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

The study author did not provide measurements of the fish at test initiation. Therefore, the weights and lengths reported in this report are from test termination, as noted above.

Mean measured concentrations ranged from 68-85% of nominal. The measured concentration for the 0.031 µg ai/L sample on Day 2 was 55% of nominal (extrapolated value of 0.0170 µg ai/L). The mean percent recovery was 68% of nominal for the 0.031 µg ai/L group, and the mean percent recovery for all other treatment groups were >70% of nominal. The Day 2 and 4 measured concentrations were 67-98% of Day 0 measured concentrations. The mean measured concentrations were reported for all results.

The in-life phase of the definitive test occurred from March 12-16, 2012.

G. CONCLUSIONS:

This study is scientifically sound and classified as acceptable. At 96 hours, mortality was 0% for the negative and solvent controls, and 0, 0, 14, 86, and 100% in the 0.021, 0.046, 0.11, 0.21, and 0.38 µg ai/L groups, respectively. Sub-lethal effects observed in the 0.11, 0.21, and 0.38 µg ai/L groups included surfacing, lethargy, erratic swimming, and/or loss of equilibrium. The 96-hour LC_{50} (with 95% CI) value was 0.15 (0.11-0.21) µg ai/L.

LC_{50}: 0.15 µg ai/L	95% C.I.: 0.11 to 0.21 µg ai/L
Probit Slope: 7.7	95% C.I.: 2.22-13.2

III. REFERENCES:

None other than standard guidelines or methodologies.

Appendix: CETIS Output Summary.

CETIS Summary Report

Report Date: 20 Jan-14 10:41 (p 1 of 2)
Test Code: 109303 48831602 | 15-9762-6306

OPPTS 850.1075 Acute Fish — Wildlife International

Batch ID:	14-0010-6380	Test Type:	Mortality (96-h)	Analyst:	
Start Date:	12 Mar-12	Protocol:	OPPTS 850.1075 Acute Fish	Diluent:	Well Water
Ending Date:		Species:	Oncorhynchus mykiss	Brine:	Not Applicable
Duration:	NA	Source:	Thomas Fish Company, Anderson CA	Age:	1.2g

Sample ID:	05-1822-5902	Code:	48831602	Client:	CDM Smith
Sample Date:	12 Mar-12	Material:	Esfenvalerate	Project:	Insecticide
Receive Date:		Source:	E.I. du Pont de Nemours and Company (EI		
Sample Age:	NA	Station:			

Batch Note: PC Code 109303 MRID 48831602
Sample Note: PC Code 109303 MRID 48831602

Point Estimate Summary

Analysis ID	Endpoint	Level	µg ai/L	95% LCL	95% UCL	TU	Method
06-5839-6975	48h Mortality Rate	LC5	0.179	0.0466	0.233		Linear Regression (MLE)
		LC10	0.198	0.0672	0.252		
		LC15	0.212	0.0857	0.266		
		LC20	0.224	0.103	0.28		
		LC25	0.235	0.121	0.294		
		LC40	0.263	0.172	0.346		
		LC50	0.283	0.204	0.397		
05-8164-0858	48h Mortality Rate	LC50	0.282	0.242	0.33		Trimmed Spearman-Kärber
01-3243-5554	96h Mortality Rate	LC5	0.0928	0.0247	0.124		Linear Regression (MLE)
		LC10	0.103	0.0353	0.134		
		LC15	0.111	0.0448	0.143		
		LC20	0.118	0.0538	0.15		
		LC25	0.124	0.0626	0.158		
		LC40	0.141	0.0889	0.186		
		LC50	0.152	0.106	0.212		
11-2151-7256	96h Mortality Rate	LC50	0.149	0.115	0.193		Spearman-Kärber

48h Mortality Rate Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	1	0			0	0	0	0		
0	Negative Control	1	0			0	0	0	0		
0.021		1	0			0	0	0	0		
0.046		1	0			0	0	0	0		
0.11		1	0			0	0	0	0		
0.21		1	0.143			0.143	0.143	0	0	0.0%	
0.38		1	0.857			0.857	0.857	0	0	0.0%	

96h Mortality Rate Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	1	0			0	0	0	0		
0	Negative Control	1	0			0	0	0	0		
0.021		1	0			0	0	0	0		
0.046		1	0			0	0	0	0		
0.11		1	0.143			0.143	0.143	0	0	0.0%	
0.21		1	0.857			0.857	0.857	0	0	0.0%	
0.38		1	1			1	1	0	0	0.0%	

CETIS Summary Report

Report Date: 20 Jan-14 10:41 (p 2 of 2)
Test Code: 109303 48831602 | 15-9762-6306

OPPTS 850.1075 Acute Fish — Wildlife International

48h Mortality Rate Detail

C-µg ai/L	Control Type	Rep 1
0	Solvent Blank	0
0	Negative Control	0
0.021		0
0.046		0
0.11		0
0.21		0.143
0.38		0.857

96h Mortality Rate Detail

C-µg ai/L	Control Type	Rep 1
0	Solvent Blank	0
0	Negative Control	0
0.021		0
0.046		0
0.11		0.143
0.21		0.857
0.38		1