Study Title

2-Ethylhexyl Paraben - Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines

Data Requirements

OPPTS Guideline 890.1350
OECD Guideline 229

Author

Joseph P. Marini

Study Completed On

30 April 2018

Submitted To

U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Performing Laboratory

Smithers Viscient
790 Main Street
Wareham, Massachusetts 02571-1037

Laboratory Project ID

Smithers Viscient Study No. 13784.6120

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The data and report presented for **"2-Ethylhexyl Paraben - Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines"** were produced and compiled in accordance with all pertinent U.S. Environmental Protection Agency (EPA) Good Laboratory Practices as set forth under the Federal Insecticide, Fungicide, and Rodenticide Act (U.S. EPA, 1989) and as accepted by the OECD Principles of Good Laboratory Practice (OECD, 1998) with the following exceptions:

- While the test substances were characterized prior to use in this study, these characterizations were not performed in accordance with GLPs.
- Routine food and water contaminant screening analyses were conducted at GeoLabs, Inc., Braintree, Massachusetts using standard U.S. EPA procedures and are considered facility records under Smithers Viscient's Standard Operating Procedures. Since the analyses were conducted following standard validated methods, this exception has no impact on the study results.
- Iodide concentrations were also analyzed for this exposure on representative samples of the well water source and were conducted by Galbraith Laboratories, Inc., Knoxville, Tennessee by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).
- The light meter used during the definitive test was past the calibration expiry date. Another light meter that was within the calibration date was used to verify the original measurements. Since the two measurements only differed by 15% of each other and the guideline does not require a light reading or provide a required range for light readings, this exception had no impact on the study results.

SMITHERS VISCIENT

Joseph P. Marini
Study Director

30 Apr 18
Date

BATTELLE

Vincent J. Brown
Sponsor Representative

30 APRIL 2018
Date

Submitter

30 APRIL 2018
Date

QUALITY ASSURANCE STATEMENT

The study conduct, raw data, and report for **"2-Ethylhexyl Paraben - Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines"** were inspected by the Quality Assurance Unit (QAU) at Smithers Viscient to determine adherence with the study protocol, amendments, laboratory standard operating procedures, and the applicable GLP regulations. This report is an accurate representation of the raw data. Dates of study inspections, study inspection types, and dates reported to the Study Director and to Management are provided below.

Inspection Date	Inspection Type	Reported to Study Director/ Management
24 April 2016	Protocol Review	24 April 2016
11 October 2016	In-Life: Biological Observations	12 October 2016
22 - 25 May 2017	Audit of Data Quality	26 May 2017
16 - 18, 21 August 2017	Audit of Data Quality	23 August 2017
16 - 17, 21 August 2017	Draft Report Audit	23 August 2017
27 September 2017	Revised Draft Report Audit	27 September 2017
6 March 2018	Appendices	6 March 2018
9 March 2018	Revised Draft Report Audit	9 March 2018
19 April 2018	Revised Draft Report Audit	19 April 2018
30 April 2018	Final Report	30 April 2018

SMITHERS VISCIENT

Robin Dyer
Quality Assurance Auditor

30 April 2018
Date

KEY STUDY PERSONNEL

The following Smithers Viscient personnel were responsible for the conduct of the work and reporting of the study results.

Joseph P. Marini	Study Director
Lee E. Sayers	Senior Research Biologist
Katherine Urann	Biologist II
Amy B. Viveiros	Biologist I
Leah Vandermeer	Biologist I
Ryan T. Higgins	Biologist I
Tabatha L. Perrone	Biologist I
Alyssa Palmer	Assistant Biologist
Natalie Boudwin	Assistant Biologist
Justin Clark	Assistant Biologist
Leonardo Fernandes	Assistant Biologist
Samantha Ferguson	Assistant Biologist
Devin Escobar	Assistant Biologist
Brynne Boyce	Assistant Biologist
Nicole M. Wolford	Assistant Chemist
Kevin N. Nelson	Chemistry Technician II
Jason O'Haver	Chemistry Technician II
Ryan Morrissey	Chemistry Technician II
Kathryn E. Brennan	Chemistry Technician II
Paul Moses	Senior Chemist
Francis J. McGuiness	Senior Chemist
Stephen R. DeVellis	Senior Chemist
Nancy L. McHale	Senior Research Chemist
Marjorie E. Dix	Research Director, Chemistry
Francesca Bagangan	Technical Report Writer
Heather Grove	Director, Ecotoxicology

TABLE OF CONTENTS

Page

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT....2
QUALITY ASSURANCE STATEMENT....3
KEY STUDY PERSONNEL....4
TABLE OF CONTENTS....5
EXECUTIVE SUMMARY....10
1.0 INTRODUCTION....17
2.0 MATERIALS AND METHODS....17
2.1 Protocol....17
2.2 Test Substance....18
2.3 Test Organism....18
2.4 Feeding....18
2.5 Dilution Water....19
2.6 Test Conditions....20
2.7 Test Substance Concentrations....20
2.8 Stock Preparation....21
2.9 Exposure System....22
2.10 Exposure Initiation....24
2.10.1 Pre-Exposure Phase....24
2.10.2 Pre-Exposure System....25
2.10.3 Selection and Weighing of Test Fish....26
2.10.4 Definitive Exposure....26
2.11 Test Monitoring....27
2.11.1 Water Quality Measurements....27
2.11.2 Analytical Measurements....27
2.12 Endpoints and Observations....29
2.12.1 Survival....29
2.12.2 Appearance of Adults....29
2.12.3 Behavior of Adults....29
2.12.4 Fecundity....29
2.12.5 Fertilization Success....30
2.13 Test Termination....30
2.13.1 Nuptial Tubercle Scoring....30

2.13.2 Gonadal Somatic Index (GSI) and Histology 31
2.13.3 Sex Steroid Analysis 31
2.13.4 Vitellogenin (VTG) Analysis 32
2.14 Performance Criteria 33
2.15 Statistical Analyses 33
3.0 RESULTS 35
3.1 Preliminary Exposure 35
3.2 Definitive Exposure 35
3.2.1 Evaluation of Test Conditions 35
3.2.2 Analytical Results 36
3.2.3 Biological Observations 37
3.2.4 Survival 37
3.2.5 Behavioral Appearance and Observations 38
3.2.6 Fertilization Success and Fecundity 39
3.2.7 Tubercle Scores 39
3.2.8 Weight Endpoints 40
3.2.9 Length Results 40
3.2.10 Gonadal Somatic Index (GSI) Endpoints 41
3.2.11 Blood Plasma Vitellogenin (VTG) Concentration 41
3.2.12 Sex Steroid Analysis 42
3.2.12.1 Male Sex Steroid Analysis 43
3.2.12.2 Female Sex Steroid Analysis 43
3.2.13 Histopathology 44
4.0 PERFORMANCE CRITERIA 45
5.0 PROTOCOL DEVIATIONS 46
6.0 DISCUSSION AND CONCLUSION 48
REFERENCES 51
Table 1. Dilution water quality measurements during the pre-exposure period and 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 53
Table 2. Water quality measurements during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 54
Table 3. Concentrations of 2-ethylhexyl paraben measured in the exposure solutions during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 55
Table 4. Survival at test termination of the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 56

Table 5. Summary of fertilization success and fecundity during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 57
Table 6. Male termination endpoint summary during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 58
Table 7. Female termination endpoint summary during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 59
Table 8. Results of VTG analysis using all data points (no outliers removed) during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 60
Table 9. Results of the sex steroid analysis following the 21 day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 61
Figure 1. Relationship between nominal and mean measured concentrations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 62
Figure 2. Mean male survival during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 63
Figure 3. Mean female survival during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 64
Figure 4. Mean combined male and female survival during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 65
Figure 5. Mean fertilization success (percentage of viable eggs) during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 66
Figure 6. Mean fecundity (number of eggs per female per reproductive day) during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 67
Figure 7. Median male tubercle score during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 68
Figure 8. Mean male weight during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 69
Figure 9. Mean female weight during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 70
Figure 10. Mean male length during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 71
Figure 11. Mean female length during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 72
Figure 12. Mean male GSI during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 73

Figure 13. Mean female GSI during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 74
Figure 14. Mean male VTG concentration using all data points (no outliers removed)during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 75
Figure 15. Mean female VTG concentration using all data points (no outliers removed)during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 76
Figure 16. Male testosterone concentration following the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 77
Figure 17. Male 17β-estradiol concentration following the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 78
Figure 18. Female testosterone concentration following 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 79
Figure 19. Female 17β-estradiol concentration following the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben. 80
APPENDIX 1 – STUDY PROTOCOL 81
APPENDIX 2 – FOOD ANALYSIS 108
APPENDIX 3 – PRELIMINARY EXPOSURE AND STUDY DATA.....118
APPENDIX 4 – ANALYTICAL METHODOLOGY 121
Table 1A. Analytical results for the recovery of 2-ethyl paraben in freshwater during the method validation. 129
Figure 1A. Representative chromatogram of a 1.50 µg/L calibration standard during the method validation with 2-ethyl paraben. 130
Figure 2A. Representative chromatogram of 125 µg/L recovery sample during the method validation with 2-ethyl paraben.....131
Figure 3A. Representative chromatogram of a control sample during the method validation with 2-ethyl paraben. 132
Figure 4A. A typical regression analysis for the calibration standards used to quantitate 2-ethyl paraben in the recovery samples.....133
APPENDIX 5 – 2-ETHYL PARABEN TESTING SUMMARY 134
APPENDIX 6 – TESTING SUMMARY – SEX STEROID ANALYSIS 139
APPENDIX 7 – STATISTICAL ANALYSIS.....157
APPENDIX 8 – STUDY DATA 183
APPENDIX 9 – EPA-PRESCRIBED DATA ENTRY SPREADSHEET TEMPLATES (DESTS).....218
APPENDIX 10 – IODIDE ANALYSIS 346

APPENDIX 11 – HISTOPATHOLOGY.....348

EXECUTIVE SUMMARY

The 21-day short-term reproduction assay (OPPTS 890.1350) of 2-ethylhexyl paraben (99.8% purity) with fathead minnow (*Pimephales promelas*) was performed under flow-through conditions. Spawning adult fish, four female and two male fish, based on secondary sexual characteristics, were placed in each of 4 replicate tanks. Adult fish were exposed to a control and mean average concentrations of 9.7, 20, 53, and 110 µg/L. The exposure was maintained for a period of 21 days. Nominal test concentrations were 14, 35, 88, and 220 µg/L 2-ethylhexyl paraben.

The following endpoints were evaluated for statistical differences:

- Fecundity (number of eggs/female/day)
- Fertilization success
- Nuptial tubercle score
- Blood plasma vitellogenin (VTG) concentration
- Gonadal Somatic Index (GSI)
- Weight (male and female)
- Survival

One other endpoint, plasma sex steroids, which is listed as optional in the OPPTS 890.1350 guidelines, was required for data collection in this fish short-term reproduction assay (FSTRA) per sponsor direction. As the guidelines suggest (pg. 21), tabulated data on biological observations of sex steroids are included in the results and in the data appendix. However, because this endpoint often shows high variability between fish, the data were evaluated empirically and not statistically. The sex steroid data are available for statistical analysis in the future.

During the exposure period, the appearance of the fish, behavior, fecundity, and fertilization success were assessed daily. At test termination, observations were made on the behavior and coloration of the fish. The fish were then euthanized with tricaine methanesulfonate, measured

for standard length and wet weight, and observed for secondary sexual characteristics. Blood samples were taken for plasma vitellogenin (VTG) and sex steroid analysis. The gonads were removed and weighed for gonadal somatic index (GSI) determination and histological evaluation. Tubercle scoring was also performed. The thyroid was evaluated histologically. Fecundity in the control was 46 eggs/female/day; fertilization success was 99.7%.

Notable observations were made with regards to coloration/banding. One female was observed to have color bars present in replicate A of the control on exposure day 7; one female in replicate D of the 9.7 µg/L treatment level on exposure days 7 and 8; and one female in replicate A of the 53 µg/L treatment level on exposure days 9 through 20. While color bars are primarily considered to be a secondary sexual characteristic for males, females can occasionally be observed to express color bars. The color bars observed in females during this exposure were transient and did not exhibit a dose-dependent trend. None of the female coloration observations described above were considered to be a result of exposure to 2-ethylhexyl paraben. A female in replicate A of the 9.7 µg/L treatment level was inadvertently injured during aquarium cleaning on test day 20. Therefore, the female mortality in this replicate on test day 21 was likely a result of this technician error and not treatment-related. All fish found dead were macroscopically examined for presence of ovaries and were appropriately preserved. Gonad and thyroid histopathology was not performed on the dead fish. The results of the statistically evaluated endpoints, sex steroid plasma concentrations, and histopathology are presented below.

Exposure to 2-ethylhexyl paraben did not result in any significant changes in male or female body weight, body length, fecundity, and fertility. Exposure to 2-ethylhexyl paraben significantly increased male VTG plasma concentration (with and without outliers removed) at the 53 and 110 µg/L concentrations. The induction of VTG in male fish is a well-established response to estrogen receptor agonists (Borgert et al., 2014). Plasma VTG was not significantly increased in females. Exposure to 2-ethylhexyl paraben appeared to increase male plasma concentrations of 17β-estradiol at the 110 µg/L concentration based on empirical evaluation, but data are highly variable.

Significant increases in male and female GSI occurred at the 110 µg/L treatment level (highest treatment level) in this study. Increases in GSI have been linked to possible endocrine disruptor activity, and may be due to the presence of intravascular and interstitial fluid in the gonads of males and females. However, the mechanisms underlying GSI responses are not well characterized and are not always specific (Borgert et al., 2014).

Responses in secondary sexual characteristics such as tubercle scores in males also provide evidence of estrogenic activity (OECD, 2007). In this study, exposure to 2-ethylhexyl paraben caused a slight, not statistically-significant, reduction in male tubercle scores at the 20 and 53 µg/L treatment levels.

Histopathological findings associated with 2-ethylhexyl paraben exposure occurred in the gonads of male and female fish and included: intravascular and interstitial proteinaceous fluid in the testes of the 53 and 110 µg/L treatment level males (minimal to mild), and in the ovaries of the 110 µg/L treatment level females (mild); increased male testis stage scores, particularly in males of the 53 and 110 µg/L treatment levels; a dose-dependent increase in the prevalence of oocyte atresia in all treatment levels (minimal to moderate); a dose-dependent increase in the severity of oocyte atresia in the 53 and 110 µg/L treatment levels (minimal to moderate); decreased post-ovulatory follicles in females of the 53 and 110 µg/L treatment levels; and shifts in ovarian stage scores in females, particularly in females of 53 and 110 µg/L treatment levels. Although mean ovarian stage scores were comparable among females in the control and the treatment levels, there was a shift away from females in the spawning or recently spawned stages (i.e., stages 4.0, 2.0, and 2.5) toward stages of oocyte maturation (i.e., stages 3.0 and 3.5). There were no thyroid findings.

The presence of proteinaceous intravascular and interstitial fluid in the gonads of male and female fathead minnows is consistent with increased hepatic VTG production as might be induced by substances with estrogenic activity. A number of *in vitro* and *in vivo* studies have demonstrated estrogen-like effects in fish exposed to various parabens, including VTG induction (Pedersen et al., 2000; Inui et al., 2003; Yamamoto et al., 2011). Although not specific for

enhanced estrogenic activity, increased oocyte (follicular) atresia is another commonly reported effect of xenoestrogen exposure (Dietrich and Krieger, 2009).

Endpoints		Mean Measured Concentration (µg/L)			
		10	20	53	110
Day 21	Combined Male and Female Survival	-	-	-	-
	Fecundity	-	-	-	-
	Fertilization Success	-	-	-	-
	Nuptial Tubercle Score	-	-	-	-
	Male Weight	-	-	-	-
	Male GSI	-	-	-	↑
	Female Weight	-	-	-	-
	Female GSI	-	-	-	↑
	Male VTG	-	-	↑	↑[a]
	Female VTG	-	-	-	-
	Male Testosterone	ND[b]	ND[b]	ND[b]	ND[b]
	Male 17β-estradiol	ND[b]	ND[b]	ND[b]	↑[c]
	Female Testosterone	ND[b]	ND[b]	ND[b]	ND[b]
	Female 17β-estradiol	ND[b]	ND[b]	ND[b]	ND[b]
- Endpoint not statistically different from controls. ↓ Statistical analysis determined endpoint to be significantly reduced ($p \leq 0.05$) compared to the control. ↑ Statistical analysis determined endpoint to be significantly increased ($p \leq 0.05$) compared to the control. [a] Statistically significant increase based on three plasma samples in this treatment level. However, the five remaining samples above detection limit (ADL) suggests a strong dose response at this endpoint. [b] No empirical difference from the control. Statistical analysis was not required by protocol. [c] Increase in plasma concentration of 17β-estradiol based on empirical evaluation of treatment means and variation among replicates.					
Male Gonadal Staging		NF	NF	F	F
Male Histopathology Findings		NF	NF	F	F
Female Gonadal Staging		NF	NF	F	F
Female Histopathology Findings		NF	NF	F	F
Thyroid Histopathology Findings		NF	NF	NF	NF
NF = No findings related to 2-ethylhexyl paraben exposure. F = Findings potentially related to 2-ethylhexyl paraben exposure. See Section 3.2.13 for summary.					

CONCLUSION:

Exposure to 2-ethylhexyl paraben caused treatment-related effects in males and females: histopathological findings in the gonads at the 53 and 110 µg/L treatment levels and increased GSI at the highest treatment level (110 µg/L), and increased plasma VTG in males. Based on these results and the decision criteria in the FSTRA test guideline (OPPTS 890.1350), 2-ethylhexyl paraben may be potentially active on the hypothalamic pituitary gonadal (HPG) axis.

The Guideline performance criteria were met, with these exceptions: a CV <20% is desired for the measured exposure concentrations and all groups were 38 to 46%. While the analytical recoveries were variable over the length of the exposure, the desired concentration gradient was maintained at each sampling interval and no overlap in concentrations was observed. Variability between replicates of the same concentration was maintained within approximately 20% at each interval. The variability in the measured concentrations between intervals was likely related to the physical properties of the test substance (e.g., logKow > 5), which indicate a propensity for adsorption to the high amount of glass and silicone surface area in the test system as well as possible uptake by the adult fathead minnows. The inherent aging of the biological exposure system (i.e., feeding, fish biomass, and microbial populations) also likely affected the recoveries of 2-ethylhexyl paraben in the aquaria samples.

Additionally, temperature in replicate D of the 220 µg/L treatment level on test day 0 was outside 25 ± 1 °C criterion (26.7 °C). This slight variation was immediately addressed on the same day and all temperatures were observed to be within the acceptable range prior to the addition of organisms. Several deviations from the study protocol were recorded during the exposure and are presented in detail in Section 5.0. These deviations did not impact the integrity or interpretation of the study.

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided. It was stated test substance characterization was not performed in accordance with Good Laboratory Practice Standards (GLPs).

TEST SUBSTANCE: 2-Ethylhexyl paraben, Lot No. VXGDC, CAS No. 5153-25-3, reported to have a purity of 99.8%, was received from Tokyo Chemical Industry America on 20 October 2015.

DEFINITIVE TEST DATES: 22 September to 13 October 2016

TEST ORGANISM: *Pimephales promelas*
Age at exposure initiation: approximately 20 weeks old
Source: Smithers Viscient culture

DILUTION WATER: Laboratory well water: parameters presented here are measured weekly at a central source and demonstrate the acceptability of the dilution water for use (Table 1). These parameters are measured in accordance with the study protocol and are maintained in the facility records.
pH: 6.8 to 7.4
Conductivity: 420 to 550 µS/cm
Total hardness as $CaCO_3$: 64 to 84 mg/L
Total alkalinity as $CaCO_3$: 18 to 24 mg/L

TEST CONDITIONS: Results presented here represent the measurements from the exposure system during the definitive exposure:

Test duration:	21 days
Temperature range:	24 to 27 °C (see Protocol Deviations)
Dissolved oxygen range:	4.6 to 8.5 mg/L (see Protocol Deviations)
pH range:	6.8 to 7.5
Light intensity range:	52 to 69 footcandles (560 to 740 lux)
Photoperiod:	Illumination of 16 hours light: 8 hours dark

NOMINAL TEST CONCENTRATIONS: 14, 35, 88, and 220 µg/L

MEAN MEASURED CONCENTRATIONS: 9.7, 20, 53, and 110 µg/L

STATISTICALLY EVALUATED ENDPOINTS:

- Fecundity (number of eggs/female/day)
- Fertilization success
- Nuptial tubercle score
- Blood plasma vitellogenin (VTG) concentration
- GSI (Gonadal Somatic Index)
- Weight (male and female)
- Survival

1.0 INTRODUCTION

The purpose of this study was to assess the potential for 2-ethylhexyl paraben to interact with the endocrine system in fathead minnows (*Pimephales promelas*) exposed under flow-through conditions. The endpoints evaluated were fecundity (number of eggs per female per reproductive day), fertilization success, secondary sex characteristics (nuptial tubercle score), blood plasma vitellogenin (VTG) concentrations, gonadal somatic index (GSI), weight, survival, blood plasma sex steroid (testosterone and 17β-estradiol), and histopathological findings. The results of this study are based on mean measured concentrations of 2-ethylhexyl paraben.

The study was initiated on 13 April 2016, the date the Study Director signed the protocol, and was completed on the day the Study Director signed the final report. The experimental phase of the study was conducted from 22 September to 13 October 2016 at Smithers Viscient (SMV), located in Wareham, Massachusetts. All raw data, the protocol, and the original final report produced during this study are stored in Smithers Viscient archives at the above location. Experimental Pathology Laboratories (EPL), Sterling, Virginia, performed the histopathology work. Specimens associated with the histopathology including all slides produced during the histopathological analysis will be stored at EPL until study finalization. After study finalization, all slides will be shipped to the Sponsor.

2.0 MATERIALS AND METHODS

2.1 Protocol

The methodology used for the short-term assay is presented in the Smithers Viscient protocol entitled “Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines” (Appendix 1). The study protocol describes standard toxicity test procedures for a short-term assay with the fathead minnow (*Pimephales promelas*) based on the protocol developed by Ankley et al., 2001 and meets the requirements of OPPTS Guideline 890.1350 (U.S. EPA, 2009) and OECD Guideline 229 (OECD, 2009) for the fish short-term reproduction assay.

2.2 Test Substance

The test substance, 2-ethyl paraben, was received on 20 October 2015 from Tokyo Chemical Industry America, Montgomeryville, Pennsylvania. The following information was provided:

Name:	2-ethyl paraben
Synonyms:	2-ethylhexyl paraben, 2-ethylhexyl 4-hydroxybenzoate
Lot No.:	VXGDC
CAS No.:	5153-25-3
Purity:	99.8%
Expiration Date:	Not Listed

Upon receipt at Smithers Viscient, the test substance (SMV No. 7928) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance.

2.3 Test Organism

The fathead minnows (*Pimephales promelas*) used during this study (SMV Lot No. 16A061) were obtained from a laboratory supply of reproductively mature animals (approximately 20 weeks old at the initiation of the pre-exposure period), in spawning condition.

2.4 Feeding

Prior to exposure initiation and throughout the exposure period (including the pre-exposure spawning qualification period), the fish were fed two feedings of frozen brine shrimp (e.g., 3.0 mL) and one feeding of fish flake food (e.g., 2.5 mL) daily. Prior to the use of a new lot of food, a representative sample of the food sources was analyzed for the presence of pesticides, PCBs, and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. Based on these analyses, which follow EPA SW-846 Test Methods for Evaluating Solid Waste, the food sources were considered to be of acceptable quality since all analyte concentrations were below levels of concern based on ASTM, 2005 (Appendix 2). Food lots were screened for metals under the SW-846 Compendium methods of the 6000 and 7000 series (i.e., 6010B and 7470A) and

pesticides/PCBs were tested under the 8000 series (i.e., 8081A, 8082, and 8141A), as per the EPA Publication SW-846. Fish were not fed during the 24-hour period prior to test termination.

2.5 Dilution Water

The dilution water used during this study consisted of a mixture of unadulterated water from an onsite 100-meter bedrock well and de-chlorinated Town of Wareham well water. This mixture was pumped into a 5700-liter polyethylene tank that was continuously circulated through de-gassing chambers to ensure proper mixing and equilibration of dissolved gases with the laboratory atmosphere.

During the study, water quality parameters of the dilution water were evaluated weekly at a central source. This evaluation was performed to demonstrate the acceptability of the dilution water for use. Weekly characterization of the well water established total hardness and alkalinity ranges as $CaCO_3$ of 64 to 84 mg/L and 18 to 24 mg/L, respectively, a pH range of 6.8 to 7.4, and a conductivity range of 420 to 550 µS/cm. These data are summarized in Table 1. Representative samples of the dilution water source were analyzed periodically for the presence of pesticides, PCBs, and toxic metals by GeoLabs, Inc., Braintree, Massachusetts. None of these compounds were detected at concentrations considered toxic in any of the water samples analyzed, in agreement with ASTM (2005) standard practices. Representative samples of the dilution water source were screened for metals under the SW-846 Compendium methods of the 6000 and 7000 series (i.e., 6010B and 7470A) and pesticides/PCBs were tested under the 8000 series (i.e., 8081A, 8082, and 8141A), as per the EPA Publication SW-846. In addition, samples of the dilution water source were analyzed monthly for total organic carbon (TOC) concentration. This analysis established a TOC concentration of 0.93 and 0.72 mg/L for the months of September and October 2016, respectively.

2.6 Test Conditions

The vessels were positioned in two (upper and lower) water baths containing circulating water. The upper bath replicates A and B and the lower bath contained replicates C and D. The system was designed to maintain the test solution temperatures at 25 ± 1 °C and dissolved oxygen concentrations at ≥60% of saturation. Daily temperature measurements taken in the exposure system ranged from 24 to 27 °C (see Protocol Deviations). Dissolved oxygen concentrations ranged from 4.6 to 8.5 mg/L (see Protocol Deviations). Aeration was added to the system prior to initiation to raise and maintain appropriate dissolved oxygen concentrations. Aeration is often required to compensate for decreasing dissolved oxygen concentrations in exposures with adult, spawning fish. The deviation in dissolved oxygen concentration (56.1%) in the 35 µg/L concentration replicate B tank occurred for a short time on test day 14 as a result of an aeration stone malfunction. This issue was immediately addressed and dissolved oxygen re-measured at 90.6% on the same day. Illumination was provided by Sylvania Octron fluorescent bulbs centrally located above the test aquaria. Sixteen hours of light at 52 to 69 footcandles (560 to 740 lux) at the exposure solution surface was provided daily. Light intensity was measured with a VWR traceable light meter. The diluter system was enclosed with black polyethylene curtains to prevent disturbance of spawning and to minimize the interference of laboratory lighting with the intended photoperiod.

2.7 Test Substance Concentrations

Nominal concentrations for the short-term reproduction assay with fathead minnow (*Pimephales promelas*) were selected based on preliminary testing (Appendix 3) and in consultation with the Study Sponsor. The nominal concentrations selected for the reproduction assay were 14, 35, 88, and 220 µg/L. Specific details regarding the selection of these nominal test concentrations are outlined in Section 3.1.

2.8 Stock Preparation

For this exposure, glass wool saturator columns were used to deliver 2-ethylhexyl paraben to the exposure system, similar to those described in Kahl, et al. 1999. The glass columns were packed with glass wool, and then coated with the test substance. The columns were designed to provide a constant flow of saturated aqueous solution (effluent) of 2-ethylhexyl paraben (6.0 mg/L) to the diluter system without the use of a carrier solvent. Columns were constructed entirely of chemical inert materials (glass and Teflon).

To construct the columns, each 60 (length) × 4.8 (diameter) centimeter column was firmly packed with glass wool, which approximated 15% of the total column volume. This provided ample surface area inside the column for the exposure substance to adhere once the column preparation was complete. After the column was packed, the end fittings were placed on the column. All fittings used to enclose the column and to attach the column to the water source were composed of Teflon.

To coat a column, approximately 15 grams of 2-ethylhexyl paraben was diluted with 50 mL of acetone, reagent grade (CAS No. 67-64-1). This solution was slowly poured into the glass column. After all of the solution was added, the column was attached to a vacuum pump. The vacuum pump was used to draw the solution evenly throughout the column to uniformly coat the wool with the test substance and evaporate the remaining acetone. After it had visually appeared that all of the glass wool was coated and all the solution was evaporated, the column was detached from the vacuum pump and attached to a FLUID Metering, Inc. (FMI) pump, which delivered a flow of dilution water through the column at 17 mL/min to the chemical's mixing chamber. The column output concentration was used to calculate the appropriate flow rate of the stock solution into the diluter system. During the exposure, saturator columns were prepared and replaced on the exposure system approximately biweekly based on previous column stability data.

2.9 Exposure System

The exposure system consisted of a modified 2-L intermittent-flow proportional diluter (Mount and Brungs, 1967) and a two-tiered water bath, consisting of an upper and a lower level water bath (one positioned over the other). The exposure system was designed to provide four concentrations of the test substance and a control (absent of 2-ethylhexyl paraben) to four replicate exposure aquaria. The upper bath contained replicates A and B and the lower bath contained replicates C and D.

Prior to exposure initiation, an FMI pump was calibrated to deliver approximately 17 mL/min (i.e., 119 mL per cycle) of the 6.0 mg/L saturator column effluent to the diluter system's mixing chamber at each cycle (as described in Section 2.8). This chamber also received 3.10 L of dilution water at each cycle for a total volume of 3.248 L. The mixing chamber was positioned over a magnetic stir plate which aided in homogenization of the exposure solutions.

Historical trends with studies that begin with actively spawning adult fathead minnows indicated that dissolved oxygen levels typically drop significantly soon after initiation (within 24 hours). Therefore, oil-free aeration was added to all aquaria with a regenerative blower and air stones during the pre-test period prior to initiation to avoid anoxic conditions during the test. Analytical data during the pre-exposure period yielded all 2-ethylhexyl paraben treatment levels at approximately 55 to 65% of nominal concentrations as a result of the aeration. The saturator column effluent flow rate to the mixing chamber was, therefore, intentionally increased to 20 mL/min on day -1. The increase in saturator column effluent flow rate was designed to dose the diluter mixing chamber at a slightly higher concentration than the highest nominal concentration (i.e., 270 µg/L vs. 220 µg/L) in order to more closely approximate nominal concentrations across the treatment range. The slightly higher concentration of the mixing chamber solution was subsequently diluted by a constant factor of approximately 2.5 to provide the remaining nominal exposure concentrations (88, 35, and 14 µg/L). On day 11 of exposure, the column effluent flow rate to the mixing chamber was intentionally increased again to 26 mL/min (e.g., 186 mL/cycle) in order to more closely approximate nominal concentrations.

This modification was employed due to the observed recoveries of 2-ethylhexyl paraben in the day 7 aquaria samples, which ranged from 20 to 40% of nominal concentrations. On day 18 of exposure, the stock delivery was intentionally increased once more to 31 mL/min (e.g., 224 mL/cycle) due to the observed recoveries of 2-ethylhexyl paraben in the day 14 aquaria samples, which ranged from 20 to 40% of nominal concentrations. The volume of dilution water delivery to the mixing chamber was adjusted from 3.10 L to 3.00 L to accommodate the day 18 toxicant delivery modification. While there were difficulties maintaining consistent recoveries of 2-ethylhexyl paraben throughout the exposure, corrective action was taken as soon as plausible following the pre-test analytical interval and test days 7 and 14. The high variability in 2-ethylhexyl paraben recovery was likely due to the physicochemical properties of the test substance (e.g., logKow > 5). Risk of analytical variability is high when chemicals with properties similar to 2-ethylhexyl paraben are delivered to diluter systems with sexually dimorphic, actively spawning fish; the high amount of glass surface area creates high binding potential for the chemical to adsorb. These adsorption rates are difficult to predict, and stock flow increases are typically performed conservatively in order to avoid potential dosing spikes.

A set of control vessels was also established, which contained the same dilution water and was maintained under the same conditions as the treatment level vessels, but contained no 2-ethylhexyl paraben.

The exposure system and exposure aquaria were constructed of glass, silicone sealant, and nylon. Each 18-L test aquarium measured 39 × 20 × 25 cm (L × W × H) with a 13-cm high side drain that maintained a constant exposure solution volume of approximately 10 L. Test aquaria were labeled to identify the nominal test substance concentration and designated replicate.

Chemical cleaning of the diluter system and aquaria was performed prior to exposure initiation. During the 21-day study, exposure aquaria were brushed and siphoned at least twice per week to remove excess food and fecal matter. At a minimum, the diluter mixing chamber, chemical cells, splitters, and delivery tubing were cleaned weekly.

Flow-splitting chambers were used between the diluter cells and the four replicate test vessels to promote mixing of the 2-ethylhexyl paraben solution and diluent water, and to equally split the test solution between the test vessels. The flow-splitting chambers promoted further mixing of the chemically-dosed water and dilution water, and equally distributed the test solutions through glass delivery tubes to the replicate exposure aquaria. During the test, 0.5 L per cycle of test solution was delivered to each aquarium at a rate of approximately 199 cycles per 24 hours. This flow provided a turnover rate of 10 volume replacements every 24 hours, or a 90% replacement time of approximately 3 hours (Sprague, 1969).

The exposure system was operating properly for eight days prior to exposure initiation to allow equilibration of the test substance in the diluter apparatus and exposure aquaria. Analysis of exposure solutions for 2-ethylhexyl paraben was performed on test days 0 (exposure initiation), 7, 14, and 21.

2.10 Exposure Initiation

2.10.1 Pre-Exposure Phase

Prior to exposure initiation, the adult fish were housed in aquaria within a pre-exposure system to evaluate reproductive performance over a 16-day period.

During the seven days prior to the pre-exposure, no mortality was observed in the population of fish used to initiate this study. Fish did not receive treatment for disease in the two weeks prior to the spawning qualification period preceding the test, or during the exposure period.

Water flowing to the pre-exposure system was from the same source as the dilution water used during the exposure. Water quality parameters of the dilution water were evaluated weekly at a central source, according to the study protocol. This evaluation was performed to demonstrate the acceptability of the dilution water for use and the results presented below are maintained as facility records. The dilution water was characterized during the timeframe of the pre-exposure

period as having total hardness and total alkalinity ranges as calcium carbonate ($CaCO_3$) of 70 to 84 mg/L and 18 to 20 mg/L, respectively, a pH range of 6.6 to 7.1, and a conductivity range of 450 to 550 µS/cm. These data are summarized in Table 1. Fish used during the definitive exposure were maintained under these conditions for 16 days prior to testing. Water quality parameters (dissolved oxygen, temperature, and pH) were also measured daily in the pre-exposure system. As the primary purpose of the pre-exposure spawning period is to establish robust spawning groups, aeration was maintained in this system during the entire qualification. Therefore, the dissolved oxygen concentration presented here for the pre-exposure period are not a predictor of the concentrations that might be observed in the actual exposure system. At the initiation of the pre-exposure, measurements were performed in each individual tank; tanks were rotated for evaluation for subsequent measurements. Dissolved oxygen levels ranged from 78 to 110% of saturation. Daily measurements of temperature in the aquaria ranged from 24 to 26 °C, and pH measurements ranged from 6.8 to 7.3 (see Protocol Deviations). The acceptable performance of the organisms during the pre-exposure period demonstrated that the water quality was appropriate for the survival and reproduction of fathead minnows. All parameters reported in this section were during the pre-exposure spawning qualification period and do not reflect parameters during the definitive exposure.

2.10.2 Pre-Exposure System

The pre-exposure system was a two-tiered water bath, consisting of an upper and a lower level. The aquaria were divided between these two water baths and were constructed entirely of glass and silicone sealant. Each 18-L test aquarium measured 39 × 20 × 25 cm with a 13-cm high side drain that maintained a constant exposure solution volume of approximately 10 L. Each test aquarium contained three spawning substrates as described in Section 2.9.

Four females and two males were impartially assigned to each of the 36 pre-exposure vessels 16 days prior to exposure initiation. Additional exposure chambers were set up during the pre-exposure phase to account for a potential lack of spawning in some chambers and/or mortality during this phase. The pre-exposure phase was conducted under test conditions similar

to those used during the chemical exposure. The animals were fed a measured amount of frozen brine shrimp twice daily (e.g., 3.0 mL) and once daily with fish flake food (e.g., 2.5 mL). Each group was monitored daily for survival and active spawning, and fecundity data was collected. During this phase, suitability for testing was established when regular spawning occurred in each replicate chamber at least twice in the immediate 7-day period preceding exposure initiation and when an egg production rate of >15 eggs/female/day/replicate was achieved.

2.10.3 Selection and Weighing of Test Fish

Fish utilized in the pre-exposure system were approximately 20 weeks old. All fish were sexually dimorphic and exhibited the appropriate secondary sexual characteristics. Prior to adding fish to the pre-exposure system, subsamples of both male and female fish in the test population were weighed. Weight measurements are presented in. A representative sample of the male (N = 30) and female (N = 30) fish from the test population selected for the 2-ethylhexyl paraben exposure had a mean wet weight of 4.3 g (range 3.4 to 5.1 g, within ± 20% of the mean) and 2.8 g (range 2.2 to 3.3 g, within ± 20% of the mean), respectively. All fish used in the pre-exposure were weighed to determine if their weight fell within the expected range for their respective sex.

2.10.4 Definitive Exposure

Once successful spawning was established during the pre-exposure phase, 20 spawn groups were added to the exposure system using a random block distribution, which divided fish into blocks based on spawning activity and then randomized the blocks to exposure vessels. The exposure was maintained for a period of 21 days. Each replicate tank contained four female and two male fish based on secondary sexual characteristics.

During the exposure period, the appearance of the fish, behavior, fecundity, and fertilization success were assessed daily. At test termination, observations were first made on the behavior and coloration of the fish. The fish were then euthanized by transfer to a buffered solution of MS-222 (tricaine methanesulfonate), measured for standard length and wet weight, and observed

for secondary sexual characteristics. Blood samples were taken for plasma VTG and sex steroid analysis. The gonads were removed in-situ and weighed for GSI determination and histological analyses. Fish carcasses were preserved for subsequent tubercle scoring.

2.11 Test Monitoring

2.11.1 Water Quality Measurements

At exposure initiation and weekly thereafter, total hardness, total alkalinity, and conductivity were measured and recorded in one replicate of one treatment level and the control alternating between treatment levels and replicate vessels (A, B, and C) each week. Dissolved oxygen, pH, and temperature were recorded in each concentration and control vessel at exposure initiation and subsequently in alternating replicates on a daily basis. Test solution temperature was continuously monitored during the exposure period in the upper and lower water baths using Fisher Scientific and VWR minimum-maximum thermometers, respectively. Dissolved oxygen concentrations and daily temperature were determined using a Yellow Springs Instrument (YSI) Model No. 550A dissolved oxygen meter/temperature probe. The pH was measured using a YSI Model pH100A pH meter. Total hardness concentrations presented in this report were measured by the EDTA titrimetric method, and total alkalinity concentrations were determined by potentiometric titration to an endpoint of pH 4.5 (APHA et al., 2005). Conductivity was measured using a YSI Model Pro 30 conductivity meter.

2.11.2 Analytical Measurements

Prior to the start of the definitive exposure, samples from the column effluent, two replicates (A and C or B and D) of each treatment level and the control were collected and analyzed for 2-ethylhexyl paraben concentration during two separate intervals. Results of the pretest analyses were used to confirm that the diluter was functioning properly.

Samples were removed at exposure initiation and weekly thereafter. On test days 0 (exposure initiation) and 14, samples were removed from replicates C and D; and on test days 7 and 21,

samples were removed from replicates A and B. The arithmetic mean concentration was calculated for each treatment. The coefficient of variance (% CV) is the standard deviation divided by the mean measured concentration expressed as a percent. Standard deviation is the square root of the variance. All exposure samples were removed from the approximate midpoint of each aquarium using a pipette. The saturator column effluent was also analyzed during the pre-test period and at each sampling interval during the exposure period.

Three quality control (QC) samples were prepared and analyzed with each set of the study samples. These QC samples were prepared in dilution water at concentrations similar to the treatment levels tested. Results of the analyses of the QC samples were used to judge the precision and quality control maintained during the analysis of exposure solution samples.

All exposure solutions and QC samples were analyzed for 2-ethylhexyl paraben using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) based on methodology validated at Smithers Viscient. The method validation study was conducted in laboratory well water and established an average recovery of 111 ± 6.98% for 2-ethylhexyl paraben. Defined limits for acceptance of quality control sample performance in subsequent studies were set at 80.0 to 120%. Analytical conditions and procedures used throughout the analysis of exposure solutions and QC samples during this study were similar to those used in the method validation study. Processing procedures for the exposure solution samples and QC samples were the same as the procedures utilized during the method validation. Additionally, the dosing procedures for QC samples were the same as the dosing procedures for the recovery samples during the method validation (Appendix 4). Samples from the exposure system were not dosed in the same manner as recovery samples. A summary of the exact procedures used during this testing can be found in Appendix 5.

2.12 Endpoints and Observations

2.12.1 Survival

An assessment of survival was conducted daily throughout the exposure to provide a base for interpretation of reproductive output. Fish were examined daily during the test, and any external abnormalities and mortalities were noted. Dead fish were removed when observed and were not replaced. Upon discovery, carcasses were dissected to macroscopically confirm presence of testis or ovaries, preserved overnight in Davidson's Fixative, then rinsed with reagent grade alcohol and placed into a container with 10% neutral buffered formalin.

2.12.2 Appearance of Adults

Secondary sexual characteristics, e.g., body color (light or dark), coloration patterns, body shape, presence of dorsal nape pad, nuptial tubercles, and ovipositor were observed, when possible, and recorded daily as described by Ankley et al., 2001.

2.12.3 Behavior of Adults

Abnormal behavior (relative to the control), such as hyperventilation, loss of equilibration, uncoordinated swimming, atypical quiescence, lethargy, and feeding abstinence were noted daily during observations. Alterations in behavior, particularly loss of territorial aggressiveness by males, were also noted.

2.12.4 Fecundity

Throughout the 21-day exposure period, egg production was determined daily. Spawning substrates and spawning trays were removed and the number of eggs present were counted, recorded, and then assessed for fertilization success. Fecundity is expressed as the number of eggs produced by surviving females per reproductive (test) day per replicate.

2.12.5 Fertilization Success

Fertilization success was determined by evaluating the embryos under the appropriate magnification after being removed from the spawning substrate. Embryos were considered fertile if undergoing late cleavage and were considered infertile if the eggs were opaque or clear with a white dot where the yolk had precipitated. The fertility rate is expressed as the number of embryos divided by the number of eggs × 100.

2.13 Test Termination

At the conclusion of the exposure (day 21), all surviving fish were euthanized with a neutral buffered solution of MS-222 and separated by sex. Observations were made on the presence of secondary sexual characteristics (i.e. fatpad, color bars, and papillae) and individual standard lengths and wet weights were determined. Lengths were measured to the nearest 0.01 mm and wet weights to 0.10 mg. As much blood as possible was then collected from the caudal artery/vein of each fish with a heparinized microhematocrit capillary tube. Plasma was separated from the blood via centrifugation (approximately 5 minutes at 15,000 *g*) and stored with protease inhibitors at -75 °C to -85 °C until analyzed for VTG and sex steroids.

2.13.1 Nuptial Tubercle Scoring

The OPPTS Guideline 890.1350 (U.S. EPA, 2009) outlines a set of procedures for the evaluation of nuptial tubercle scoring (i.e. Appendix B, contained within the 890.1350 test guideline). Nuptial tubercles were counted, mapped, and scored on preserved fish according to these procedures (see Protocol Deviations). Six specific areas were identified for assessment of tubercle presence and development. A template was developed to map the location and quantity of tubercles present. The tubercles were rated using a 1, 2, or 3 as follows: 1 = tubercles were present, 2 = tubercles are present and enlarged, and 3 = tubercles are present and pronounced.

The mapping regions were as follows:

A. Tubercles located around the eye. Mapped dorsal to ventral around anterior rim of eye. There are commonly multiple tubercles in mature males and none present in control females.
B. Tubercles located between nares (sensory canal pores). These are normally present in pairs for control males at more elevated levels of development and they are not present in control females.
C. Tubercles located immediately anterior to nares, parallel to mouth. These are generally enlarged or pronounced in mature control males.
D. Tubercles located parallel along the mouth line. These are generally developed in control males and absent in control females.
E. Tubercles located on lower jaw, close to the mouth, usually small and commonly in pairs. These vary in control males.
F. Tubercles located ventral to "E". They are commonly small and paired in control males.

2.13.2 Gonadal Somatic Index (GSI) and Histology

Gonads were removed from the fish by making an incision from the vent forward through the pelvic girdle to the opercula. Gonads were fixed using Davidson's fixative to prevent autolysis and cellular deterioration before removal. Gonads were transferred to tared tissue cassettes and weighed to the nearest 0.10 mg for calculation of the GSI. Each fish carcass and cassette was placed into a labeled container of Davidson's fixative. After overnight fixing, the specimens were rinsed with alcohol and placed into a container of 10% neutral buffered formalin. The specimens were shipped to Experimental Pathology Laboratories (EPL), Sterling, Virginia for histopathological analysis.

2.13.3 Sex Steroid Analysis

Per Study Sponsor direction, plasma concentrations of 17β-estradiol and testosterone were determined using liquid chromatography with mass spectrometry (LC-MS/MS) methodology

optimized for the relatively small sample volumes obtained from the fathead minnow. The analytical methodology for sex steroid analysis was established prior to evaluation of sex steroids. Since this endpoint often shows high variability between fish, and because it is listed as optional in the OPPTS 890.1350 guidelines, the sex steroid data were evaluated empirically and not statistically. Due to the small volume of plasma available, priority was given to the analysis of 17β-estradiol for females and testosterone for males. A summary of the exact processing and analysis procedures used during this testing can be found in Appendix 6. If any analytical result was below the limit of quantification (LOQ), one-half of the LOQ was used for the calculation of the treatment mean values.

2.13.4 Vitellogenin (VTG) Analysis

Plasma samples were analyzed for VTG using an enzyme-linked immunosorbent assay (ELISA). The VTG ELISA kits were manufactured by BioSense Laboratories, Bergen, Norway. The analysis was performed according to the instructions provided by BioSense Laboratories. For the ELISA, monoclonal fathead minnow (*Pimephales promelas*) VTG antibody and purified VTG protein, also from the fathead minnow, were utilized. The plasma samples were diluted three times prior to analysis (i.e., 1:50, 1:5000, and 1:500,000).

Quality of the procedures was evaluated through the use of a standard curve which contained a minimum of 6 calibration standards expected to cover the range of the VTG results and a non-specific binding assay blank. The assay blank is designed to determine background signal in the samples. A fortified QC sample was prepared on each day of analysis by rehydrating a lyophilized VTG standard with culture male plasma and following the dilution scheme used for the test samples. The batch of VTG standard used for this fortification was purchased independently from the batch utilized to create the standard curve as recommended by OPPTS (2009). An unfortified subsample of the culture male plasma was also evaluated as a comparison. The standard curve, all samples, and the QC's were analyzed in duplicate. The standard curve was considered to be acceptable if the correlation coefficient (R^2) was greater than 0.99. Based on the standard curve, a limit of quantification was calculated for each plate. If

a sample resulted in a value below detectable limits, then one half the LOQ was used as the result for that sample in statistical analysis. As part of the ELISA procedure, absorbance values at 450 nm were evaluated with a microtiter plate reader and used to calculate the concentration of VTG in each sample.

2.14 Performance Criteria

The following criteria are required for the exposure to be acceptable based on the study protocol:

Performance Criteria (Expected Results)	Expected Results
Survival	Survival of 90% or greater in controls. Survival was 100% in the control.
Fecundity	Evidence that fish were actively spawning in all replicates prior to initiating the chemical exposure and in the control replicates during the test (e.g., spawning occurred at least every four days in each control replicate, or an average of at least 15 eggs/female/day/replicate). This exposure met all criteria listed here.
Fertilization Success	Control fertilization should be ≥95%. Fertilization success was 99.4% for the control.
Dissolved Oxygen	Dissolved oxygen will be ≥60% of saturation. During this exposure, dissolved oxygen levels ranged from 56 to 100% of saturation (see Protocol Deviations).
Temperature	Water temperature will not differ by more than 1 °C between test tanks at any one time during the exposure period and will be maintained within ± 1 °C of the 25 °C temperature. This exposure did not meet all criteria listed here (see Protocol Deviations).
Analytical Samples	Measured exposure concentration CV values will not exceed 20% for all replicates. During this exposure, CV values were 340, 44, 38, and 46%, respectively, for the 9.7, 20, 53, and 110 µg/L mean measured concentrations. Given the difficult nature of this material, the CV values are considered to be acceptable (see Section 3.2.2).

2.15 Statistical Analyses

A complete summary of the statistical analysis performed for this study is in Appendix 7. Statistical analyses were performed consistent with OPPTS Guideline 890.1350 (U.S. EPA, 2009), OECD Guideline 229 (OECD, 2009), and the Task Order 14 QAPP (2015).

Preliminary analyses (i.e., descriptive statistics such as means, standard errors, and percent coefficient of variation) were performed separately on continuous quantitative endpoints: fecundity, fertilization success, nuptial tubercle score, female/male VTG, female/male GSI, and

female/male body weight. Concentration-response monotonicity was assessed visually via plots of replicate means and the group medians of the replicate means.

To determine if significant differences in mortality existed between treatment groups, survival data were evaluated with the Cochran-Armitage test with survival results pooled across replicates within treatment or control group if data were considered to be monotonic. If monotonicity was not observed, Fisher's Exact test with a Bonferroni-Holm adjustment was performed.

The treatment effect assessment was performed using data from the control group and the four treatment groups. The treatment effect for nuptial tubercle score was determined using the Jonckheere-Terpstra test. For other continuous quantitative endpoints that followed a monotonic concentration-response, the Jonckheere-Terpstra test was applied in a step-down manner. For endpoints that were not consistent with a monotonic concentration-response, the data were evaluated for normality (Shapiro-Wilk's test) and homogeneity of variance (Levene's test). If a data set was found to have a non-normal distribution or a heterogeneous distribution of variance, a normalizing, variance stabilizing transformation was used. If data sets were normally distributed with homogeneous variance following transformation, the data set was evaluated using Dunnett's test. If the data set was normally distributed with heterogeneous variance following data transformation, the Mann-Whitney-Wilcoxon test (with Bonferroni-Holm adjustment) was used to evaluate the data. Where no normalizing transformation was found, the Mann-Whitney-Wilcoxon test with a Bonferroni-Holm adjustment to the p-values was used to evaluate the data sets. The Jonckheere-Terpstra test and Mann-Whitney-Wilcoxon test with a Bonferroni-Holm adjustment to the p-values were performed on replicate medians, and Dunnett's test was performed on replicate means.

Potential statistical outliers were assessed for all treatment and control data for all continuous quantitative endpoints. An analysis of variance model was fit to the data separately for each endpoint. Model residuals were estimated for each observation. If the residual value was greater than the median residual plus three times the residual interquartile range (i.e., the difference between the 75^{th} and 25^{th} percentiles), then the observed value was flagged as a potential

statistical outlier. Analyses were performed with and without the potential outliers. Analyses in this report present results using all data, while Appendix B contains the results when potential outliers were removed.

The statistical analysis software package SAS (version 9.4) was used in all statistical analyses, with statistical significance assessed at the two-sided 0.05 level.

3.0 RESULTS

3.1 Preliminary Exposure

A 96-hour range-finding acute exposure was conducted at Smithers-Viscient exposing fathead minnow under flow through conditions to nominal 2-ethylhexyl paraben concentrations of 0.062, 0.19,0.56, 1.7, and 5.0 mg/L a control and solvent (triethylene glycol) control. The results of the non-GLP preliminary exposure are presented in Appendix 3.

3.2 Definitive Exposure

Results and EPA-prescribed Data Entry Spreadsheet Templates (DESTs) are reported in Appendix 8 and Appendix 9, respectively.

3.2.1 Evaluation of Test Conditions

A summary of the water quality parameters measured during the 21-day exposure is presented in Table 2. Dissolved oxygen levels ranged from 56 to 100% of saturation throughout the exposure period (see Protocol Deviations). Daily measurements of temperature in the test aquaria and continuous temperature monitoring established that the exposure solution temperatures ranged from 24 to 27 °C in the upper and lower levels during the exposure period (see Protocol Deviations). Iodide concentrations were also analyzed on samples removed at test initiation and test termination by Galbraith Laboratories, Inc., Knoxville, Tennessee. Iodide samplings performed on the dilution water resulted in concentrations of 9.5 and 4.9 µg/L

(Appendix 10). The acceptable performance of the control organisms demonstrated that the water quality was appropriate for the survival and reproduction of fathead minnows.

3.2.2 Analytical Results

The diluter system which prepared and delivered the test solutions to the exposure aquaria functioned properly during the pretest period and throughout the study. Throughout the exposure, all exposure solutions were observed to be clear and colorless. No undissolved test substance was observed in the diluter system.

The results of the analysis of the exposure solutions for 2-ethylhexyl paraben during the in-life portion of the definitive study are presented in Table 3 and illustrated in Figure 1. Results of the analyses established that the measured concentrations maintained the expected concentration gradient but were relatively variable. Recoveries on day 7 were lower than expected, ranging from 22 to 40% of nominal concentrations. While a direct cause cannot be established, this drop in recoveries may have been related to the additional biomass associated with actively spawning, adult fish that were not present in the system during the analytical pretest period. Therefore, column effluent delivery was immediately adjusted on day 11 of exposure and recoveries were slightly closer to the desired nominal concentrations on day 14, ranging from 27 to 63%. The toxicant delivery was adjusted again on day 18 (see Section 2.9 for more information) to avoid further concentration decline. While the analytical recoveries were somewhat variable over the length of the exposure (with coefficients of variance (CV) ranging from 38 to 46%), the desired concentration gradient was maintained at each sampling interval and no overlap in concentrations was observed. Variability between replicates of the same concentration was maintained within approximately 20% at each interval. The variability in the measured concentrations between intervals was likely related to the physical properties of the test substance (e.g., $\log K_{ow} > 5$), which indicates a propensity for adsorption to the high amount of glass and silicone surface area in the test system as well as possible uptake by the adult fathead minnows. The inherent aging of the biological exposure system (i.e., feeding, fish biomass, and microbial populations) also likely affected the recoveries of 2-ethylhexyl paraben in the aquaria

samples. Therefore, the higher than expected variability did not significantly impact the interpretation of the study results.

Mean measured concentrations defined the treatment levels tested as 9.7, 20, 53, and 110 µg/L (69, 56, 60 and 50% of nominal concentrations, respectively).

Analysis of the quality control samples resulted in measured concentrations that were consistent with the predetermined recovery range (Appendix 4) and ranged from 83.2 to 117% (N = 12) of the nominal fortified concentrations (6.25, 37.5, and 225 µg/L). Based on these results, it was determined that the appropriate quality control was maintained during the analyses of the exposure solutions.

3.2.3 Biological Observations

Raw data for the definitive study is presented in (Appendix 8). Treatment mean values in the report tables (Table 4 through Table 9) are the means of the four treatment replicates, with the exception of male tubercle score which is presented as the median values (Table 6). No abnormal observations in behavior such as hyperventilation, uncoordinated swimming, atypical quiescence, and feeding abstinence were noted in any treatment levels tested during daily observations.

3.2.4 Survival

Percent survival following 21 days of exposure to 2-ethylhexyl paraben is presented in Table 4. Following 21 days of exposure, mean percent survival among male fish exposed to the control was 100%. Mean percent survival among male fish exposed to all treatment levels tested (9.7, 20, 53, and 110 µg/L) was 100% (Figure 2). Statistical analysis was not required for this endpoint. No dose-related trend was empirically determined.

Mean percent survival among female fish exposed to the control was 100%. Mean percent survival among female fish exposed to the 9.7, 20, 53, and 110 µg/L treatment levels was 88, 94,

94, and 100%, respectively (Figure 3). Statistical analysis was not required for this endpoint. No dose-related trend was empirically determined. A female in replicate A of the 9.7 µg/L treatment level was inadvertently injured during aquarium cleaning on test day 20. Therefore, the female mortality in this replicate on test day 21 was likely a result of this technician error and not treatment-related. All fish found dead were macroscopically examined for presence of ovaries and were appropriately preserved. Gonad and thyroid histopathology was not performed on the dead fish.

Mean percent survival among male and female fish combined exposed to the control was 100%. Mean percent survival among male and female fish exposed to the 9.7, 20, 53, and 110 µg/L treatment levels was 92, 96, 96, and 100%, respectively (Figure 4). Statistical analysis (Fisher's Exact Test with Bonferroni-Holm's Adjustment) determined no significant reduction in mean percent survival among male and female fish combined in any of the treatment levels tested compared to the control (i.e., 100%).

3.2.5 Behavioral Appearance and Observations

Notable physiological observations were made with regards to coloration/banding. One female was observed to have color bars present in replicate A of the control on exposure day 7; one female in replicate D of the 9.7 µg/L treatment level on exposure days 7 and 8; and one female in replicate A of the 53 µg/L treatment level on exposure days 9 through 20. While color banding is considered to be a secondary sexual characteristic for male fish it is not uncommon to observe transient color banding in female fish. This is not considered to be an effect of exposure to the test substance. No abnormal secondary sexual characteristics (e.g., body color (light or dark), coloration patterns, body shape, size of dorsal nape pad) were observed in males. No abnormal observations of ovipositor size in females were made during the exposure period or at study termination. Normal male territorial behavior was observed daily in all replicates from all treatment levels and the control.

3.2.6 Fertilization Success and Fecundity

A summary of the fertilization success and fecundity during this study is presented in Table 5 and illustrated in Figure 5 and Figure 6, respectively.

The mean percentage of fertilized eggs in the control was 99.4%. The mean percentage of fertilized eggs in the 9.7, 20, 53, and 110 µg/L treatment levels was 99.5, 98.6, 99.5, and 99.4%, respectively (Figure 5). Statistical analysis (Mann-Whitney-Wilcoxon's Test with Bonferroni-Holm's Adjustment) determined no significant reduction in the percentage of fertilized eggs among fish exposed to the any of the treatment levels tested when compared to control data (i.e., 99.4%).

The mean number of eggs per female per reproductive day for the control was 48. The mean number of eggs per female per reproductive day for the 9.7, 20, 53, and 110 µg/L treatment levels was 35, 41, 43, and 37, respectively (Figure 6). Statistical analysis (Dunnett's Multiple Comparison Test) determined no significant reduction in the mean number of eggs per female per reproductive day among fish exposed to the any of the treatment levels tested when compared to control data (i.e., 48 eggs per female per reproductive day).

3.2.7 Tubercle Scores

Tubercles were not observed in females at any concentration. The median male tubercle scores are presented in Table 6. The median tubercle score in the control was 22. Median tubercle scores in the 9.7, 20, 53, and 110 µg/L treatment levels were 20, 16, 18, and 19, respectively (Figure 7). Statistical analysis (Jonckheere-Terpstra's Step-Down Test) determined no significant difference in median tubercle score in fish exposed to any of the treatment levels compared to the control data (i.e., 22).

3.2.8 Weight Endpoints

The results for male and female body weights (wet weights) are presented in Table 6 and Table 7, respectively.

The mean male wet weight in the control was 4.3577 g. The mean male weight among fish exposed to the 9.7, 20, 53, and 110 µg/L treatment levels was 4.2063, 4.6273, 4.1175, and 4.0573 g, respectively (Figure 8). Statistical analysis (Dunnett's Multiple Comparison Test) determined no significant difference in mean male weight in fish exposed to any of the treatment levels tested compared to the control data (i.e., 4.3577 g).

The mean female wet weight in the control was 2.2539 g. The mean female wet weight among fish exposed to the 9.7, 20, 53, and 110 µg/L treatment levels was 2.2986, 2.4256, 2.2726, and 2.3553 g, respectively (Figure 9). Statistical analysis (Jonckheere-Terpstra's Step-Down Test) determined no significant difference in mean female wet weight in fish exposed to any of the treatment levels tested compared to the control data (i.e., 2.2539 g).

3.2.9 Length Results

Length data were not statistically analyzed as adult (i.e., sexually mature) fish were used to initiate this exposure.

The mean male body length for fish exposed to the control was determined to be 53.39 mm. Mean male body length for fish exposed to the 9.7, 20, 53, and 110 µg/L treatment levels was 53.28, 55.58, 52.03, and 51.89 mm, respectively (Figure 10).

The mean female body length for fish exposed to the control was determined to be 45.10 mm. Mean female body length for fish exposed to the 9.7, 20, 53, and 110 µg/L treatment levels was 46.15, 46.72, 45.85, and 46.21 mm, respectively (Figure 11).

3.2.10 Gonadal Somatic Index (GSI) Endpoints

The results for male and female GSI are presented in Table 6 and Table 7, respectively.

The mean male GSI in the control was 1.2%. The mean male GSI among fish exposed to the 9.7, 20, 53, and 110 µg/L treatment levels was 1.6, 1.3, 1.5, and 2.1%, respectively (Figure 12). Statistical analysis (Jonckheere-Terpstra's Step-Down Test) determined a significant increase ($p = 0.0053$) in mean male GSI among fish exposed to the 110 µg/L treatment level when compared to the control data (i.e., 1.2%).

The mean female GSI in the control was 15%. The mean female GSI among fish exposed to the 9.7, 20, 53, and 110 µg/L treatment levels was 13, 15, 17, and 18%, respectively (Figure 13). Statistical analysis (Jonckheere-Terpstra's Step-Down Test) determined a significant increase ($p = 0.0463$) in mean female GSI among fish exposed to the 110 µg/L treatment level when compared to the control data (i.e., 15%).

3.2.11 Blood Plasma Vitellogenin (VTG) Concentration

All VTG results are presented in Appendix 8. Potential outliers to the data set were evaluated and identified. Five and seven data points (i.e., individual fish plasma concentrations) were identified as statistical outliers during analysis of male and female VTG, respectively. However, since there was no significant difference in response between analyses with and without potential statistical outliers, all samples are being presented for both male and female VTG.

Subsamples of unfortified control male plasma and fortified control male plasma were analyzed in two duplicate wells during each day of VTG analysis for QC analysis. The mean quantity of VTG measured in the unfortified control male plasma samples was added to a known quantity of VTG added to the fortification samples to determine an expected concentration. A standard concentration of 5.0 µg/mL was used for the lyophilized fortified sample of the QCs. The fortification concentrations attained resulted in 98 to 152% of the expected fortified concentration.

The results for male VTG analysis are presented in Table 8. The mean male VTG concentration in the control was 1.64×10^2 ng/mL. The mean male VTG concentration in the 9.7, 20, 53, and 110 µg/L treatment levels was 2.84×10^4, 1.29×10^4, 8.48×10^6, and 1.87×10^7 ng/mL, respectively (Figure 14). Statistical analysis (Jonckheere-Terpstra's Step-Down Test) determined a significant increase ($p = 0.0015, 0.0001$) in mean male VTG among fish exposed to the 53 and 110 µg/L treatment levels when compared to the control data (i.e., 1.64×10^2 ng/mL). All eight male plasma samples in the 53 and 110 µg/L treatment levels had elevated VTG concentrations. However, five of the eight male plasma samples in the 110 µg/L treatment level were excluded from statistical analysis because they were above detectable limits at the maximum standard dilution. Overall, means from the 53 and 110 µg/L treatment levels demonstrate a dose response when compared to the controls. These data provide evidence that strong male VTG induction was observed in these treatment levels.

The results for female VTG analysis are presented in Table 8. The mean female VTG concentration in the control was 1.52×10^6 ng/mL. The mean female VTG concentration in the 9.7, 20, 53, and 110 µg/L treatment levels was 2.89×10^6, 4.19×10^6, 1.34×10^6, and 1.94×10^6 ng/mL, respectively (Figure 15). Statistical analysis (Dunnett's Multiple Comparison Test) determined no significant difference in mean female VTG among fish exposed to any of the treatment levels tested compared to the control data (i.e., 1.52×10^6 ng/mL).

3.2.12 Sex Steroid Analysis

The results of the sex steroid analysis during this study are presented in Table 9 and Figure 16 through Figure 19, respectively. Since this endpoint often shows high variability between fish, the sex steroid data were evaluated empirically and not statistically. The sex steroid data are available for statistical analysis in the future. All steroid analyses were evaluated empirically and were not subject to statistical evaluation. The results of the analyses are below.

3.2.12.1 Male Sex Steroid Analysis

The mean male testosterone concentration in the control was 1.11 µg/L. The mean male testosterone concentrations in the 9.7, 20, 53, and 110 µg/L treatment levels were 1.06, 2.30, 1.76, and 2.55 µg/L, respectively (Figure 16). No substantial differences in male testosterone plasma concentration were observed based on empirical evaluation.

The mean male 17β-estradiol concentration in the control was 0.872 µg/L. The mean male 17β-estradiol concentrations in the 9.7, 20, 53, and 110 µg/L treatment levels were 0.257, 1.03, 0.429, and 2.21 µg/L, respectively (Figure 17). An increase in plasma concentration of 17β-estradiol was observed based on empirical evaluation of the treatment and control means as well as variation among replicates. However, no clear linear trend for sex steroids resulted from exposure to 2-ethylhexyl paraben.

3.2.12.2 Female Sex Steroid Analysis

The mean female testosterone concentration in the control was 8.56 µg/L. The mean female testosterone concentrations in the 9.7, 20, 53, and 110 µg/L treatment levels were 2.37, 1.94, 2.64, and 2.96 µg/L, respectively (Figure 18). No substantial differences in female testosterone plasma concentration were observed based on empirical evaluation.

The mean female 17β-estradiol concentration in the control was 16.2 µg/L. The mean female 17β-estradiol concentrations 9.7, 20, 53, and 110 µg/L treatment levels were 14.9, 7.73, 5.67, and 10.3 µg/L, respectively (Figure 19). Female 17β-estradiol plasma concentration means at the 20, 53, and 110 µg/L treatment levels were lower than control means based on empirical evaluation. However, no clear linear trend for sex steroids resulted from exposure to 2-ethylhexyl paraben.

3.2.13 Histopathology

Histopathology reports for this study are presented in Appendix 11. It should be noted that the histology report is presented with nominal exposure concentrations which were updated to mean measured concentrations for presentation in this report. Hematoxylin and eosin (H & E)-stained tissue-sections of testis, ovary, and thyroid were examined from control and 2-ethylhexyl paraben-exposed fathead minnows. There were no test compound-related histopathological observations for the thyroid in this study. The statistical report is presented in Appendix 7. A description of the statistical procedures is in Section 2.15.

Findings associated with 2-ethylhexyl paraben exposure occurred in the gonads of male and female fish and included: intravascular and interstitial proteinaceous fluid in the testes of the 53 and 110 µg/L treatment level males (minimal to mild), and in the ovaries of the 110 µg/L treatment level females (mild); increased male testis stage scores, particularly in males of the 53 and 110 µg/L treatment levels; a dose-dependent increase in the prevalence of oocyte atresia in all treatment levels (minimal to moderate); a dose-dependent increase in the severity of oocyte atresia in the 53 and 110 µg/L treatment levels (minimal to moderate); decreased post-ovulatory follicles in females of the 53 and 110 µg/L treatment levels; and shifts in ovarian stage scores in females, particularly in females of 53 and 110 µg/L treatment levels. Although mean ovarian stage scores were comparable among females in the control and the treatment levels, there was a shift away from females in the spawning or recently spawned stages (i.e., stages 4.0, 2.0, and 2.5) toward stages of oocyte maturation (i.e., stages 3.0 and 3.5). That shift is consistent with the decreased post-ovulatory follicles observed in females of the 53 and 110 µg/L treatment levels, because stage 3.0 and 3.5 ovaries typically contain few, if any, of those residual structures. An unusual finding that was not related to test substance exposure was the presence of multiple testicular oocytes in the collecting ducts of a control male. This finding is not expected to impact the results or interpretation of this study. A rare background incidence of testicular oocytes has been reported in untreated fathead minnows and in other common test species such as Japanese medaka (*Oryzias latipes*), and the incidence can vary according to test facility (Grim et al., 2007 and U.S. EPA, 2009). For further details, see Appendix 11.

The relatively few additional histopathological diagnoses recorded in this study were background types of findings observed typically in fathead minnow gonads, and were present in comparable numbers of control and 2-ethylhexyl paraben-treated fish generally at low prevalence and severity; thus, none were considered to be treatment-related.

Statistical analysis of the histopathology data using the RSCABS (Rao-Scott Cochran-Armitage by Slices) method was considered for this exposure, but in consultation with the Study Sponsor and the Study Director, it was not determined to be justified, based on the pathologist's view of the quantity and nature of the histopathological findings.

4.0 PERFORMANCE CRITERIA

The following criteria are required for the exposure to be acceptable based on the study protocol:

Performance Criteria (Expected Results)	Expected Results
Survival	Survival of 90% or greater in controls. Mean percent survival in the control was 100% for males, females, and male/female combined. For further information, see Section 3.2.4 and Table 4.
Fecundity	Evidence that fish were actively spawning in all replicates prior to initiating the chemical exposure and in the control replicates during the test. The mean number of eggs per female per reproductive day for the control was 48 and for the 9.7, 20, 53, and 110 µg/L treatment levels was35, 41, 43, and 37, respectively. For further information, see Section 3.2.6 and Table 5.
Fertilization Success	Control fertilization should be ≥95%. The mean percentage of fertilized eggs in the control was 99.4%. For further information, see Section 3.2.6 and Table 5.
Dissolved Oxygen	Dissolved oxygen will be ≥60% of saturation. During this exposure, dissolved oxygen levels ranged from 56 to 100% of saturation. For further information, see Protocol Deviations, Section 3.2.1 and Table 2.
Temperature	Water temperature will not differ by more than 1 °C between test tanks at any one time during the exposure period and will be maintained within ± 1 °C of the 25 °C temperature. This exposure did not meet all criteria listed here. For further information, see Protocol Deviations, Section 3.2.1 and Table 2.
Analytical Samples	Measured exposure concentration CV values will not exceed 20% for all replicates. During this exposure, CV values were 340, 44, 38, and 46%, respectively, for the 9.7, 20, 53, and 110 µg/L mean measured concentrations. Given the difficult nature of this material, the CV values are considered to be acceptable. For further information, see Section 3.2.2 and Table 3.

5.0 PROTOCOL DEVIATIONS

1. The protocol states that temperature, dissolved oxygen concentration, and pH will be measured in every aquarium on day 0 and in alternating replicates thereafter. On 20 September 2016 (pre-exposure day 14), a pH value of 1.14 was entered into the water quality data. Based on all other pH values throughout the exposure period and historical water quality parameters of Smithers Viscient laboratory well water, this was likely a typographical error; a pH of 1.12 would have resulted in overt fish mortality. However, the single aquarium pH value cannot be verified for that day since this error was removed from the data. Since the fish did not display any lethal or sub-lethal effects in that tank, it can be reasonably concluded that the pH value was likely between 6.5 and 9. Therefore, this deviation had did not have a negative impact on the results or interpretation of this study.

2. The protocol states that nuptial tubercles will be counted, mapped, and scored according to methods recommended in OECD Guideline 229, Annex 5A (OECD, 2009) and OPPTS Guideline 890.1350, Appendix B (U.S. EPA, 2009). All female fish were observed to have no tubercles based on biological observations; however, after termination tubercles were only counted, mapped and scored for the male fish. Since all female fish were observed to have no tubercles on a daily basis throughout the exposure, this deviation did not have a negative impact on the results or interpretation of this study.

3. The protocol states that total dissolved oxygen concentrations will be maintained at >60% of saturation during the exposure. On test day 14 (6 October 2016), the 35 µg/L concentration replicate B tank had a dissolved oxygen level of 56.1% due to a malfunction with the aeration stone in the aquarium. The aeration stone was, therefore, immediately replaced. The dissolved oxygen level was re-measured later in the day and recorded as 90.6%. As the dissolved oxygen level was only slightly out of range temporarily and the fish did not show any signs of stress or mortality, this deviation did not have a negative impact on the results or interpretation of this study.

4. The protocol states that water temperature will not differ by more than 1 °C between test tanks at any one time during the exposure period and will be maintained within ±1 °C of the 25 °C temperature. On day 0, the 14 µg/L treatment level replicate A aquarium was measured at 24.4°C. This temperature was >1°C different from several other aquaria. Also, the 220 µg/L treatment level replicate D aquarium was measured at 26.7 °C on day 0. This measurement was >1 °C different from 25 °C. Additional circulation pumps were placed in the water bath and the heaters were immediately reset to adjust the temperatures and equilibrate water flow within the bath. All water quality measurements were then re-measured prior to the addition of organisms on day 0. Since these deviations were temporary, corrective action was taken immediately, and the temperatures were adjusted prior to the addition of the organisms, these deviations did not have a negative impact the results or interpretation of this study.

5. The protocol states that the measured exposure concentration CV will not exceed 20% for all replicates. Concentration CV's were 38 to 46%. While the analytical recoveries were variable over the length of the exposure, the desired concentration gradient was maintained at each sampling interval and no overlap in concentrations was observed. Variability between replicates of the same concentration was maintained within approximately 20% at each interval. The variability in the measured concentrations between intervals was likely related to the physical properties of the test substance (e.g., logKow > 5), which indicate a propensity for adsorption to the high amount of glass and silicone surface area in the test system as well as possible uptake by the adult fathead minnows. The inherent aging of the biological exposure system (i.e., feeding, fish biomass, and microbial populations) also likely affected the recoveries of 2-ethylhexyl paraben in the aquaria samples. Risk of analytical variability is high when chemicals with properties similar to 2-ethylhexyl paraben are delivered to diluter systems with sexually dimorphic, actively spawning fish; the high amount of glass surface area creates high binding potential for the chemical to adsorb. These adsorption rates are difficult to predict, and stock flow increases, which occurred as quickly as plausible during this study, are typically performed conservatively in order to avoid potential

dosing spikes. Therefore, the higher than expected variability did not significantly impact the interpretation of the study results.

6.0 DISCUSSION AND CONCLUSION

As presented above, several deviations from the study protocol occurred during the conduct of this study. None of these deviations had any impact of the integrity or interpretation of the exposure. The results of the statistical analyses, gonadal staging, and histopathology evaluation are presented below.

Endpoints		Mean Measured Concentration (µg/L)			
		10	20	53	110
Day 21	Combined Male and Female Survival	-	-	-	-
	Fecundity	-	-	-	-
	Fertilization Success	-	-	-	-
	Nuptial Tubercle Score	-	-	-	-
	Male Weight	-	-	-	-
	Male GSI	-	-	-	↑
	Female Weight	-	-	-	-
	Female GSI	-	-	-	↑
	Male VTG	-	-	↑	↑[a]
	Female VTG	-	-	-	-
	Male Testosterone	ND[b]	ND[b]	ND[b]	ND[b]
	Male 17β-estradiol	ND[b]	ND[b]	ND[b]	↑[c]
	Female Testosterone	ND[b]	ND[b]	ND[b]	ND[b]
	Female 17β-estradiol	ND[b]	ND[b]	ND[b]	ND[b]

- Endpoint not statistically different from controls.
↓ Statistical analysis determined endpoint to be significantly reduced ($p \leq 0.05$) compared to the control.
↑ Statistical analysis determined endpoint to be significantly increased ($p \leq 0.05$) compared to the control.
[a] Statistically significant increase based on three plasma samples in this treatment level. However, the five remaining samples above detection limit (ADL) suggests a strong dose response at this endpoint.
[b] No empirical difference from the control. Statistical analysis was not required by protocol.
[c] Increase in plasma concentration of 17β-estradiol based on empirical evaluation of treatment means and variation among replicates.

Male Gonadal Staging	NF	NF	F	F
Male Histopathology Findings	NF	NF	F	F
Female Gonadal Staging	NF	NF	F	F
Female Histopathology Findings	NF	NF	F	F
Thyroid Histopathology Findings	NF	NF	NF	NF

NF = No findings related to 2-ethylhexyl paraben exposure.
F = Findings potentially related to 2-ethylhexyl paraben exposure. See Section 3.2.13 for summary.

The 21-day short-term reproduction assay of 2-ethylhexyl paraben with fathead minnow (*Pimephales promelas*) was performed under flow-through conditions. Adult fish were exposed to a control and mean measured concentrations of 9.7, 20, 53, and 110 µg/L. Notable physiological observations were made with regards to coloration/banding. One female was observed to have color bars present in replicate A of the control on exposure day 7; one female in replicate D of the 9.7 µg/L treatment level on exposure days 7 and 8; and one female in replicate A of the 53 µg/L treatment level on exposure days 9 through 20. While color bars are primarily considered to be a secondary sexual characteristic for males, females can occasionally be observed to express color bars. The color bars observed in females during this exposure were transient. None of the observations described above were considered to be a result of exposure to 2-ethylhexyl paraben. No other abnormal observations were made in regards to coloration/banding or behavior. Fecundity in the control was 48 eggs/female/day; fertilization success was 99.7%. Exposure to 2-ethylhexyl paraben slightly reduced nuptial tubercle scores in male fish at the 20 and 53 µg/L concentrations, but this difference was not statistically significant. Exposure to 2-ethylhexyl paraben significantly increased male and female GSI at the 110 µg/L concentration and male VTG plasma concentration (with and without outliers removed) at the 53 and 110 µg/L concentrations. Plasma VTG was not significantly increased in females. Exposure to 2-ethylhexyl paraben also increased male plasma concentrations of 17β-estradiol at the 110 µg/L concentration based on empirical evaluation.

Histopathological findings associated with 2-ethylhexyl paraben exposure occurred in the gonads of male and female fish and included: intravascular and interstitial proteinaceous fluid in the testes of the 53 and 110 µg/L treatment level males (minimal to mild), and in the ovaries of the 110 µg/L treatment level females (mild); increased male testis stage scores, particularly in males of the 53 and 110 µg/L treatment levels; a dose-dependent increase in the prevalence of oocyte atresia in all treatment levels (minimal to moderate); a dose-dependent increase in the severity of oocyte atresia in the 53 and 110 µg/L treatment levels (minimal to moderate); decreased post-ovulatory follicles in females of the 53 and 110 µg/L treatment levels; and shifts in ovarian stage scores in females, particularly in females of 53 and 110 µg/L treatment levels. Although mean ovarian stage scores were comparable among females in the control and the treatment

levels, there was a shift away from females in the spawning or recently spawned stages (i.e., stages 4.0, 2.0, and 2.5) toward stages of oocyte maturation (i.e., stages 3.0 and 3.5). There were no thyroid findings.

The presence of proteinaceous intravascular and interstitial fluid in the gonads of male and female fathead minnows is consistent with increased hepatic VTG production as might be induced by substances with estrogenic activity. A number of *in vitro* and *in vivo* studies have demonstrated estrogen-like effects in fish exposed to various parabens, including VTG induction (Pedersen et al., 2000; Inui et al., 2003; Yamamoto et al., 2011). Although not specific for enhanced estrogenic activity, increased oocyte (follicular) atresia is another commonly reported effect of xenoestrogen exposure (Dietrich and Krieger, 2009).

The histopathological findings in males and females stated above and increased male VTG in the 53 and 110 µg/L treatment levels suggest 2-ethylhexyl paraben may have estrogenic activity. The induction of VTG in male fish is a well-established response to estrogen receptor agonists (Borgert et al., 2014). Responses in secondary sexual characteristics such as tubercle scores in males also provide evidence of estrogenic activity (OECD, 2007). Exposure to 2-ethylhexyl paraben caused a slight, not statistically-significant, reduction in male tubercle scores at the 20 and 53 µg/L treatment levels. Significant increases in male and female GSI occurred at the 110 µg/L treatment level (highest treatment level) in this study. Increases in GSI have been linked to possible endocrine disruptor activity; however, the mechanisms underlying these responses are not well characterized and are not always specific (Borgert et al., 2014). Exposure to 2-ethylhexyl paraben caused treatment-related effects in males and females: histopathological findings in the gonads at the 53 and 110 µg/L treatment levels and increased GSI at the highest treatment level (110 µg/L), and increased plasma VTG in males. Based on these results and the decision criteria in the FSTRA test guideline (OPPTS 890.1350), 2-ethylhexyl paraben may be potentially active on the hypothalamic pituitary gonadal (HPG) axis.

REFERENCES

Ankley, G.T., Jensen, K.M., Kahl, M.D., Korte, J.J., and Makynen E.A., 2001. Description and evaluation of a short-term reproduction test with the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 20:1276-1290.

APHA, AWWA, WPCF, 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edition, Washington, DC.

ASTM, 2005. Committee E-47 on Biological Effects and Environmental Fate. Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes. ASTM Designation E: 1241-05. American Society for Testing and Materials, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, Pennsylvania.

Borgert, C.J., Stuchal, L.D., Mihaich, E.M., Becker, R.A., Bentley, K.S., Brausch, J.M., et al. 2014. Relevance weighting of Tier 1 endocrine screening endpoints by rank order. Birth Defects Res B, 101, 90–113.

Dietrich, D.R. and Krieger, H.O., 2009. Histological Analysis of Endocrine Disruptive Effects in Small Laboratory Fish. John Wiley & Sons, Inc., Hoboken, NJ, 121 pp.

DeVellis, S.R., 2017. Testosterone and Estradiol - Validation of the Analytical Method for the Determination of a Test Substance in Serum. Smithers Viscient, Wareham, Massachusetts. Study No. 13836.6114.

Grim, K.C., Wolfe, M., Hawkins, W., Johnson, R., Wolf, J., 2007. Intersex in Japanese Medaka (*Oryzias latipes*) Used as Negative Controls in Toxicologic Bioassays: A Review of 54 Cases from 41 Studies. Environ. Toxicol. Chem. 26, 1636–1643.

Inui, M., Adachi, T., Takenaka, S., Inui, H., Nakazawa, M., Ueda, M., Watanabe, H., Mori, C., Iguchi, T., and Miyatake, K., 2003. Effect of UV screens and preservatives on vitellogenin and choriogenin production in male medaka (*Oryzias latipes*). Toxicology, 194(1-2):43-50.

Kahl, M.D., Russom, C.L., DeFoe, D.L., and Hammermeister, D.E., 1999. Saturation Units for Use in Aquatic Bioassays. Chemosphere 39: 539-551.

Mount, D. I. and Brungs, W. A., 1967. A simplified dosing apparatus for fish toxicology studies. Water Research 1: 21-29.

OECD, 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris, France. 41 pp.

OECD, 2000. Official Journal of the European Communities. OECD Series on Testing and Assessment Number 23. Guidance document on Aquatic Testing of Difficult Substances and Mixtures. ENV/JM/MONO(2000)6. Organization for Economic Cooperation and Development. Paris, France.

OECD, 2007. Final report of the validation of the 21-day fish screening assay for the detection of endocrine active substances. Series on testing and assessment number 78. ENV/JM/MONO(2007)25.

OECD, 2009. OECD Guideline for the testing Chemicals. Fish Short Term Reproduction Assay. Guideline #229. Paris, France. 40 pp.

Pedersen, K.L., Pedersen, S.N., Christiansen, L.B., Korsgaard, B., and Bjerregaard, P., 2000. The preservatives ethyl-, propyl- and butylparaben are oestrogenic in an in vivo fish assay. Pharmacol Toxicol. Mar;86(3):110-113.

Sprague, J.B., 1969. Measurement of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. Water Res. 3: 793-821.

Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, EPA publication SW-846, Third Edition, Final Updates I (1993), II (1995), IIA (1994), IIB (1995), III (1997), IIIA (1999), IIIB (2005), IV (2008), and V (2015).

U.S. EPA, 1989. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160); FR: 8/17/89; pp. 34052. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA, 2009. Office of Prevention, Pesticides and Toxic Substances. Fish Short-Term Reproduction Assay OPPTS Guideline 890.1350. Endocrine Disruptor Screening Program. EPA 740-C-09-007. October 2009. U.S. Environmental Protection Agency. Washington, D.C.

U.S. EPA, 2011. Office of Prevention, Pesticides and Toxic Substances. Fish Short-Term Reproduction Assay OPPTS Guideline 890.1350. Standard Evaluation Procedure (SEP). Endocrine Disruptor Screening Program. October 2011. U.S. Environmental Protection Agency. Washington, D.C.

Yamamoto, H., Tamura, I., Hirata, Y., Kato, J., Kagota, K., Katsuki, S., Yamamoto, A., Kagami, Y., and Tatarazako, N., 2011. Aquatic toxicity and ecological risk assessment of seven parabens: Individual and additive approach. Sci Total Environ, 410-411:102-111.

Table 1. Dilution water quality measurements during the pre-exposure period and 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

Test Phase	Date	Total Hardness (mg/L as $CaCO_3$)	Total Alkalinity (mg/L as $CaCO_3$)	pH	Conductivity (µS/cm)
Pre-exposure period	1-Sep-16	70	19	7.1	450
	7-Sep-16	80	20	6.6	530
	15-Sep-16	78	18	6.9	510
	22-Sep-16	84	18	6.8	550
21-day exposure	22-Sep-16	84	18	6.8	550
	28-Sep-16	64	22	7.1	520
	5-Oct-16	72	23	7.1	530
	12-Oct-16	70	24	7.4	500
	19-Oct-16	76	21	7.3	420

NOTE: Measurements have been rounded to two significant figures.

Table 2. Water quality measurements during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

Nominal Concentration (µg/L)	Ranges						
	Dissolved Oxygen[a]		Temperature[ab] (°C)	pH[a]	Total Hardness[c] (mg/L as $CaCO_3$)	Total Alkalinity[c] (mg/L as $CaCO_3$)	Conductivity[c] (µS/cm)
	mg/L	% Saturation					
Control	5.9 - 8.3	71 - 100	25 - 26	7.0 - 7.5	68 - 88	20 - 26	490 - 710
14	5.6 - 8.3	68 - 100	24 - 26	7.0 - 7.5	72	24	460
35	4.6 - 8.5	56[d] - 100	25 - 26	6.8 - 7.5	72	24	530
88	6.6 - 8.1	80 - 99	25 - 26	7.0 - 7.5	68	20	490
220	6.8 - 8.4	82 - 100	25 - 27[d]	7.1 - 7.5	88	20	460

[a] N = 25

[b] Continuous temperature monitoring of the upper water bath (control replicate A) and the lower water bath (control replicate C) established a temperature range of 24 to 26 °C throughout the exposure period.

[c] N = 4. Samples were taken from one replicate of one treatment level and the control alternating between treatment levels and replicate vessels (A, B and C) at each sampling interval. The control measurements represent a range of the three measurements. All other values are discrete measurements.

[d] Value was out of the required range. See Protocol Deviations.

NOTE: Measurements have been rounded to two significant figures.

Table 3. Concentrations of 2-ethylhexyl paraben measured in the exposure solutions during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

Nominal Concentration (µg/L)	Measured Concentration (µg/L)					Percent of Nominal (%)[a]	%CV
	Day 0	Day 7	Day 14	Day 21	Mean Measured Concentration (SD)[a]		
Control	< 1.1[b]	< 1.0	< 1.4	< 1.1	NA[c]	NA	NA
	< 1.1	< 1.0	< 1.4	< 1.1	(NA)		
14	16	4.8	8.8	11	9.7	69	40
	12	5.6	6.8	12	(3.8)		
Mean	**14**	**5.2**	**7.8**	**12**			
35	26	12	14	28	20	56	44
	27	8.5	12	30	(8.7)		
Mean	**26**	**10**	**13**	**29**			
88	60	34	51	80	53	60	38
	51	34	32	83	(20)		
Mean	**55**	**34**	**41**	**81**			
220	110	58	100	160	110	50	46
	160	49	60	170	(50)		
Mean	**140**	**54**	**80**	**167**			
QC[d]#1	5.84	5.20	6.62	6.36			
6.25	(93.4)	(83.2)	(106)	(102)			
QC #2	36.8	39.8	34.7	38.9			
37.5	(98.1)	(106)	(92.5)	(104)			
QC #3	230	261	202	264			
225	(102)	(116)	(89.7)	(117)			

[a] Mean measured concentration values, percent of nominal, and standard deviations (SD) were calculated using the actual analytical results and not the rounded values (two significant figures) presented in this table.

[b] Concentrations expressed as less than values were below the method detection limit (MDL). The MDL is dependent upon the lowest concentration calibration standard and the dilution factor of the controls.

[c] NA = Not Applicable

[d] QC = Quality Control sample. Percent recovery for each QC sample is presented in parentheses.

Table 4. Survival at test termination of the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

Mean Measured Concentration (µg/L)	Replicate	Mean Male Survival (%)	Mean Female Survival (%)	Mean Male/Female Combined Survival (%)
Control	A	100	100	100
	B	100	100	100
	C	100	100	100
	D	100	100	100
	Mean (CV[a])	**100 (0)**	**100 (0)**	**100 (0)**
9.7	A	100	75	83
	B	100	75	83
	C	100	100	100
	D	100	100	100
	Mean (CV)	**100 (0)**	**88 (17)**	**92 (11)**
20	A	100	75	83
	B	100	100	100
	C	100	100	100
	D	100	100	100
	Mean (CV)	**100 (0)**	**94 (13)**	**96 (8.7)**
53	A	100	75	83
	B	100	100	100
	C	100	100	100
	D	100	100	100
	Mean (CV)	**100 (0)**	**94 (13)**	**96 (8.7)**
110	A	100	100	100
	B	100	100	100
	C	100	100	100
	D	100	100	100
	Mean (CV)	**100 (0)**	**100 (0)**	**100 (0)**

[a] CV = Coefficient of variation ((standard deviation/mean) × 100).

NOTE: Values presented in this table have been rounded to two significant figures. All calculations were made using the actual (unrounded) data.

Table 5. Summary of fertilization success and fecundity during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

Mean Measured Concentration (µg/L)	Replicate	Mean Fertilization Success (% Fertilized)	Mean Fecundity (Eggs/Female/Day)
Control	A	99.6	46
	B	99.7	59
	C	99.6	48
	D	99.7	39
	Mean (CV[a])	**99.4 (0.48)**	**48 (18)**
9.7	A	98.9	29
	B	99.6	31
	C	99.6	43
	D	99.9	38
	Mean (CV)	**99.5 (0.43)**	**35 (19)**
20	A	97.9	43
	B	99.7	47
	C	99.5	32
	D	97.5	44
	Mean (CV)	**98.6 (1.1)**	**41 (16)**
53	A	99.4	58
	B	98.7	44
	C	100.0	26
	D	99.9	44
	Mean (CV)	**99.5 (0.58)**	**43 (30)**
110	A	99.6	38
	B	99.6	42
	C	99.2	29
	D	99.4	38
	Mean (CV)	**99.4 (0.18)**	**37 (15)**

[a] CV = Coefficient of variation ((standard deviation/mean) × 100).

NOTE: Values presented in this table have been rounded. All calculations were made using the actual (unrounded) data.

Table 6. Male termination endpoint summary during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

Mean Measured Concentration (µg/L)	Replicate	Median Tubercle Score	Mean Wet Body Weight (g)	Mean GSI (%)[a]
Control	A	23	4.3649	1.2
	B	21	5.1055	0.83
	C	25	4.5737	1.0
	D	20	3.3869	1.6
	Mean (CV[b])[c]	**22 (10.5)**	**4.3577 (16)**	**1.2 (29)**
9.7	A	18	4.0615	1.7
	B	24	4.4244	1.4
	C	14	4.1407	1.3
	D	25	4.1985	1.9
	Mean (CV)	**20 (25)**	**4.2063 (3.7)**	**1.6 (18)**
20	A	17	4.6283	1.1
	B	17	4.8946	1.7
	C	14	4.8912	1.4
	D	18	4.0952	1.1
	Mean (CV)	**16 (12)**	**4.6273 (8.1)**	**1.3 (23)**
53	A	18	4.0019	1.3
	B	23	4.1957	1.6
	C	19	4.4630	1.5
	D	12	3.8095	1.6
	Mean (CV)	**18 (26)**	**4.1175 (6.8)**	**1.5 (8.7)**
110	A	22	4.0433	1.9
	B	17	3.6927	2.3
	C	20	4.5100	2.3
	D	18	3.9832	1.9
	Mean (CV)	**19 (11)**	**4.0573 (8.3)**	**2.1[c] (11)**

[a] GSI = gonad weight/body weight × 100

[b] CV = Coefficient of variation ((standard deviation/mean) × 100).

[c] Mean (CV) is the mean of replicate means and CV is variation between means.

[d] Significantly increased (p = 0.0053) compared to the control data, based on Jonckheere-Terpstra's Step-Down Test.

NOTE: Tubercle scores and GSI values presented in this table have been rounded to two significant figures. All calculations were made using the actual (unrounded) data.

Table 7. Female termination endpoint summary during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

Mean Measured Concentration (µg/L)	Replicate	Mean Wet Body Weight (g)	Mean GSI (%)[a]
Control	A	2.3213	14
	B	2.3857	17
	C	2.1192	14
	D	2.1897	16
	Mean (CV[b])	**2.2539 (5.4)**	**15 (10)**
9.7	A	1.9247	11
	B	2.1512	13
	C	2.6321	15
	D	2.4864	15
	Mean (CV)	**2.2986 (14)**	**13 (15)**
20	A	2.5605	15
	B	2.2418	15
	C	2.2612	15
	D	2.6390	14
	Mean (CV)	**2.4256 (8.4)**	**15 (4.4)**
53	A	2.3974	16
	B	2.3069	21
	C	2.0512	12
	D	2.3348	18
	Mean (CV)	**2.2726 (6.7)**	**17 (22)**
110	A	2.2431	17
	B	2.2678	20
	C	2.3692	19
	D	2.5413	16
	Mean (CV)	**2.3553 (5.8)**	**18[c] (10)**

[a] GSI = gonad weight/body weight × 100

[b] CV = Coefficient of variation ((standard deviation/mean) × 100).

[c] Significantly increased (p = 0.0463) compared to the control data, based on Jonckheere-Terpstra's Step-Down Test.

NOTE: GSI values presented in this table have been rounded to two significant figures. All calculations were made using the actual (unrounded) data.

Table 8. Results of VTG analysis using all data points (no outliers removed) during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

Mean Measured Concentration (µg/L)	Replicate	Mean Male VTG Concentration (ng/mL)	Mean Female VTG Concentration (ng/mL)
Control	A	8.46×10^{1}	1.22×10^{6}
	B	3.90×10^{2}	1.57×10^{6}
	C	9.95×10^{1}	9.54×10^{5}
	D	8.00×10^{1}	2.32×10^{6}
	Mean (CV[a])	**1.64×10^{2} (9.2×10^{1})**	**1.52×10^{6} (3.9×10^{1})**
9.7	A	1.04×10^{5}	3.29×10^{6}
	B	1.15×10^{3}	8.48×10^{5}
	C	8.26×10^{3}	3.79×10^{6}
	D	8.99×10^{1}	3.62×10^{6}
	Mean (CV)	**2.84×10^{4} (1.78×10^{2})**	**2.89×10^{6} (4.8×10^{1})**
20	A	1.75×10^{3}	9.18×10^{5}
	B	4.07×10^{4}	1.96×10^{6}
	C	9.07×10^{3}	1.08×10^{7}
	D	5.20×10^{1}	3.05×10^{6}
	Mean (CV)	**1.29×10^{4} (1.47×10^{2})**	**4.19×10^{6} (1.08×10^{2})**
53	A	1.10×10^{7}	3.39×10^{6}
	B	7.69×10^{6}	1.05×10^{6}
	C	1.48×10^{7}	7.82×10^{5}
	D	4.33×10^{5}	1.19×10^{5}
	Mean (CV)	**8.48×10^{6}[c] (7.2×10^{1})**	**1.34×10^{6} (1.07×10^{2})**
110	A	2.42×10^{7}	5.95×10^{5}
	B	ADL[b]	1.04×10^{6}
	C	1.33×10^{7}	3.30×10^{6}
	D	ADL	2.82×10^{6}
	Mean (CV)	**1.87×10^{7}[c] (4.1×10^{1})**	**1.94×10^{6} (6.8×10^{1})**

[a] CV = Coefficient of variation ((standard deviation/mean) × 100).

[b] ADL = Above Detectable Limit.

[c] Significantly increased (p = 0.0015, 0.0001) compared to the control data, based on Jonckheere-Terpstra's Step-Down Test. Statistically significant increase based on three plasma samples in 110 µg/L treatment level. However, the five remaining samples above detection limit (ADL) suggests a strong dose response at this endpoint. Study data provided in Appendix 8.

NOTES: Values presented in this table have been rounded to three significant figures. All calculations were made using the actual (unrounded) data.

VTG data were analyzed for potential outliers. Statistical analyses on the data excluding the potential outliers were not different from the analyses with all data points. Therefore, analysis including all data points is reported. Variability in the VTG data is within the expected range.

Table 9. Results of the sex steroid analysis following the 21 day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

Mean Measured Concentration (µg/L)	Replicate	Sex Steroid Concentration (µg/L)[ab]			
		Male		Female	
		Testosterone	17β-Estradiol	Testosterone	17β-Estradiol
Control	A	0.406	1.24	1.74	14.4
	B	1.686	0.594	0.952	7.05
	C	1.32	0.741	1.48	11.6
	D	1.053	0.920	30.1	31.6
	Mean (SD)	**1.11 (0.539)**	**0.872 (0.276)**	**8.56 (14.3)**	**16.2 (10.7)**
9.7	A	0.515	0.327	1.17	10.2
	B	1.66	0.511	2.47	16.8
	C	1.11	0.065	2.67	22.4
	D	0.950	0.126	3.17	10.2
	Mean (SD)	**1.06 (0.473)**	**0.257 (0.203)**	**2.37 (0.854)**	**14.9 (5.89)**
20	A	0.289	0.408	0.801	2.51
	B	1.79	0.400	1.94	6.56
	C	6.28	3.03	4.27	11.10
	D	0.852	0.294	0.755	10.8
	Mean (SD)	**2.30 (2.72)**	**1.03 (1.33)**	**1.94 (1.65)**	**7.73 (4.05)**
53	A	2.60	0.097	3.76	9.66
	B	0.261	0.172	1.73	2.12
	C	1.99	0.217	0.833	3.39
	D	2.20	1.23	4.23	7.54
	Mean (SD)	**1.76 (1.03)**	**0.429 (0.536)**	**2.64 (1.62)**	**5.68 (3.52)**
110	A	0.528	0.708	3.60	19.0
	B	0.355	2.61	3.67	9.50
	C	8.75	2.74	1.73	7.29
	D	0.551	2.79	2.83	5.47
	Mean (SD)	**2.54 (4.14)**	**2.21 (1.01)**	**2.96 (0.90)**	**10.3 (6.02)**

[a] Mean measured values and standard deviations (SD) were calculated using the actual analytical results and not the rounded values (three significant figures) presented in this table.

[b] The limit of quantitation (LOQ) changed from analysis to analysis. Based on recommendations in the OECD document entitled "Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures" (OECD, 2000), a value of one-half the LOQ was used for the analytical result for the determination of the measured concentration in treatment levels where the results were below the LOQ.

NOTE: No empirical difference from the controls was observed for sex steroid analysis (estradiol or testosterone) at any treatment.

Figure 1. Relationship between nominal and mean measured concentrations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

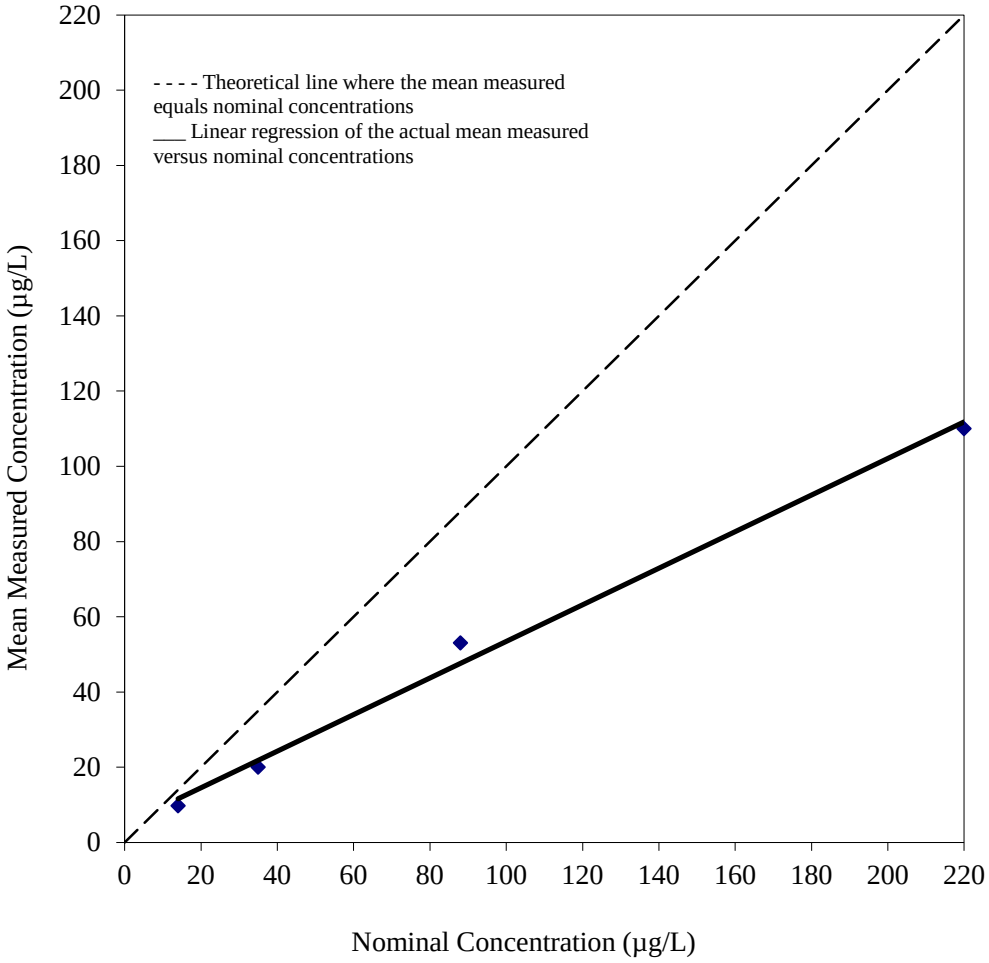


Figure 2. Mean male survival during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

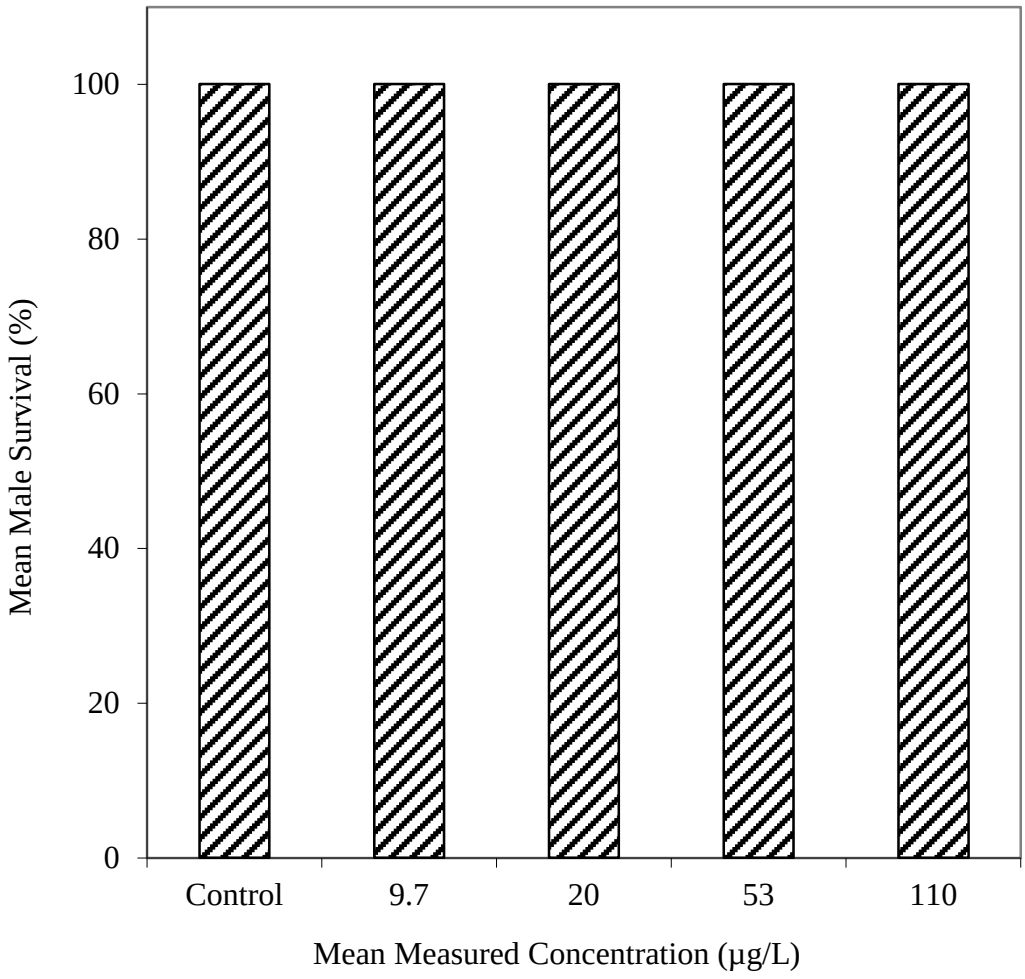
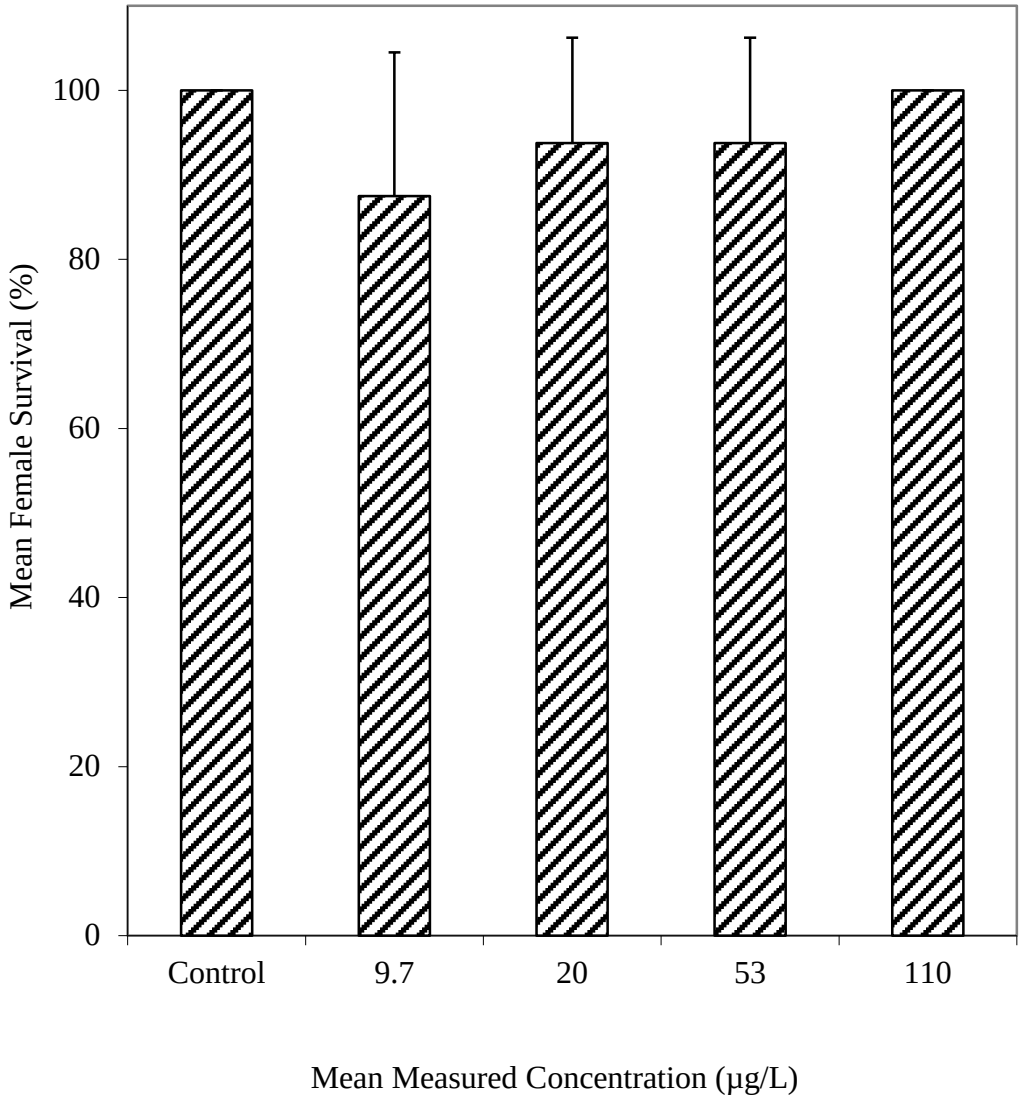
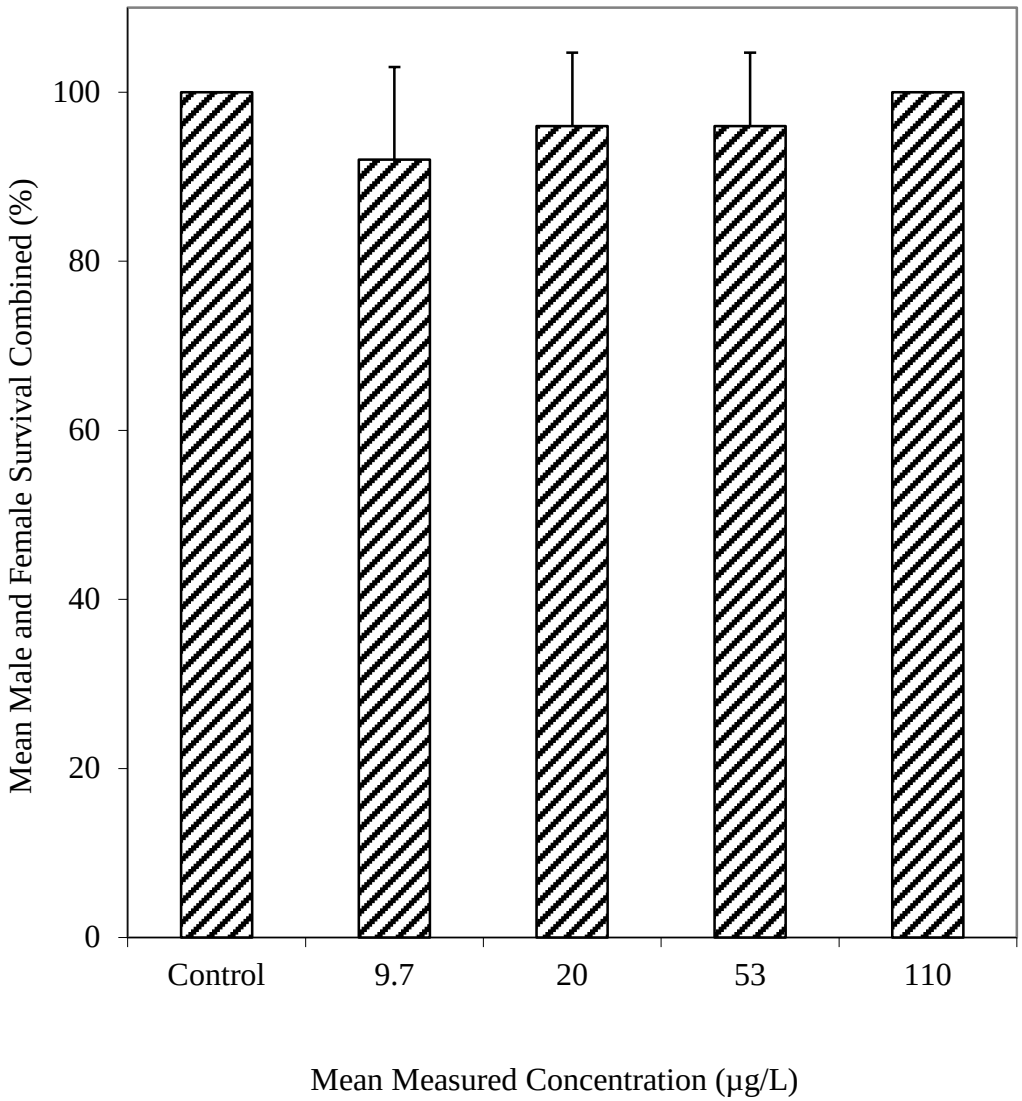


Figure 3. Mean female survival during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



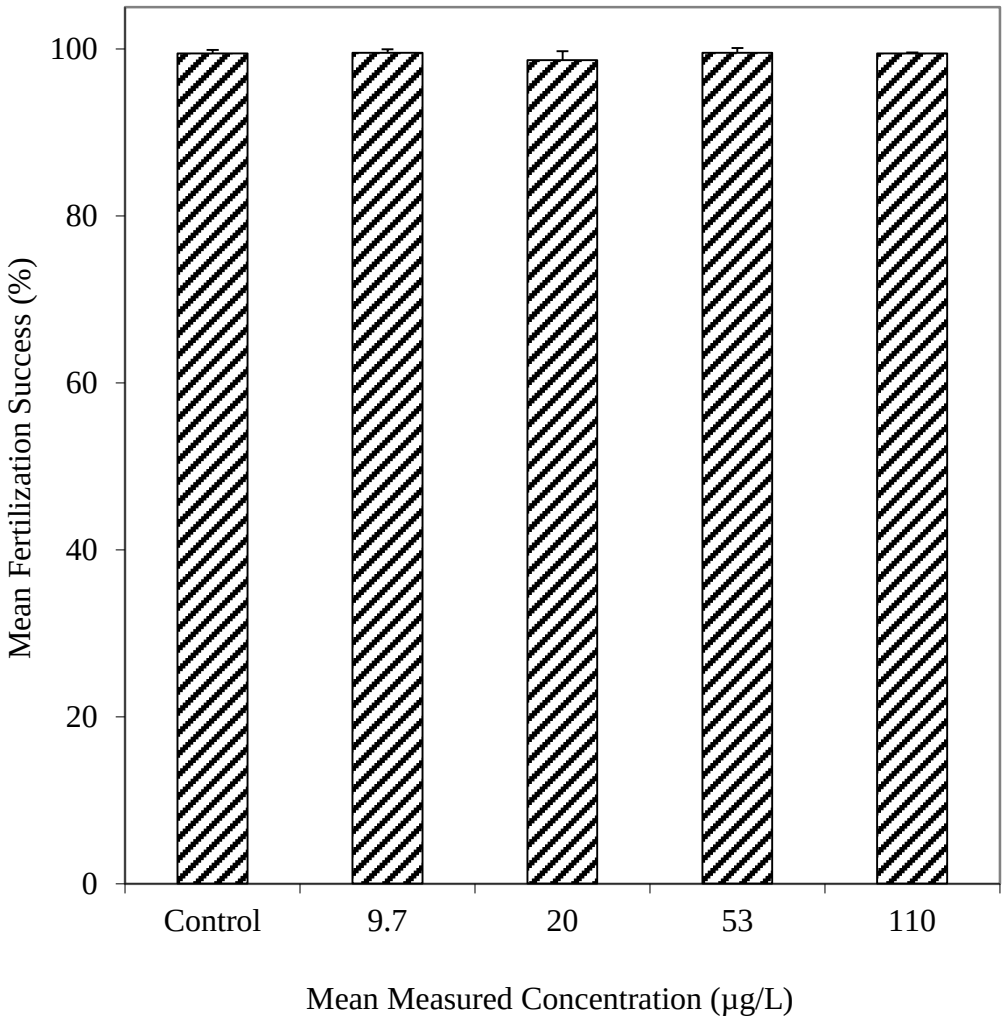
NOTE: Error bars represent the coefficient of variation.

Figure 4. Mean combined male and female survival during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



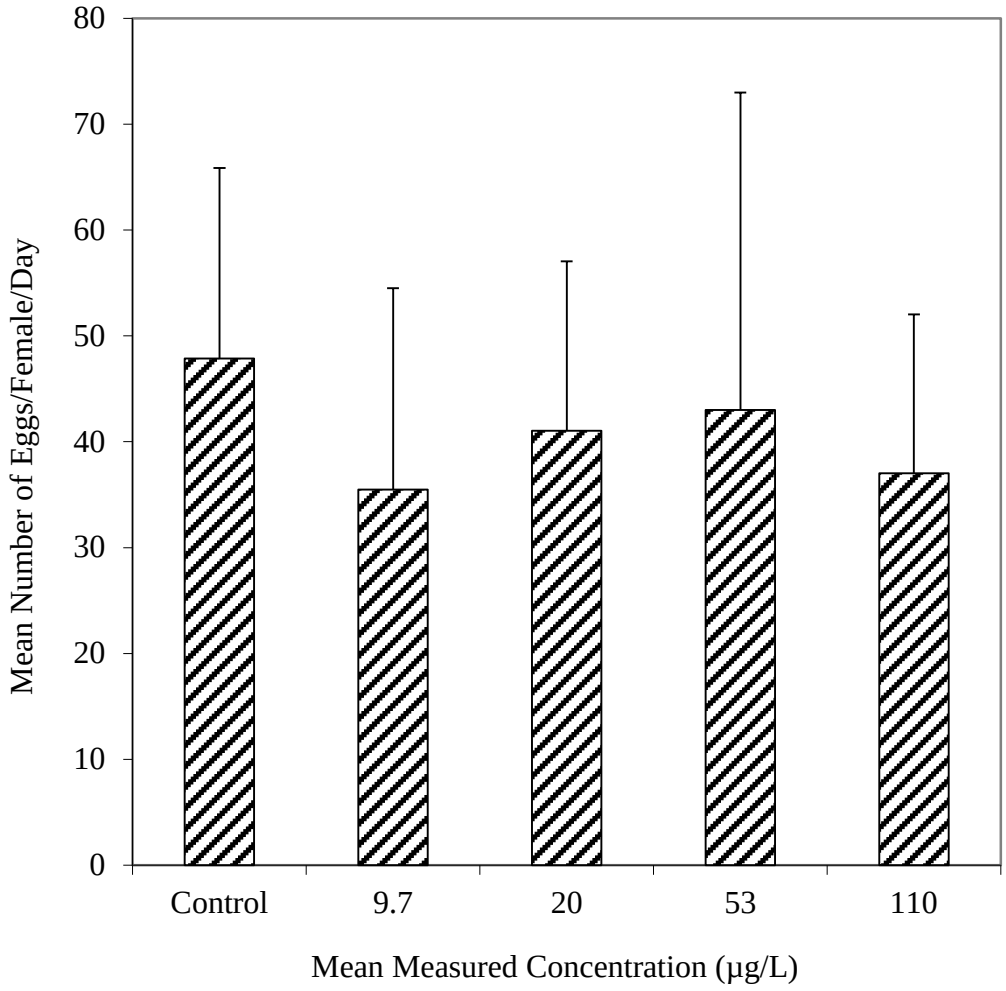
NOTE: Error bars represent the coefficient of variation.

Figure 5. Mean fertilization success (percentage of viable eggs) during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



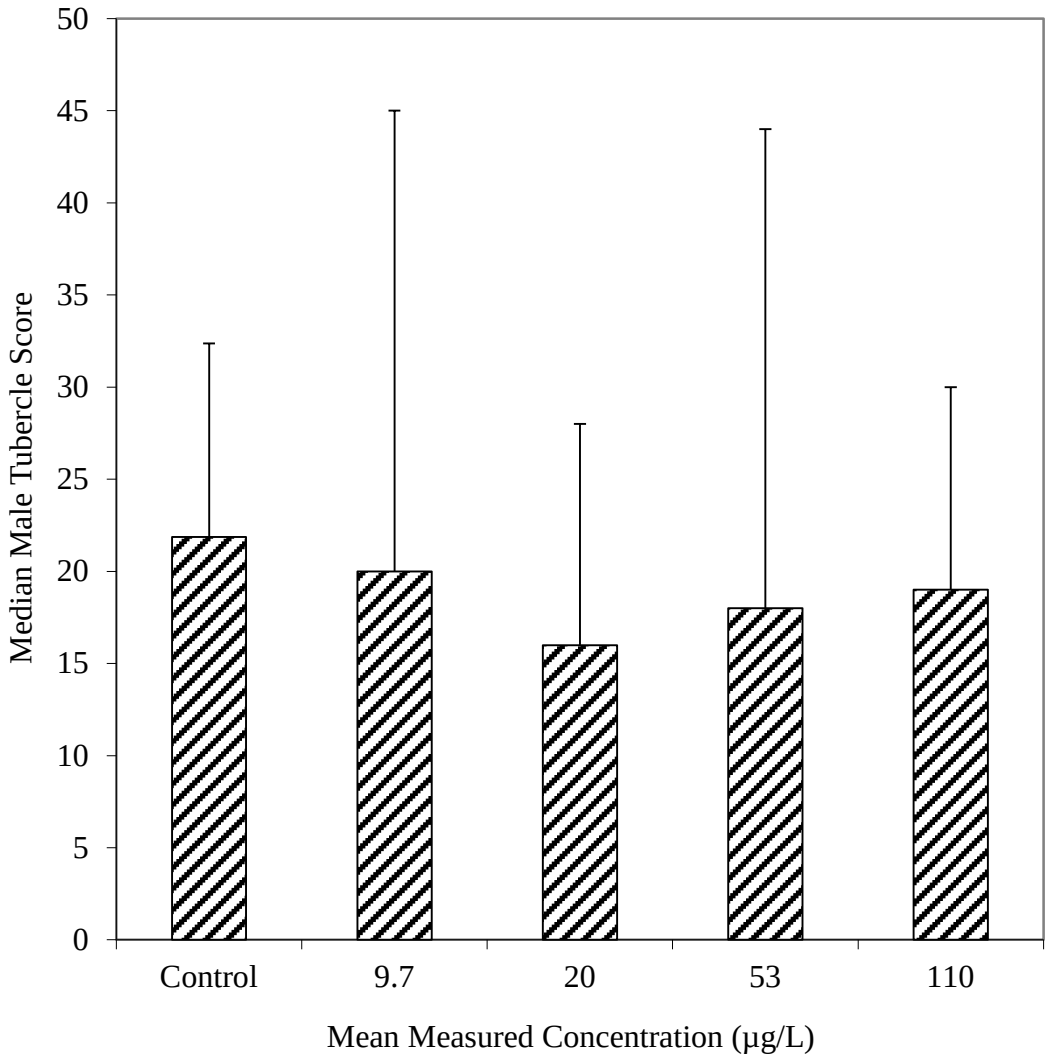
NOTE: Error bars represent the coefficient of variation.

Figure 6. Mean fecundity (number of eggs per female per reproductive day) during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



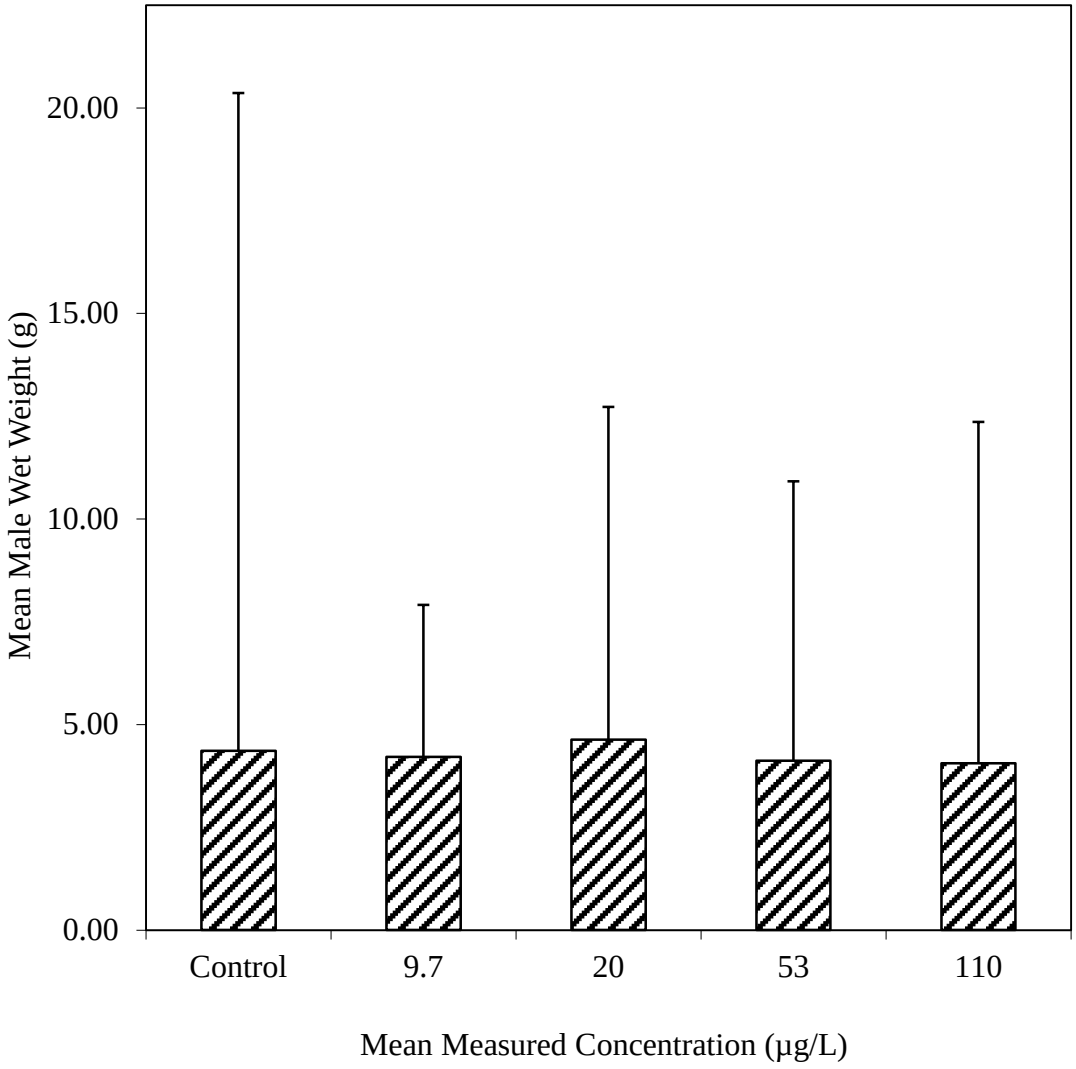
NOTE: Error bars represent the coefficient of variation.

Figure 7. Median male tubercle score during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



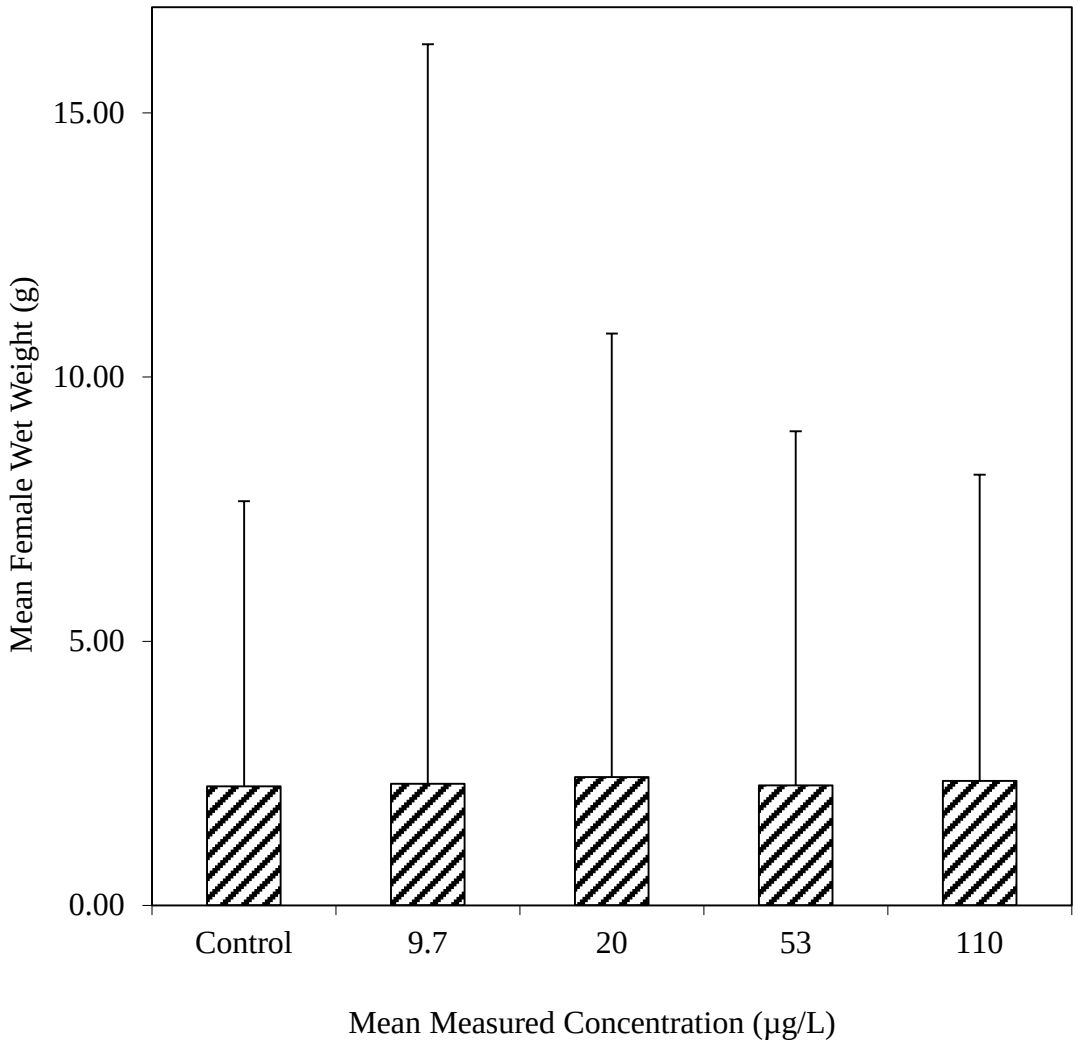
NOTE: Error bars represent the coefficient of variation.

Figure 8. Mean male weight during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



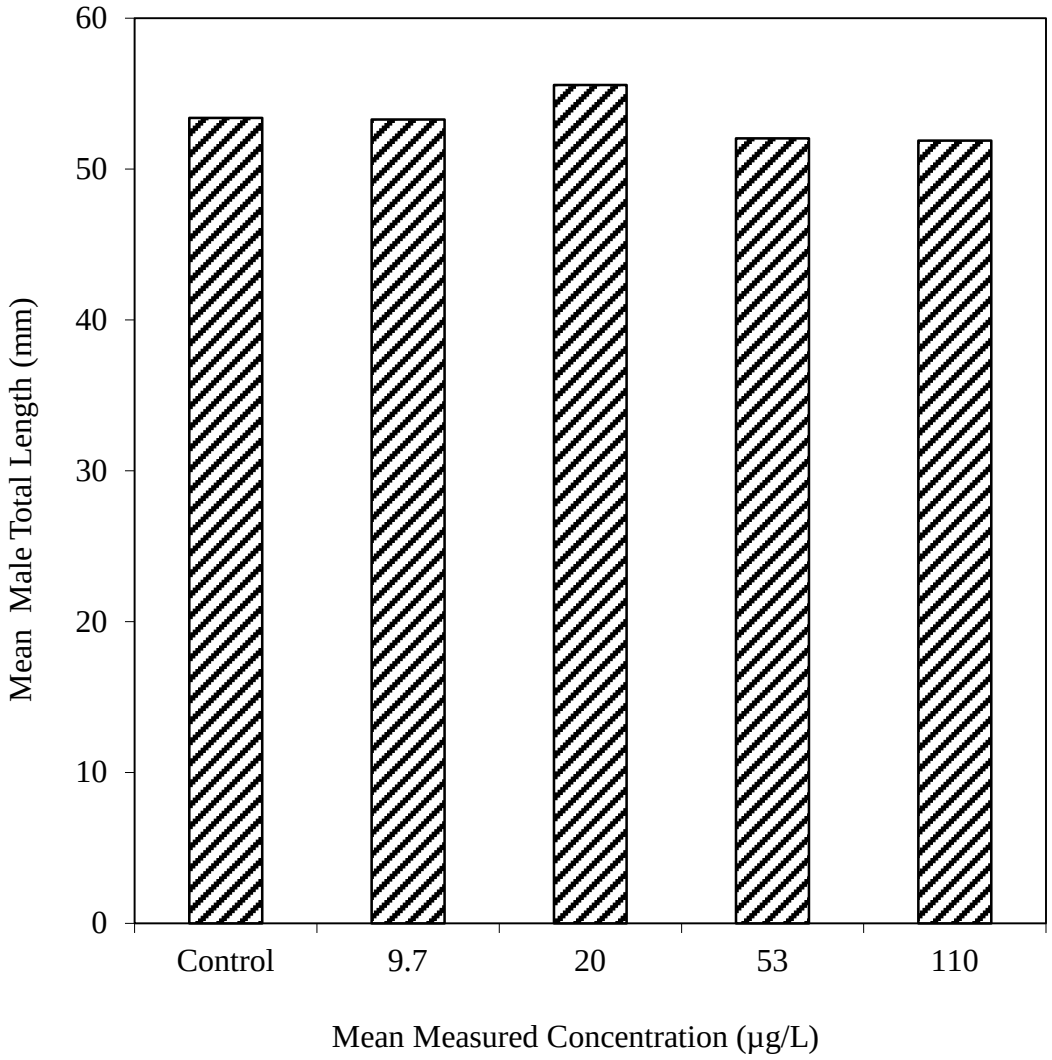
NOTE: Error bars represent the coefficient of variation.

Figure 9. Mean female weight during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



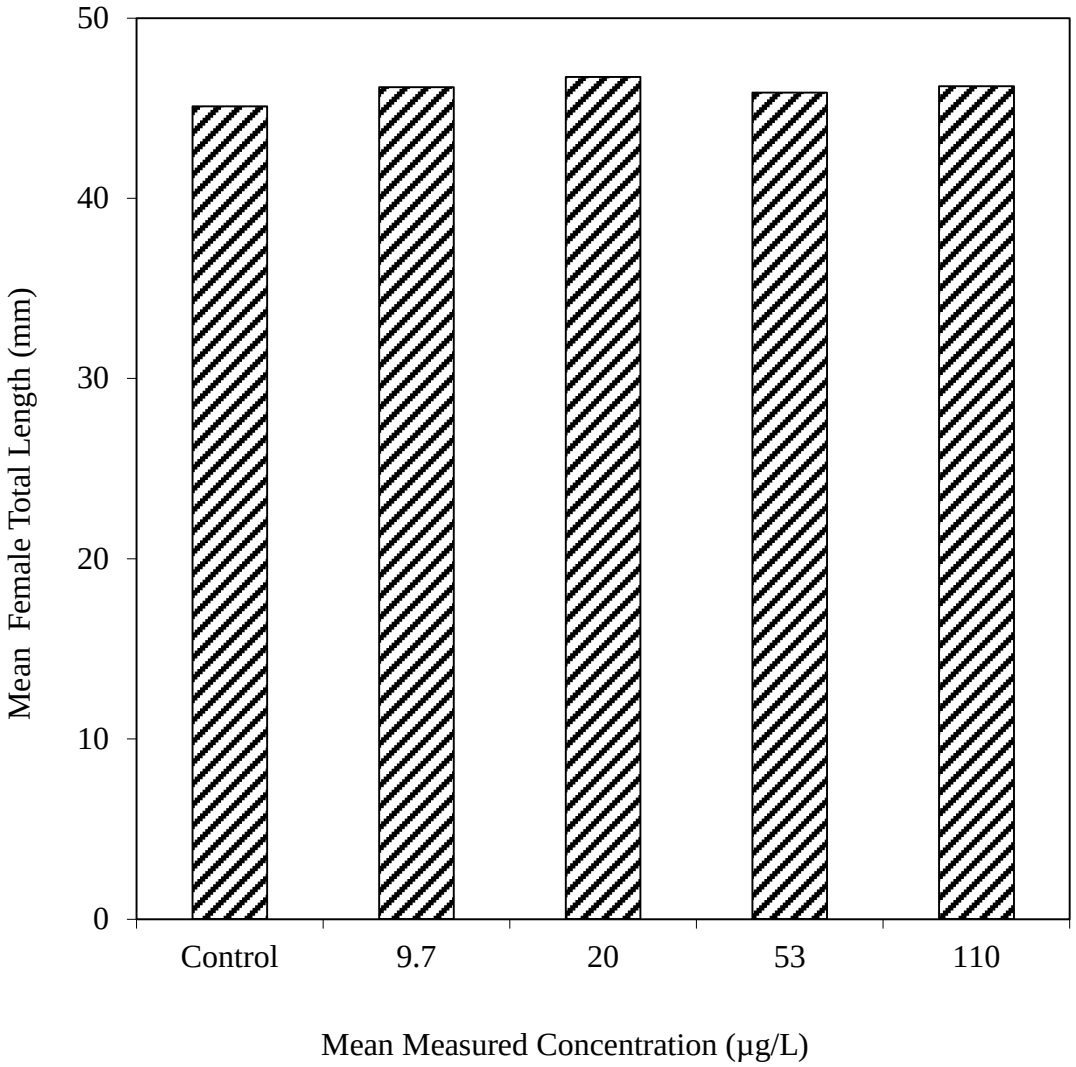
NOTE: Error bars represent the coefficient of variation.

Figure 10. Mean male length during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



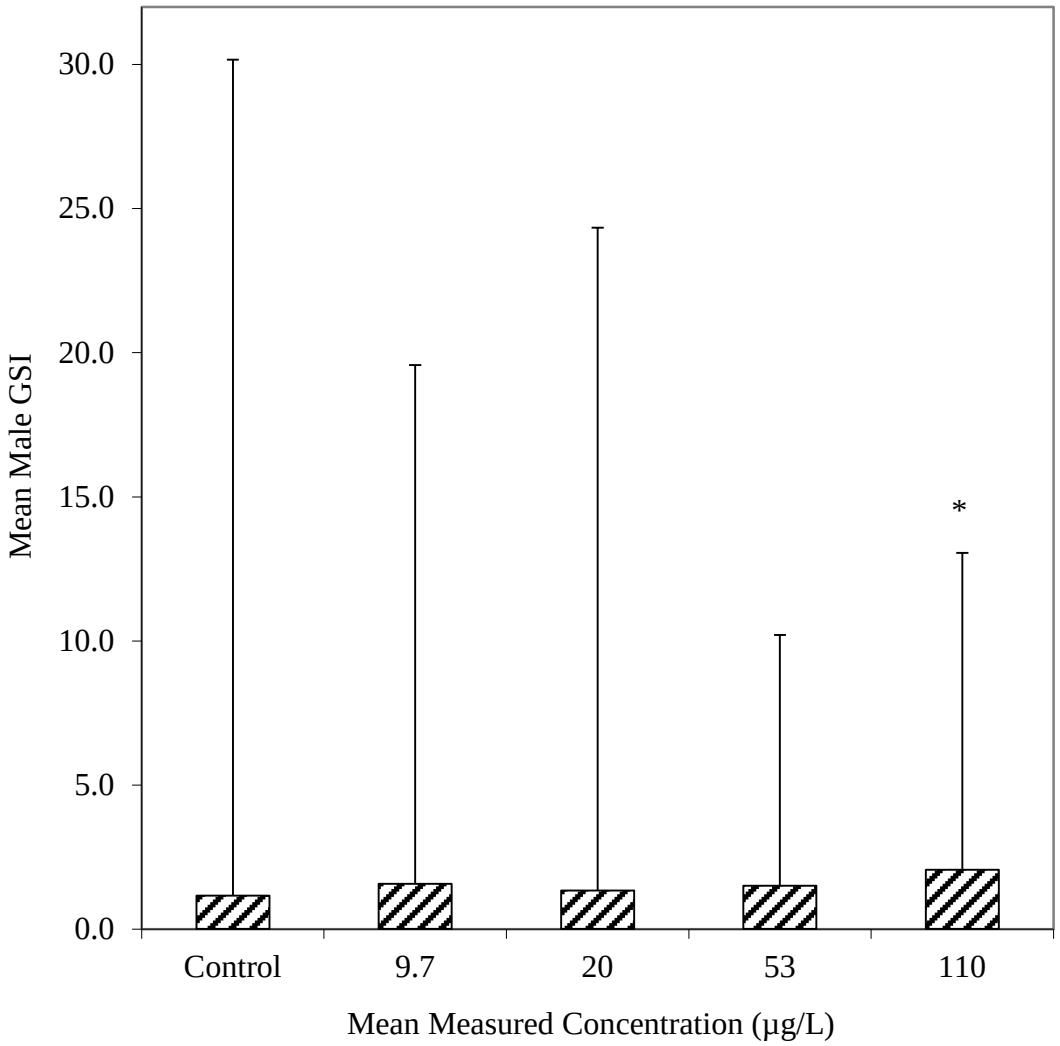
NOTE: Length data were not statistically analyzed as adult (i.e., sexually mature) fish were used to initiate this exposure.

Figure 11. Mean female length during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



NOTE: Length data were not statistically analyzed as adult (i.e., sexually mature) fish were used to initiate this exposure.

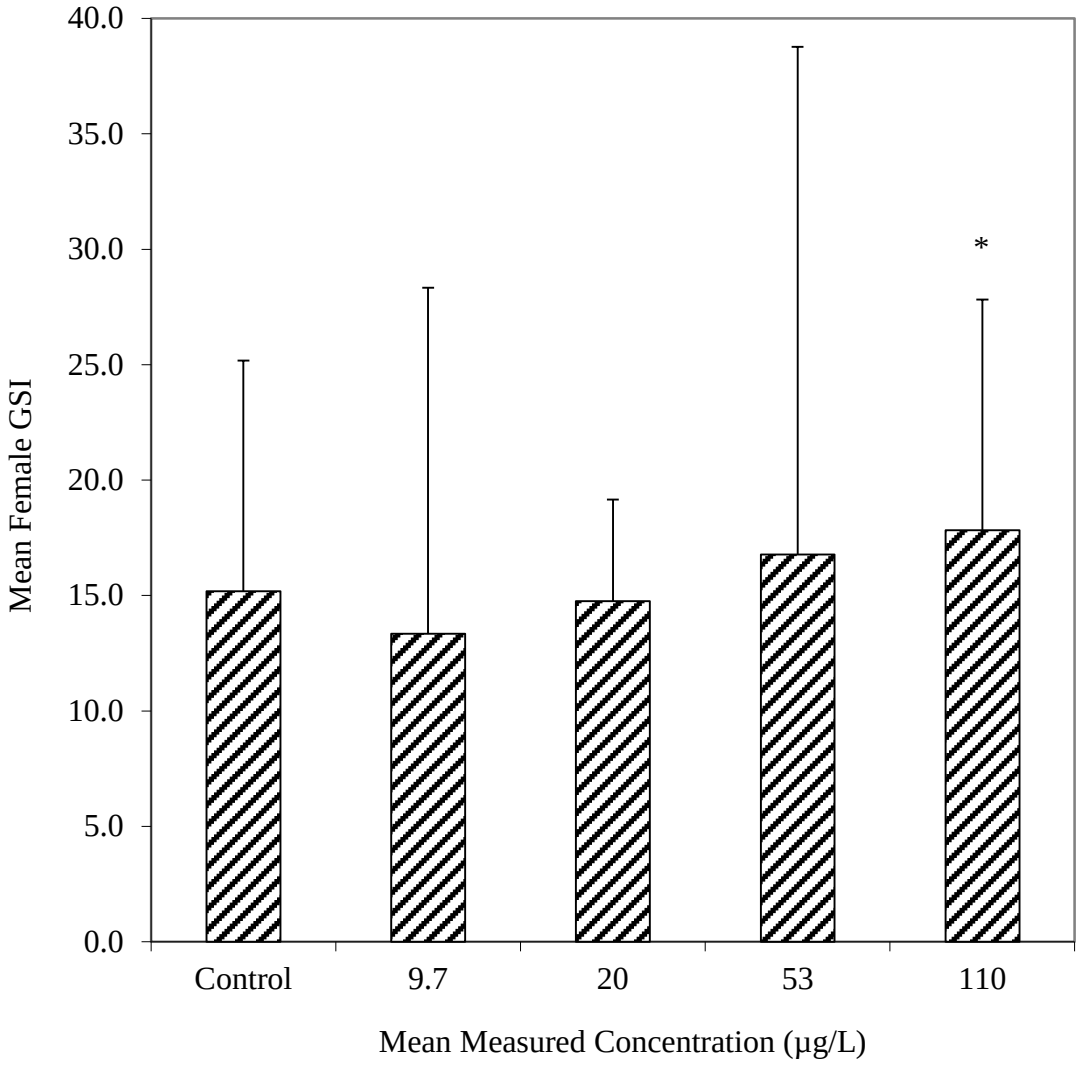
Figure 12. Mean male GSI during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



*Significantly increased compared to the control data, based on Jonckheere-Terpstra's Step-Down Test.

NOTE: Error bars represent the coefficient of variation.

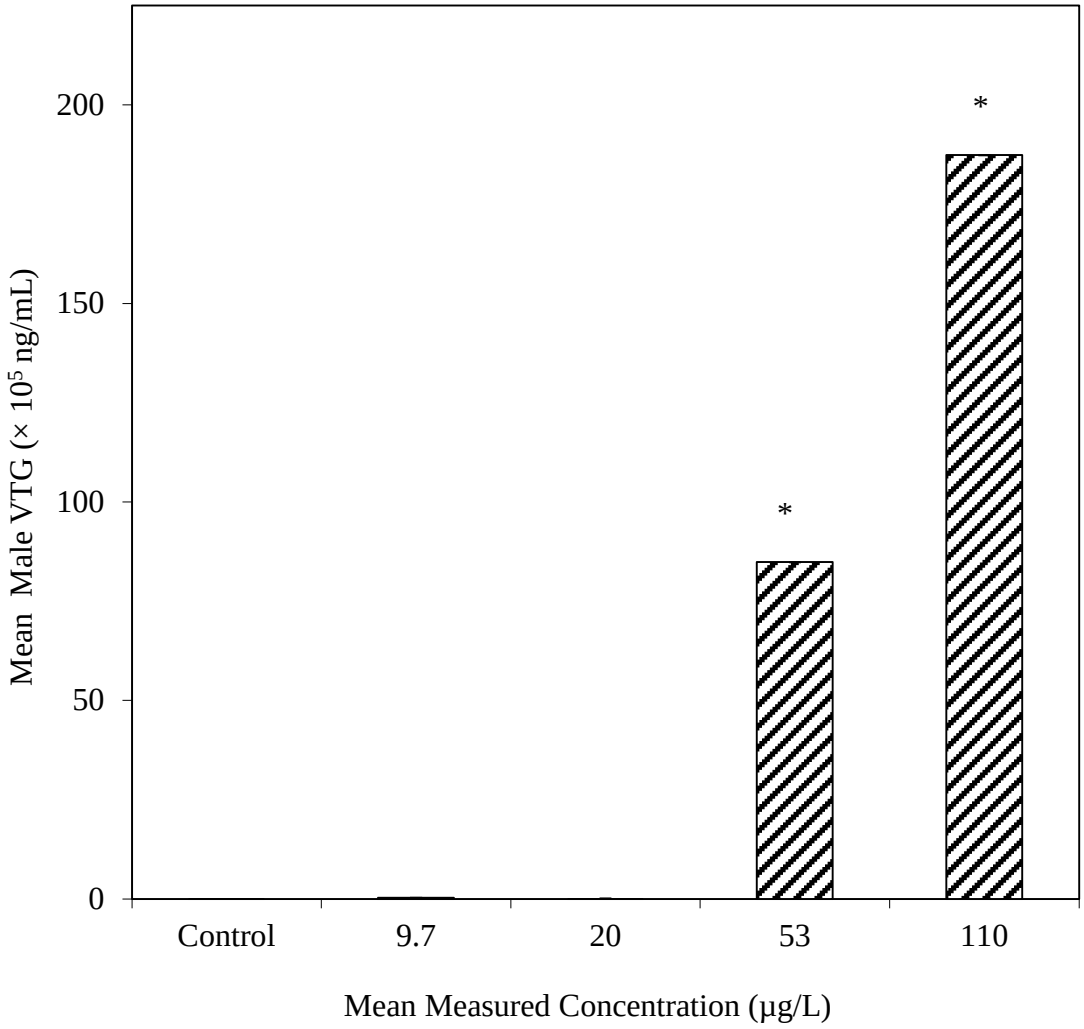
Figure 13. Mean female GSI during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



*Significantly increased compared to the control data, based on Jonckheere-Terpstra's Step-Down Test.

NOTE: Error bars represent the coefficient of variation.

Figure 14. Mean male VTG concentration using all data points (no outliers removed)during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



* Significantly increased compared to the control data, based on Jonckheere-Terpstra's Step-Down Test. Statistically significant increase based on three plasma samples in 110 µg/L treatment level. However, the five remaining samples above detection limit (ADL) suggests a strong dose response at this endpoint. Study data provided in Appendix 8.

Figure 15. Mean female VTG concentration using all data points (no outliers removed)during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.

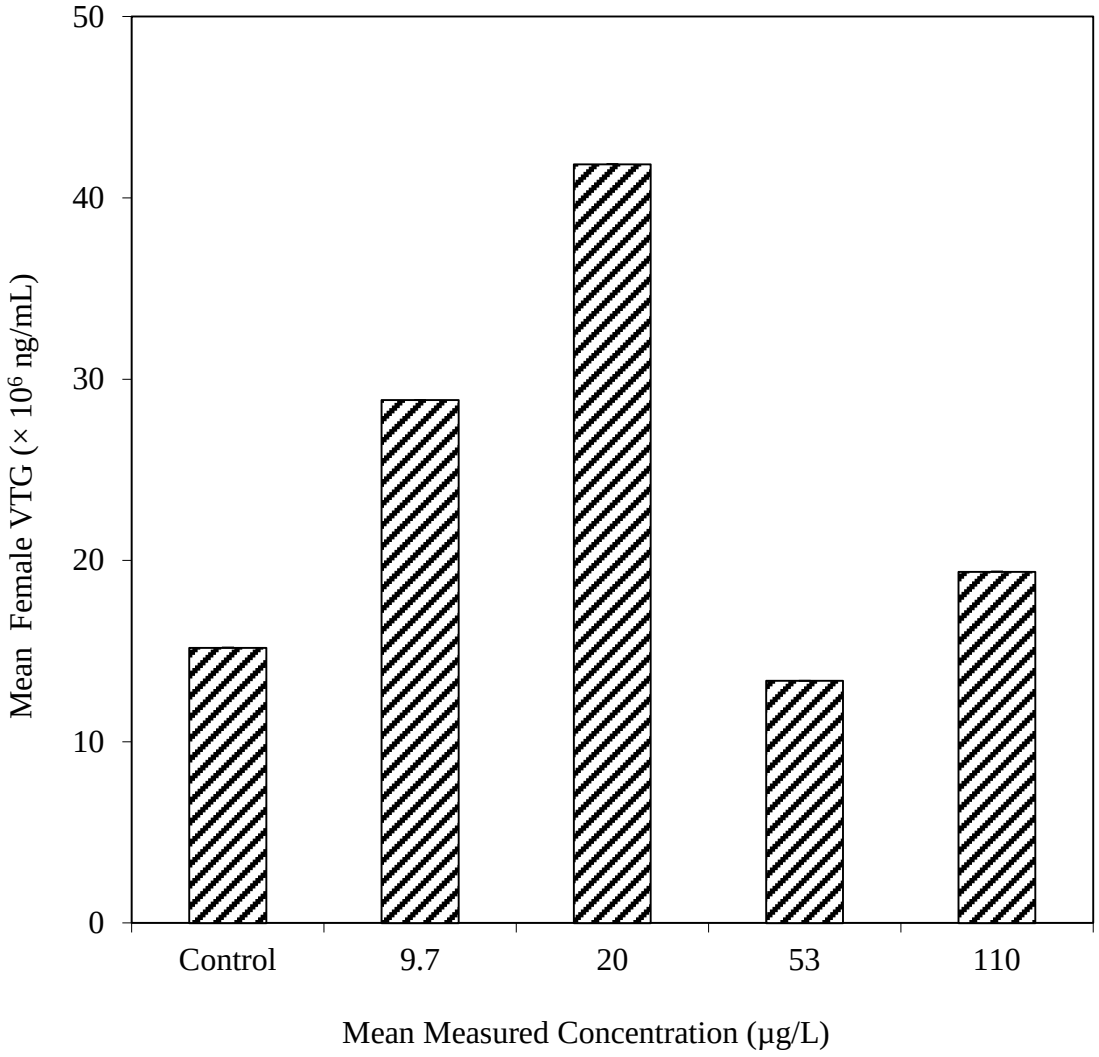
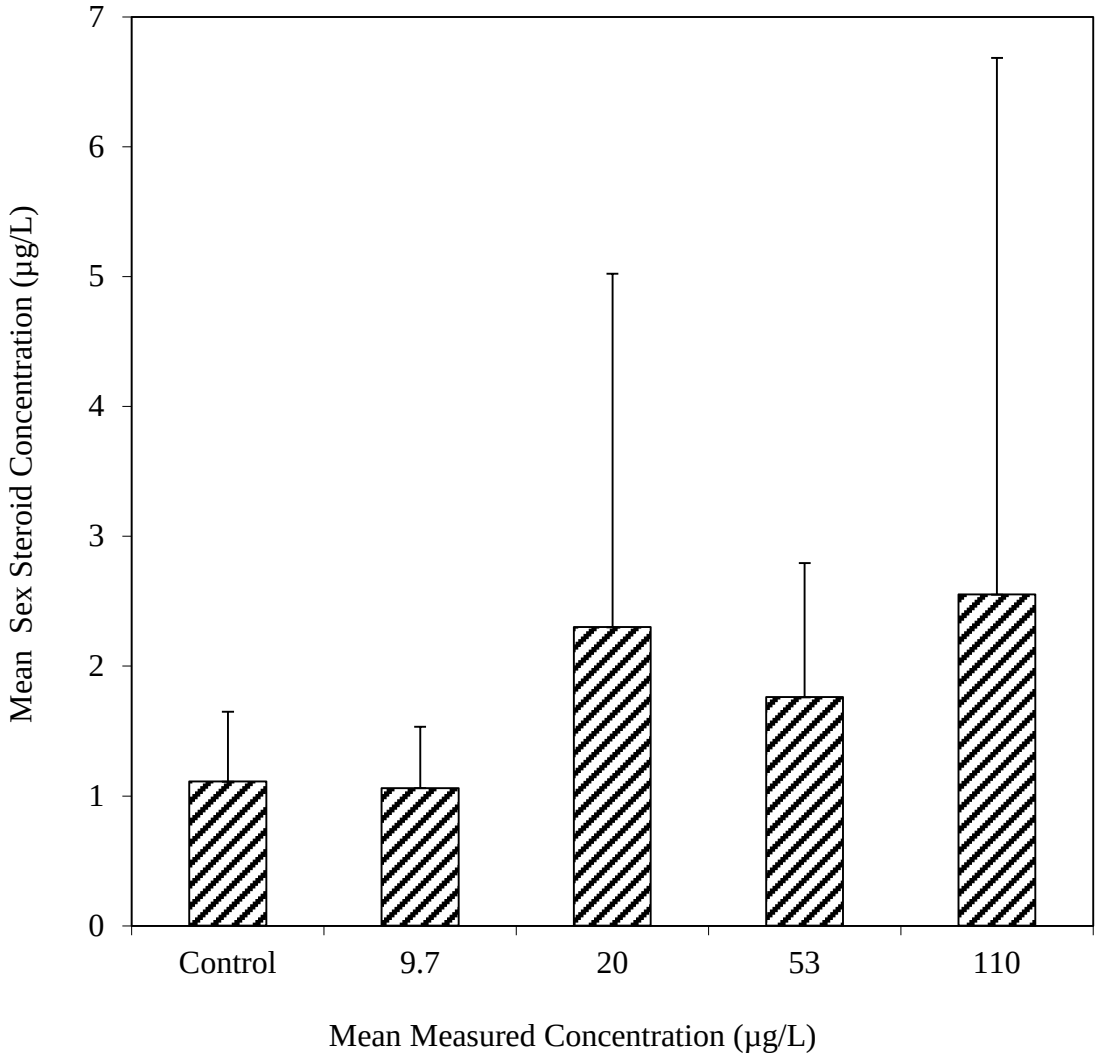
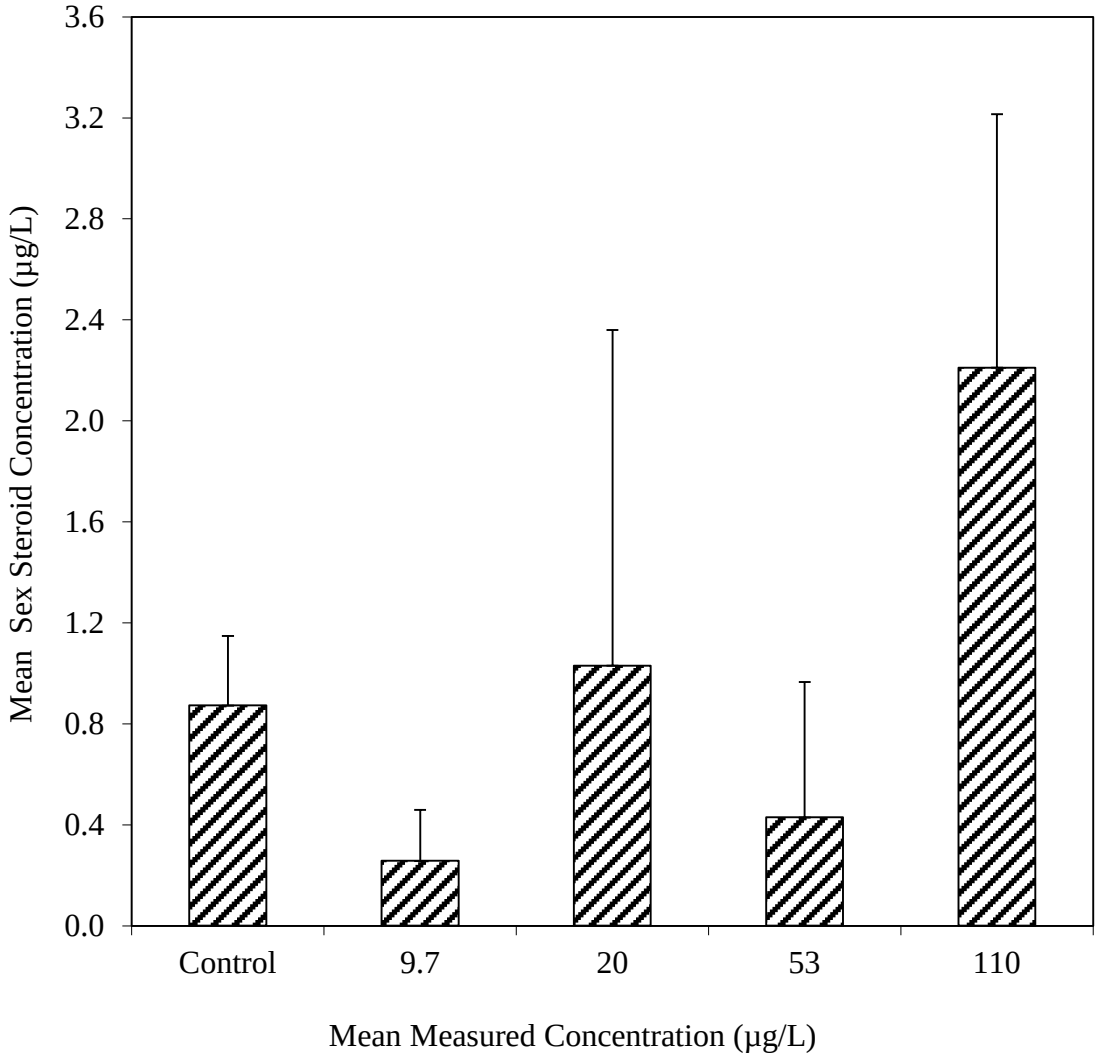


Figure 16. Male testosterone concentration following the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



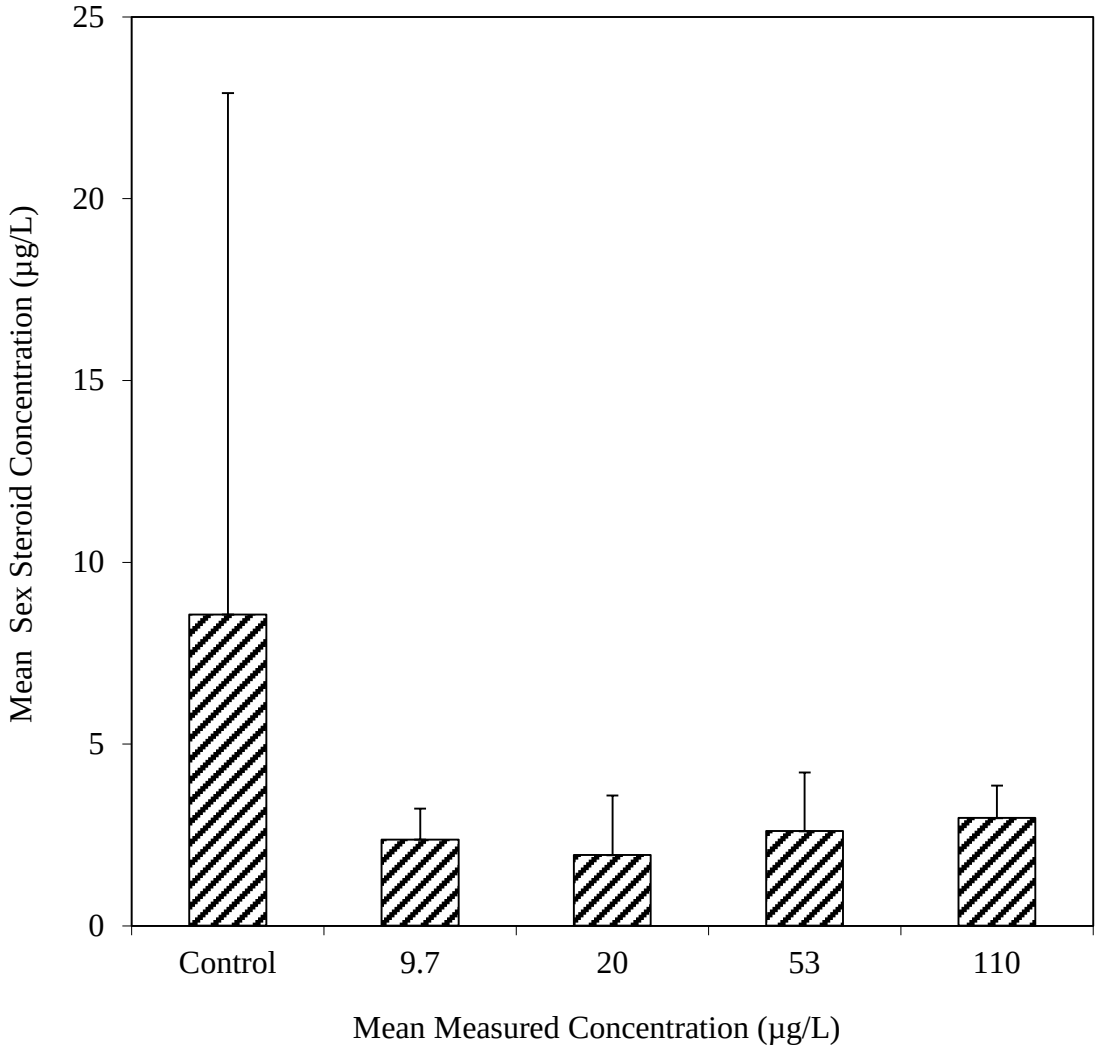
NOTE: Error bars represent the standard deviation.

Figure 17. Male 17β-estradiol concentration following the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



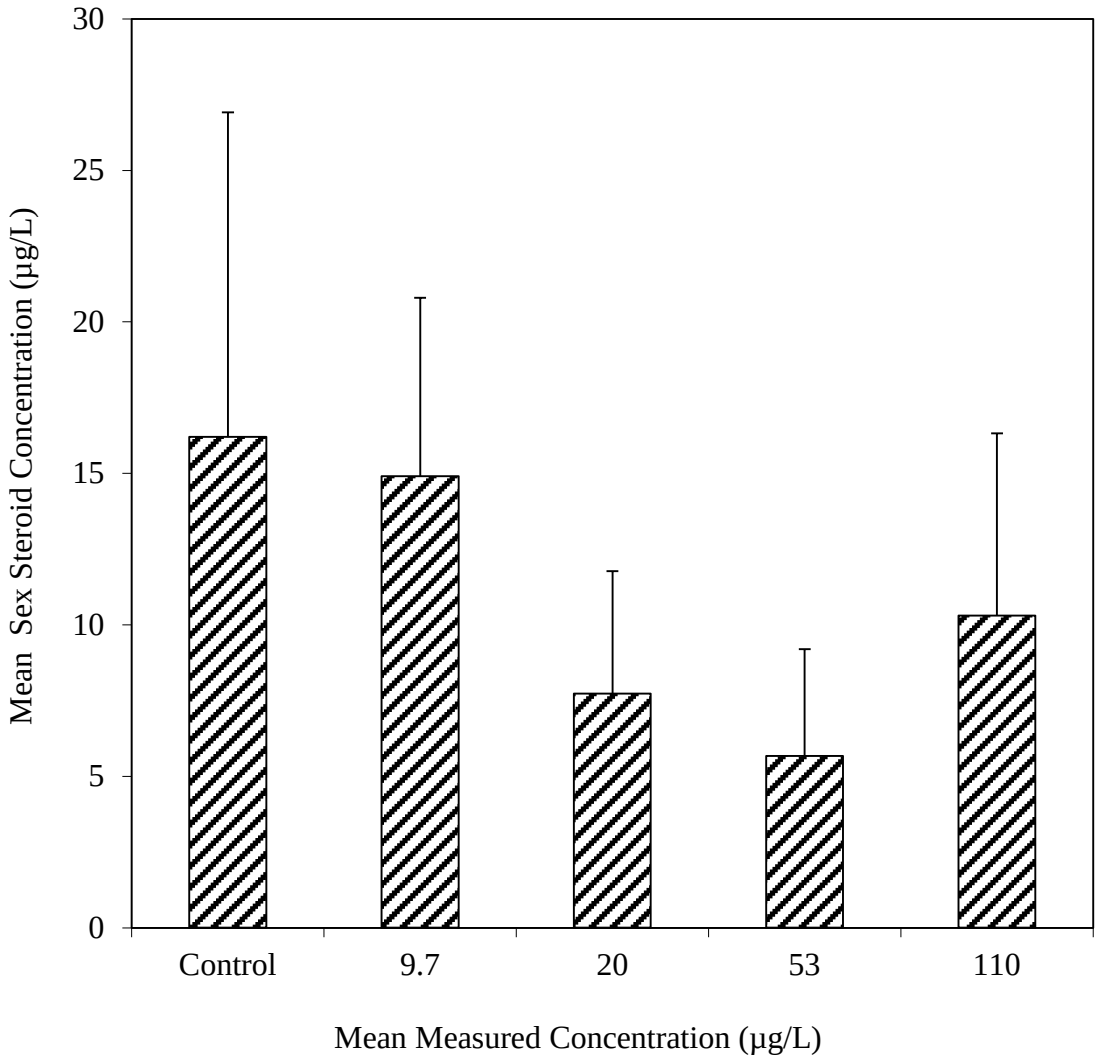
NOTE: Error bars represent the standard deviation.

Figure 18. Female testosterone concentration following 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



NOTE: Error bars represent the standard deviation.

Figure 19. Female 17β-estradiol concentration following the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben.



NOTE: Error bars represent the standard deviation.

APPENDIX 1 – STUDY PROTOCOL

SMITHERS VISCIENT

TEST PROTOCOL

Title: Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines

Test Substance(s):
Name: 2-ethylhexyl paraben
Purity: 99.8%
Batch or Lot #: VXGDC

Analytical Standard:
Name: Same as test substance
Purity: Same as test substance
Batch or Lot #: Same as test substance

Study Sponsor: U.S. Environmental Protection Agency
Address: 1200 Pennsylvania Ave., NW
Washington, DC 20460

Study Monitor: Vincent J. Brown, Battelle
Email / Phone Number: brownv@battelle.org / 614-424-5928
Sponsor Protocol/Project No. (when applicable): NA

Testing Facility: Smithers Viscient
790 Main Street
Wareham, Massachusetts 02571

Histopathology: Experimental Pathology Laboratories, Inc.
45600 Terminal Drive
Sterling, VA 20166
Point of contact: Jeff Wolf

Study Director: Joseph Marini

Smithers Viscient Study No.: 13784.6120

Test Concentration(s): *

Proposed Experimental Dates *

* To be provided by protocol amendment, if applicable.

Vincent J. Brown — 4/12/2016

Sponsor Representative (Study Monitor) Approval — **Date**

13 Apr 16

Study Director Signature — **Study Initiation Date**

Smithers Viscient LLC
www.smithersviscient.com
790 Main Street | Wareham, MA 02571 | p 508.295.2550
2900 Quakenbush Road | Snow Camp, NC 27349 | p 336.376.0141

Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines

1.0 OBJECTIVE

This protocol describes the procedures to be used in the performance of a short-term assay with fathead minnow (*Pimephales promelas*) based on the protocol developed by Ankley et al. (2001) and meets the requirements of the OPPTS 890.1350 and OECD 229 guidelines for the fish short-term reproduction assay. The purpose of this test is to assess the potential for the test substance to interact with the endocrine system in fathead minnow exposed under flow-though conditions.

2.0 MATERIALS AND METHODS

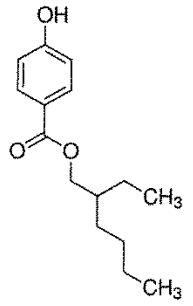
2.1 Chemical System

2.1.1 Test Substance

Upon arrival at Smithers Viscient, the test and reference substance(s) will be received by the Test Material Center. Records will be maintained in accordance with GLP requirements, and a Chain-of-Custody established. The condition of the external packaging of the test substance will be recorded and any damage noted. The packaging will be removed, the primary storage container inspected for leakage or damage, and the condition recorded. Any damage will be reported to the Sponsor and/or manufacturer.

Each sample will be given a unique sample ID number and stored under the conditions specified by the manufacturer. The following information should be provided by the test substance manufacturer or supplier, if applicable: test substance lot or batch number, test substance purity, water solubility (pH and temperature of solubility determination), vapor pressure, storage stability, methods of analysis of the test substance in water, MSDS, and safe handling procedures, and a verified expiration or reanalysis date.

The 2-ethylhexyl paraben to be used in this assay has the following structure and properties:



Molecular/Empirical formula:	$C_{15}H_{22}O_3$
CAS number:	5153-25-3
Chemical Name:	2-ethylhexyl 4-hydroxybenzoate
Trade name:	2-Ethylhexyl Paraben
Molecular weight:	250.33 g/mol
Class of compound:	Preservative
Water solubility:	2.4 mg/L
LogP:	5.36

2.1.2 Test Concentration Selection

Test concentrations will be selected based on previously conducted exposures of fathead minnow to the test substance during preliminary testing conducted at Smithers Viscient. A 96-hour range finding will be conducted to determine the LC50 for fathead minnow of similar age and size to those used during the screening assay. The range finding assay will test up to the water solubility limit of the test substance under testing conditions and will not exceed 100 mg/L as the high exposure concentration. The preliminary test will be conducted with fish of a similar age and size as the fish that are to be used in the definitive exposure. A minimum of five treatment levels and a control will be employed and each exposure vessel will contain a total of six fish, four female fish and two male fish. To help reduce mortalities based on aggressive male fish, spawning tiles will be added to the exposure vessels at initiation.

For the definitive assay, four test substance concentrations and a negative control will be used. The EPA FSTRA Test Guideline (OPPTS 890.1350, page 3ff) specifies three concentrations; however, the EPA Statement of Work for Task Order 14 (5/22/2015, page 6) amends this to require four (4) treatment concentration groups. Test concentrations will be decreased by a factor not to exceed 2.5. A negative control will consist of dilution water without test substance. The definitive concentrations will be added to the protocol by amendment.

2.1.3 Solvent Control

An organic solvent is not expected to be utilized during this testing, however solvent may, as the last resort, be used as a carrier to solubilize the test substance. In such a case, a solvent control will be included in the test, and consists of dilution water plus the highest concentration of solvent that occurs in any of the test solutions. The solvent concentration will be kept as low as possible, and will not be allowed to exceed 0.100 mL/L. The concentration of solvent in each test solution (except the negative control) will be the same.

2.1.4 Stock Solution Preparation

The test substance will be weighed on an analytical balance for which a calibration log is maintained. A Chemical Usage Log will also be maintained in which the amount, the date, the intended use and the user's initials will be recorded each time the test substance is used. The stock solution will be prepared according to the following formula:

$$\text{Stock Concentration (e.g., mg/mL)} = \frac{\text{H.C.} \times \text{M.C.}}{\text{T.D.}}$$

where:

H.C. = high concentration (e.g., mg/L)
M.C. = mixing chamber volume (L/cycle)
T.D. = toxicant or syringe delivery (mL/cycle)

Based on the physiochemical properties of the test substance, a solvent carrier may not be required for dosing. Stock solutions may be prepared using glass wool saturator columns, similar to those described in Kahl et al., 1999. Stock solution concentration will be derived from measured values. Delivery of the stock solution will be based on the high test concentration and the stock concentration.

If the saturator column method is not considered to be appropriate for the test substance or if the method is unsuccessful, other standard methods will be employed (e.g., water stocks or delivery utilizing a co-solvent).

2.2 Test System

2.2.1 Species

Reproductively mature, sexually dimorphic, fathead minnow (*Pimephales promelas*) will be used. Typically, fathead minnow will reach sexual maturity at approximately 20-26 weeks of age. Preference in determining the suitability of a population for testing will be given based on evidence of sexual maturity (i.e., observations of secondary sexual characteristics) and spawning qualification over age.

2.2.2 Justification of Test System

Characteristics which make the test species, fathead minnow, suitable are their ease of handling, their known sensitivity to a variety of toxicants, the ready availability of eggs, and the extensive existing data base for this common fish species.

2.2.3 Origin and Acclimation

Fathead minnows (*Pimephales promelas*) will be obtained from in-house cultures. Fish will be reproductively mature, possess clear secondary sexual characteristics and be actively spawning. For at least two weeks prior to the test (initiation of the pre-exposure period; see below), the population will be held under similar conditions of water quality and lighting as those used in the test (note, this acclimation period is not the *in situ* pre-exposure period). Mortalities during these two weeks will be recorded and the following criterion applied to the batch: <5% batch mortality in the seven days preceding the exposure will be considered acceptable.

It is important to minimize variation in weight of the fish at the beginning of the assay. For the whole batch of fish used in the test, the range in individual weights by sex at the

start of the test will be kept, if possible, within ±20% of the arithmetic mean weight of the same sex. A subsample of fish will be weighed before the test in order to estimate the mean weight by gender.

2.2.4 Feeding

During the pre-exposure and exposure periods, the spawning fish will be fed three times daily. Two feedings will consist of frozen brine shrimp with one feeding consisting of a commercially available flake fish food. The amount fed will be measured and recorded. The fish will not be fed 24 hours prior to test termination. Periodic analyses of representative samples of the food will be conducted using U.S. EPA standard methods (ASTM, 2007) by GeoLabs, Inc., Braintree, Massachusetts, in accordance with Smithers Viscient's standard operating procedure 07.24 (Monitoring of Feed, Soil and Source Water Contamination), to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to fish.

2.2.5 Physical System

The physical exposure system consists of diluter cells attached to a headboard, and two tiers or levels of exposure aquaria behind and below the headboard, which will provide four replicates of each treatment level and control(s). All aquaria will be labeled with treatment and replicate designation.

2.2.6 Exposure System

Each test vessel will be constructed of glass and silicone adhesive and will measure 39 x 20 x 25 centimeters (L x W x H). The test chambers will be chemically cleaned before the test is started. The aquaria will be washed with hot water and a detergent, rinsed with acetone, and then rinsed extensively with water. In addition, all test chambers will be brushed and siphoned to remove detritus and uneaten food as needed (at least twice weekly) during the test. Water depth will be maintained by a constant level overflow drain 13 cm from the bottom of each test aquarium. The total test solution volume in each aquarium will be thus maintained at 10 L. Aquaria will be labeled to identify the treatment/control and the replicate designation.

2.2.7 Spawning Substrates

During the reproductive phase, substrates will be provided on which the female will deposit adhesive embryos. The substrates are composed of two separate pieces of equipment. The primary substrate will be constructed from ~80 mm length of semi-circular guttering, that will act as an "arch" under which spawning will take place. Each primary substrate will sit on a secondary substrate. The secondary substrate will be a lipped tray of ~100 mm in length. The tray will capture embryos which do not adhere to the primary substrate. The tiles and trays will be abraded to improve adhesion of the eggs. Each spawning group will have three spawning substrates.

2.2.8 Replication and Control of Bias

Four replicates will be included for each treatment level and control. Each test vessel will contain 2 males and 4 females for a total of 8 males and 16 females per treatment. Test vessels will be randomly placed within the respective water bath. All fish will be

impartially assigned to tanks prior to pre-exposure and then will be randomly assigned to the exposure treatments within a block after successful spawning has been established. The blocks will be filled in random order, with the four tanks with the highest per-female fecundity (established during pre-exposure) being assigned first, followed by the second-highest spawners, etc.

2.2.9 Dilution Water

Dilution water will be a mixture of unadulterated on-site well water and de-chlorinated Town of Wareham, Massachusetts, well water. The on-site well water will be obtained from a 100-meter bedrock well. The Town of Wareham well water will be de-chlorinated by use of UV-filtration and activated carbon filtration. The dilution water will be pumped to a 5700 liter polyethylene tank where it will be continuously aerated to ensure proper equilibration of dissolved gases with the laboratory atmosphere. The water will then be pumped through aged PVC piping to a heat exchanger unit which will heat the water to test temperature and then pumped to the exposure system.

The pH, total hardness, alkalinity and conductivity of this water will be measured and recorded weekly. Total hardness and alkalinity will be determined according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 2005). The water is characterized as being "soft" with a typical total hardness of ≤180 mg/L as $CaCO_3$.

Periodic analyses of representative samples of dilution water will be conducted using U.S. EPA standard methods (ASTM, 2007) by GeoLabs, Inc., Braintree, Massachusetts, in accordance with Smithers Viscient's standard operating procedures, to ensure the absence of potential toxicants, including pesticides, PCBs and selected toxic metals, at concentrations which may be harmful to the fish. Dilution matrices will be sampled for analysis twice per year, typically every six months. TOC analyses will be conducted once a month in the dilution water. Guidance for acceptable dilution water parameter values are as follows:

Alkalinity > 20 mg/L (as $CaCO_3$)
Total organic carbon ≤2 mg/L
Unionized Ammonia ≤ 1µg/L
Residual chlorine < 10 µg/L

2.2.10 Diluter

A 2-liter proportional diluter (e.g., Mount and Brungs, 1967) will be employed to deliver four test substance concentrations and a negative control to replicate aquaria. As noted above, four (4) test substance concentrations is an EPA-requested departure from the FSTRA Test Guidelines will be used. Based on the solubility of the test substance in the dilution water, the stock solution stability and the range of test concentrations, one of the following test substance delivery systems will be used: a gas-tight syringe with an injector or pump metering device, a metering pump/pre-dilution chamber system (Lemke et al., 1978) or a saturator column.

A flow-splitting chamber will be used between the diluter cells and the aquaria to promote mixing of the test substance solution and diluent water. In each chamber, four separate standpipes will be employed to split the test solution equally between test aquaria. The volume of test solution delivered to each of 4 replicate aquaria (upper and lower level) will be 500 mL, at a minimum turnover rate of 6 and a 90% replacement time in ≤ 9 hours.

The calibration of the diluter system will be checked and recorded prior to test initiation. If there is any indication during the test that the diluter calibration has changed (e.g., diluter malfunction or unexplained differences in water quality or measured concentrations in the aquaria), calibration of the necessary diluter components will be checked. During the test, the diluter will be visually inspected at least twice daily, at the beginning and end of the work day, or at approximately 8:00 AM and 4:30 PM, including weekends and holidays. On weekends and holidays, the interval between inspections will be as long as possible based on the presence of laboratory personnel. Standard practice is for diluters to be inspected once upon staff arrival and again just prior to staff departure to ensure proper functioning, including weekends. A test will not be started until the diluter and test substance delivery device have been observed to be properly functioning for at least 48 hours prior to the test. Prior to initiation of the chemical exposure, the diluter should be tested for at least one week for its ability to maintain the desired concentration. Flow rates of diluent should be checked at least once per week throughout the test, and should not vary by more than 20%. Flow rates will be determined by the confirmation of the cycle rates each day.

2.2.11 Cleaning

Chemical cleaning of the test diluter system and aquaria will be done prior to the initiation of each test. At a minimum, all aquaria will be scraped and siphoned twice weekly during the test with additional cleaning done if deemed necessary by the study director. The diluter mixing chamber, chemical cells and splitters will be cleaned weekly, at a minimum, and the diluter delivery tubing will be cleaned as necessary. Any dead fish will be recorded and removed as soon as possible upon observation.

2.3 Test Conditions

2.3.1 Temperature

Water temperature will be maintained at 25 ± 1 °C by delivering pre-heated dilution water to the test system and maintaining a heated water bath using thermostatically regulated heaters.

2.3.2 Lighting

A photoperiod of 16 hours light: 8 hours dark will be employed. The diluter system will be enclosed with black polyethylene curtains to prevent disturbance of spawning and to minimize the interference of laboratory lighting with the intended photoperiod. Light intensity at the water surface will be 50 - 100 footcandles and will be measured once during the test.

2.3.3 Dissolved Oxygen

Total dissolved oxygen concentrations will be maintained at ≥ 60% of saturation during the exposure. Aeration will be initiated, if necessary, to raise and maintain the dissolved oxygen concentration to the desired level.

2.3.4 Test Initiation and Termination

The exposure will begin when the actively spawning groups have been placed in the exposure vessels and will terminate following 21 days of exposure. If there is excessive mortality during the pre-exposure or in the control tanks during exposure, the cause will be investigated and the exposure should be reinitiated.

2.4 Biological Methods

FSTRA Exposure and Endpoint Timeline

Study Week	1	2	3	4	5	Termination
Exposure Stage Key	Pre-Exposure		Spawning			
Endpoints						
Fecundity	x	x	x	x	x	
Fertilization Success			x	x	x	
Adult Survival			x	x	x	x
GSI						x
Length						x
Nuptial Tubercle Scores						x
Vitellogenin (VTG concentration)						x
Study Week	1	2	3	4	5	Data Compilation

2.4.1 Pre-Exposure Period

The pre-exposure phase will last a minimum of 14 days and will assess spawning activity. In a separate system, four females and two males will be grouped and placed in tanks under conditions identical to those in the test (e.g., temperature, lighting, feeding, etc.). Additional chambers should be set up to account for a lack of spawning in some chambers and/or mortality during the pre-exposure period. Up to eight additional tanks may be set up to ensure an adequate supply of spawning groups with the correct sex ratio. Spawning activity will be monitored daily and fecundity will be assessed and recorded. For the assay, successful pre-exposure (suitability for testing) is established when regular spawning occurs in each replicate chamber at least twice in the immediate preceding 7 days to test (exposure) initiation and egg production exceeds 15 eggs/female/day/replicate. If this criterion is established, then the spawning group will be suitable for use in the chemical exposure test.

2.4.2 Chemical Exposure

After successful spawning is verified during the pre-exposure period, the spawning phase of the test will be initiated and will be 21 days in duration. During that time, the appearance of the fish, behavior, fecundity and fertilization success will be assessed daily.

2.5 Sampling and Observations

2.5.1 Sampling

Prior to test initiation, water samples will be taken from the exposure system to confirm that the test substance is in equilibrium and the proper functioning of the diluter. The number of samples taken and the number of sampling intervals will be described in the study report. Samples from two replicates of each concentration and control will be taken at the initiation of the test (day 0) and weekly thereafter for determination of test substance concentration. Replicates sampled will be alternated at each sampling interval. A sample of the stock solution used to prepare the test solutions will be sampled and analyzed for test substance concentration if deemed necessary by the Study Director.

Water samples will be taken from a point approximately midway between the surface, bottom and sides of each test vessel and either analyzed immediately after sampling or appropriately preserved and stored until analysis can be performed. At the discretion of the Study Director, archive samples may be removed and stored for potential analysis.

Three quality control (QC) samples will be prepared at each sampling interval and stored and analyzed with the set of study samples. The QC samples will be prepared in dilution water at test substance concentrations similar to the treatment level range. Results of these analyses indicate the accuracy of the analytical method for measuring test substance concentration at each sampling period. The analytical method used to measure test substance concentration in the exposure solutions will be validated at Smithers Viscient at the expected nominal concentration range prior to test initiation (Dix 2016).

2.5.2 Water Quality Parameters

The temperature, dissolved oxygen concentration and pH will be measured in every aquarium on day 0 and in alternating replicates thereafter. The specific conductance, hardness and alkalinity will be measured in one replicate of one test concentration and control on day 0 and weekly thereafter. Measurements will be alternated between treatment levels and replicates each week. Temperature will be monitored continuously in one representative test aquarium of each diluter level (i.e., upper level and lower level) by using a minimum-maximum thermometer. Readings of temperature extremes will be recorded daily. Guidance for acceptable water quality parameters are as follows:

Water Quality Parameters	Acceptable Parameters
Dissolved Oxygen	≥60%
Temperature	25±1 °C
pH	6.5 - 9

2.6 Biological Observations of Spawning Groups

2.6.1 Survival

Daily assessment of survival will be made to provide a basis for interpretation of reproductive output (i.e., number of eggs/female/day). Fish will be examined daily during the test and any external abnormalities noted.

2.6.2 Behavior of Adults

Abnormal behavior (relative to controls), such as hyperventilation, loss of equilibrium, uncoordinated swimming, atypical quiescence and feeding abstinence will be noted during the daily observations. Alterations in behavior, particularly loss of territorial aggressiveness by males, will also be noted. Observations of secondary sex characteristics will also be made daily on all adult fish, including at test termination prior to removal of fish from the test system.

2.6.3 Fecundity

Egg production will be monitored daily. Because fathead minnows spawn within a few hours after the lights are turned on, they will not be disturbed (except for feeding) until late morning. This allows time for spawning and fertilization to be completed and for the eggs to water-harden. The spawning substrates will be removed from the tanks daily and any eggs present will be counted. Once the eggs are removed, the spawning substrate will be returned to the respective tank. Fecundity will be expressed as the number of eggs per female per reproductive test day.

2.6.4 Fertilization Success

After the eggs have been removed from the spawning substrate, they will be visually inspected under appropriate magnification for determination of fertilization success. Infertile eggs are opaque or clear with a white dot where the yolk has precipitated. Viable embryos remain clear (until reaching the eyed stage). Fertilization rate will be expressed as the number of embryos divided by the total number of eggs x 100.

2.6.5 Termination

Food will be withheld for the 24 hours prior to the termination of the test. At the end of the 21-day exposure period, fish will be euthanized with MS-222 (tricaine methane-sulfonate at 100 mg/L buffered with 200 mg $NaHCO_3$/L).

The following order of activities will be performed on each individual fish:

- Blotted dry and weighed (to 0.10 mg)
- Standard length measurement (to 0.01 mm)
- As much blood as possible will be sampled from the caudal artery/vein with a heparinized microhematocrit capillary tube
- In-situ fixation of the gonads, followed by removal and weighing to 0.1 mg. Right and left gonads will be placed in pre-labeled plastic tissue cassette which will be placed into an individual container of Davidson's fixative. The remaining carcass will be placed into an additional container of Davidson's fixative for future tubercle mapping.
- Evaluation of secondary sex characteristics—nuptial tubercles will be counted, mapped, and scored according to methods recommended in OECD (2009) Annex 5A and OPPTS 890.1350 Appendix B (EPA, 2009).

Blood and fish whole body samples will be labeled throughout with a unique identification code. This code will link blood plasma and carcass samples together enabling both sample types from individual fish to be evaluated in context with one another and the sex of that fish (if required).

2.6.6 Preservation of Carcasses

Each fish carcass and gonad will be placed into separate labeled containers of Davidson's fixative. After overnight fixing, the specimens will be rinsed with alcohol and placed into a container of 10% neutral buffered formalin. After evaluation of the applicable study endpoints (i.e. tubercle mapping), preserved carcasses will be destroyed, shipped to the Study Sponsor or shipped to a storage facility of the Study Sponsor's discretion.

2.6.7 Blood Preparation and Vitellogenin Analysis

Plasma will be separated from the blood via centrifugation (approximately 5 minutes at 15,000 x g) and stored with protease inhibitors at -75°C to -85°C until analyzed for VTG and sex steroids, as appropriate. Plasma volume will be recorded for each fish. Each sample will be normalized (for the volume of plasma) by the initial dilution during sample processing during the vitellogenin analysis.

The measurement of vitellogenin levels will be performed using a commercially available enzyme-linked immunoabsorbant assay (ELISA) raised to fathead minnow (*Pimephales promelas*) VTG (homologous ELISA kit) from BioSense Laboratories, Bergen, Norway. The VTG-analysis is based on a sandwich assay utilizing specific binding between antibodies and VTG. The wells of micro-titer plates are coated with a specific capture antibody that binds to the VTG in samples added to the wells. Unbound components are washed out, and a different VTG-specific antibody (detecting antibody) is added. Unbound detecting antibody is washed out, and an enzyme-labeled secondary antibody is added. After a last wash, the enzyme activity is determined by adding a substrate, which is metabolized to a colored product. The enzyme activity (color intensity) measured by a microplate reader is directly proportional to the concentration of VTG in the sample. The assay is calibrated using purified VTG from fathead minnow as standard, which will be provided with the ELISA kit. Any non-detect samples will be recorded as ½ the appropriate limit of quantification (LOQ).

2.6.8 Sex Steroids (Optional Endpoint)

Sex steroid analysis is an option for this assay. If this option is selected in consultation with the Study Sponsor, plasma concentrations of 17β-estradiol and testosterone will be determined using LC/MS/MS methodology optimized for the relatively small sample volumes obtained from the fathead minnow. Plasma priority will be given to 17β-estradiol for females and testosterone in males, then 17β-estradiol for males and testosterone in females. Any non-detect samples will be recorded as ½ the appropriate limit of quantification (LOQ). Details of these analyses will be detailed in the raw data and presented in the report.

2.6.9 Histopathology of Fish Gonads

Histopathology will be conducted on all fish of each treatment and controls. All specimens will be shipped in Nalgene containers filled with 10 % formalin fixative to the histopathology laboratory. Gonads will be removed and fixed at Smithers Viscient prior to shipment for histopathology. Additional preparation required for histology (i.e. impregnation with paraffin and microtomy) will be performed by the histology laboratory. Histopathological procedures and evaluations will be conducted by Experimental Pathology Laboratories (EPL), Sterling, VA, USA according to Wolf (2015).

2.6.10 Performance Criteria

Survival will be ≥ 90% in the controls over the duration of the 21-day spawning period.

Evidence that fish were actively spawning in all replicates prior to initiating the chemical exposure and in the control replicates during the test (e.g., spawning occurred at least every 4 days in each control replicate, or an average of at least 15 eggs/female/day/replicate).

Control fertilization should be ≥ 95%.

Dissolved oxygen will be ≥ 60% of saturation. Water temperature will not differ by more than 1°C between test tanks at any one time during the exposure period and will be maintained within ±1°C of the 25°C temperature. Measured exposure concentration CV will not exceed 20% for all replicates.

3.0 STATISTICS

3.1 Endpoints

The end points to be used for determination of significant effect by statistical evaluation will include the following measured or calculated variables.

(a) Fecundity (#eggs/female/day)
(b) Fertilization success
(c) Nuptial Tubercle Score
(d) Blood plasma vitellogenin (VTG) concentration
(e) GSI (Gonadal Somatic Index)
(f) Weight
(i) Survival

3.2 Statistical Methods

General guidance on statistical methods is outlined below and is based on OECD, 2006.

The intent of the assay is to conduct the exposure at concentrations which do not elicit a significant response in survival. If necessary, survival will be evaluated with the Cochran-Armitage test if data are considered to be monotonic. If monotonicity is not observed, Fisher's Exact test with a Bonferroni-Holm adjustment will be considered the appropriate statistical test.

For all continuous quantitative endpoints (fecundity, VTG, GSI, fertilization, weight) consistent with a monotonic dose-response, the Jonckheere-Terpstra (on medians) test will be used to evaluate the result. For continuous endpoints that are not monotonic, the data should be assessed for normality using the Shapiro-Wilks or Anderson-Daring test and variance homogeneity, preferably using the Levene test. If the data fails these tests a normalizing, variance stabilizing transformation should be used. If the data passes these qualifiers or can pass after transformation, it will be assessed using Dunnett's test (on replicate means). If the data passes the normality test but fails the homogeneity qualifier, it will be assessed using Tamhane-Dunnett or T3 test or the Mann-Whitney-Wilcoxon U test. If no normalizing transformation can be found, it will be assessed using Mann-Whitney-Wilcoxon U test using a Bonferroni-Holm adjustment to the p-values.

The treatment effect for tubercle score is determined using the multi-quantal Jonckheere test, or the Jonckheere-Terpstra test applied to the replicate medians. The appropriate unit for statistical analysis is the replicate. Statistical tests will be evaluated at the 0.05 significance level.

4.0 RECORDS TO BE MAINTAINED

Records to be maintained will include, but are not limited to correspondence and other documents relating to the interpretation and evaluation of data as well as all raw data and documentation generated as a result of the study. This also includes the completion of the FSTRA data entry spreadsheet template, which is a Statement of Work requirement.

5.0 REPORTING

The raw data generated at Smithers Viscient will be peer-reviewed and the final report will be reviewed by the Study Director. All values will be reported to various levels of significance depending on the accuracy of the measuring devices employed during any one process. The Quality Assurance Unit will inspect the final report to confirm that the methods, procedures, and observations are accurately and completely described, that the reported results accurately and completely reflect the raw data generated at Smithers Viscient and to confirm adherence with the study protocol. All participating facilities (e.g. Battelle and EPL) have active Quality Assurance units which will partake in the data and report generation process, as appropriate. A single copy of the draft report will be submitted to the Sponsor Representative for review. The report will be finalized according to Standard Operating Procedures. The final report will meet the formatting

requirements of EPA's PR Notice 2011-3. All reports will include, but will not be limited to, the following information:

- The study number from Smithers Viscient and Sponsor Study number (if any).
- Laboratory and site, dates of testing and personnel involved in the study, i.e., Program Coordinator (if applicable), Study Director and Principal Investigator.
- Identification of the test substance which may include chemical name, additional designations (e.g., trade name), chemical designation (CAS number), empirical formula, molecular structure, manufacturer, lot or batch number, water solubility, vapor pressure, degree of purity of test substance (percent test chemical) (Sponsor supplied, if available).
- Characterization and origin of the dilution water.
- Scientific name of the test organisms, source, percent mortality of the adult fish population 7 days prior to testing, culturing information (pretreatment, if any), and water quality.
- Exposure system description, dilution water volume and characteristics, number of adults per treatment, and test conditions.
- Detailed information on feeding, including amount given, food source, lot #, and analysis for relevant contaminants.
- Tabular presentation of all measured and calculated endpoints.
- Description of stock solution preparation.
- Ranges of water quality variables during the test.
- Results of analytical measurements of test solutions, and a detailed description of the analytical procedure(s) used, provided as an appendix.
- Evidence that controls met the validity criterion, and incidence of any unusual observations.
- The statistical evaluation of relevant endpoints and the procedures used to establish these values will be reported.
- Good Laboratory Practice (GLP) compliance statement signed by the Study Director.
- Date(s) of Quality Assurance reviews, and dates reported to the Study Director and management, signed by the Quality Assurance Unit.
- Location of the protocol, raw data and final report.
- GLP phase report for the histopathology component of the study.

6.0 PROTOCOL CHANGES

All amendments to the approved protocol must be documented in writing and signed by both the Study Director and Study Sponsor or Representative. All deviations must be documented by the Study Director. Protocol amendments and deviations will include the reasons for the change and the impact of the change on results of the study, if any.

7.0 GOOD LABORATORY PRACTICES

All test procedures, documentation, records and reports will comply with the U.S. Environmental Protection Agency's Good Laboratory Practices as set forth under the Federal Insecticide, Fungicide and Rodenticide Act (40 CFR, Part 160) and as accepted by OECD Principles of Good Laboratory Practice. Methods of euthanasia used during this study will be in conformance with the above referenced regulation and are consistent with the EPA Test Guidelines (OPPTS 890.1350).

8.0 REFERENCES

Ankley, G.T., Jensen, K.M., Kahl, M.D., Korte, J.J., Makynen, E.A. 2001. Description and evaluation of a short-term reproduction test with the fathead minnow (*Pimephales promelas*). *Environmental Toxicology & Chemistry,* 20, 1276-1290.

APHA. 2005. Standard Methods for Examination of Water and Wastewater (21st ed.) American Public Health Association, Washington, DC.

ASTM. 1985. Proposed New Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes. ASTM Committee E-47 on Biological Effects and Environmental Fate, Draft No. 10, July, 1986. 91 pp.

ASTM, 2007. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, Pennsylvania.

Battelle. 29 Sep 2015. Quality Assurance Project Plan (QAPP) for the Endocrine Disruptor Screening Program (EDSP) Tier 1 and Tier 2 In Vivo Testing of Selected Chemicals for Potential Endocrine Effects in Non-Mammals. U.S. EPA Contract No. EP-W-11-063, Task Order 14.

Benoit, D.A. 1981. User's Guide for Conducting Chronic Toxicity Tests with Fathead Minnows (*Pimephales promelas*). EPA-600/8-81/011.

Denny, J.S. 1987. Guidelines for the culture of fathead minnows *Pimephales promelas* for the use in toxicity tests. EPA/600/3-87/001.

Dix, M. 2016. 2-Ethylhexyl Paraben – Validation of the Analytical Method for the Determination of a Test Substance in Aqueous Solutions. Smithers Viscient, Wareham, Massachusetts. 13784.6118.

Kahl MD, Russom CL, DeFoe DL, Hammermeister DE. 1999. Saturation units for use in aquatic bioassays. Chemosphere 39:539-551.

Lemke, A. E., W. A. Brungs and B. J. Halligan. 1978. Manual for construction and operation of toxicity-testing proportional diluters. EPA 600/3-78-072.

McKim, J.N. and D.A. Benoit. 1971. Effect of long-term exposure to copper on survival, reproduction and growth of brook trout (*Salvelinus fontinalis*). *J. Fish. Tes. Bd. Can.* 28: 655-662.

Mount, D. I. 1968. Chronic toxicity of copper to fathead minnow (*Pimephales promelas*, Rafinesque). *Water Research.* 2: 215-223.

Mount D.I. & Brungs, W.A. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1:21-9.

OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring. Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997). Environment Directorate Chemicals Group and Management Committee. ENV/MC/CHEM(98)17. OECD Paris. France. 41 pp.

OECD. 2000. Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Environmental Health and Safety Publications, Series on Testing and Assessment No. 23 Organization for Economic Cooperation and Development. Document# ENV/JM/MONO(2000)6. Paris, France. 53 pp.

OECD. 2006. Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application. Environmental Health and safety Publications. Series on testing and Assessment, No. 54. Paris, France.

OECD. 2007. Preliminary Draft Guideline – The Fish Screening Assay for Endocrine Active Substances. As of the OECD EDTA VMG-Eco (23-24 January 2007).

OECD. 2009. Fish Short Term Reproduction Assay. OECD Guideline for the Testing of Chemicals: Test No. 229. Paris, France. 40 pp.

OECD. 2010. Guidance Document on the Diagnosis of Endocrine-Related Histopathology in Fish Gonads. OECD Environment, Health and Safety Publications, Series on Testing and Assessment: No. 123. Paris, France. 114 pp.

Olson, D. 1974. Fathead minnow methods study 1973-74 Parts I and II. Part I. Fathead minnow egg production as a function of the number of females per spawning chamber. Unpublished report. EPA/ERL Duluth. May 31, 1974. Available through Jeffrey Denny at EPA, Duluth.

Rexrode, M. and T.M. Armitage. 1986. Standard Evaluation Procedure; Fish Life-Cycle Toxicity Tests. Hazard Evaluation Division, Office of Pesticide Programs. Washington, D.C. 20460. EPA 540/9-86-137. July, 1986.

Smithers Viscient. Standard Operating Procedure 07.24, Monitoring of Feed, Soil and Source Water Contamination.

Sprague, J. B. 1969. Measurements of pollutant toxicity to fish. I.Bioassay methods for acute toxicity. *Water Research* 3: 793-831.

U.S. EPA. 40 CFR, Part 160. Federal Insecticide, Fungicide, and Rodenticide Act. Good Laboratory Practices Standards; Final Rule. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, D.C.

U.S. EPA. 1973. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. EPA-670/4-73-001. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1987. Guidelines for the culture of fathead minnows (*Pimephales promelas*) for use in toxicity tests. EPA/600/3-87/001.

U.S. EPA. 1996. Office of Chemical Safety and Pollution Prevention. Ecological Effects Test Guideline, OCSPP 850.1500. Fish Life Cycle Toxicity. "Public Draft". EPA 712-C-96-122. April 1996. U.S. Environmental Protection Agency. Washington, D.C.

U.S. EPA. 2002. A Short term Method for Assessing the Reproductive Toxicity of Endocrine Disrupting Chemicals Using the Fathead Minnow (*Pimephales promelas*) EPA/600/R-01/067.

U.S. EPA. 2006. Histopathology guidelines for Fathead Minnow (*Pimephales promelas*) 21-day Reproduction Assay. Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics*, 27: 103-117.

U.S. EPA. 2009. Endocrine Disruptor Screening Program Test Guidelines: OPPTS 890.1350: Fish Short-Term Reproduction Assay, Office of Prevention, Pesticides and Toxic Substances (7101) EPA 740-C-09-007. 93 pp.

U.S. EPA. 6 October 2011. Fish Short-Term Reproduction Assay OSCPP Guideline 890.1350 Standard Evaluation Procedure (SEP). Endocrine Disruptor Screening Program. 22 pp.

Williams, D.A. 1972. A comparison of several dose levels with a zero dose control. *Biometrics*, 28: 519-531.

Wolf, J. 12 Aug 2015. Draft Procedures for EDSP Studies. EPL (Experimental Pathology Laboratories, Inc.) internal document.

SMITHERS VISCIENT

PROTOCOL AMENDMENT

Amendment No.: 1

Protocol Title: Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OCSPP 890.1350 and OECD 229 Guidelines

Study Sponsor: U.S. Environmental Protection Agency

Test Substance: 2-ethylhexyl paraben

Study No.: 13784.6120

Amendment:
Protocol Cover Page:
The following is being added on the protocol cover page under the testing facility:

Iodide Analysis: Galbraith Laboratories, Inc.
2323 Sycamore Dr.
Knoxville, TN 37921-1700

The following is being provided as requested on the protocol cover page:

Test Concentrations: 220, 88, 35, 14 µg/L

Proposed Experimental Dates: Start: 22 September 2016 Termination: 13 October 2016

Section 2.2.9 – Dilution Water
The following paragraph is being added to the end of this section:

In addition to the aforementioned analyses, a sample of the dilution water will be taken at initiation and at test termination and analyzed for iodide content. The analyses will be conducted by Galbraith Laboratories, Inc located in Knoxville, Tennessee. Samples will be stored frozen at Smithers Viscient throughout the exposure and shipped to the testing facility after test termination.

Section 2.4 Biological Methods
The endpoint timeline in this section is being updated to the following:

Smithers Viscient LLC 790 Main Street | Wareham, MA 02571 | p 508.295.2550
www.smithersviscient.com 2900 Quakenbush Road | Snow Camp, NC 27349 | p 336.376.0141

FSTRA Exposure and Endpoint Timeline						
Study Week	1	2	3	4	5	Termination
Exposure Stage Key	Pre-Exposure	Spawning				
Endpoints						
Fecundity	x	x	x	x	x	
Fertilization Success			x	x	x	
Adult Survival			x	x	x	x
GSI						x
Length						x
Nuptial Tubercle Scores						x
Vitellogenin (VTG concentration)						x
Histopathology						X
Study Week	1	2	3	4	5	Data Compilation

This change is being made to include the endpoint of histopathology of the gonads and thyroids of the fish.

Section 2.5.2 – Water Quality Parameters
The protocol states that the specific conductance, hardness and alkalinity will be measured in one replicate of one test concentration and control on day 0 and weekly thereafter.

This is being revised to state that the conductivity, hardness and alkalinity will be measured in one replicate of one test concentration and control on day 0 and weekly thereafter.

This change is being made to have conductivity rather than specific conductance measured.

Section 2.6.9 – Histopathology of Fish Gonads
This entire section is being replaced with the following:

Section 2.6.9 - Histopathology of Fish Gonads and Thyroids

Histopathology will be conducted on all fish of each treatment and controls. All specimens will be shipped in Nalgene containers filled with 10 % formalin fixative to the histopathology laboratory. Gonads and thyroids will be fixed at Smithers Viscient prior to shipment for histopathology. Additional preparation required for histology (i.e. impregnation with paraffin and microtomy) will be performed by the histology laboratory. Histopathological procedures and evaluations will be conducted by Experimental Pathology Laboratories (EPL), Sterling, VA, USA according to Wolf (2015).

This change is being made to include the histopathology of the thyroids.

None of the above changes will have a negative impact on the study.

Approval Signatures:

Joseph Marini
Smithers Viscient Study Director

2 Sept 16
Effective Date

Vincent J. Brown
Study Sponsor Representative

2 SEP 2016
Date

SMITHERS VISCIENT

PROTOCOL AMENDMENT

Amendment No.: 2

Protocol Title: Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OCSPP 890.1350 and OECD 229 Guidelines

Study Sponsor: U.S. Environmental Protection Agency

Test Substance: 2-ethylhexyl paraben

Study No.: 13784.6120

Amendment:

Section 2.6.9 - Histopathology of Fish Gonads and Thyroids

The protocol states histopathology will be conducted on all fish of each treatment and controls. All specimens will be shipped in Nalgene containers filled with 10% formalin fixative to the histopathology laboratory. Gonads and thyroids will be fixed at Smithers Viscient prior to shipment for histopathology. Additional preparation required for histology (i.e., impregnation with paraffin and microtomy) will be performed by the histology laboratory. Histopathological procedures and evaluations will be conducted by Experimental Pathology Laboratories (EPL), Sterling, VA, USA according to Wolf (2015).

This section will be amended to:

Histopathology on fish gonads will be conducted on all fish of each treatment and controls. Histopathology of fish thyroids will be performed on four males and four female fish from each concentration. One male and one female from each of the four replicate tanks will be examined for thyroid histopathology.

All specimens will be shipped in Nalgene containers filled with 10% formalin fixative to the histopathology laboratory. Gonads and thyroids will be fixed at Smithers Viscient prior to shipment for histopathology. Additional preparation required for histology (i.e., impregnation with paraffin and microtomy) will be performed by the histology laboratory. Histopathological procedures and evaluations will be conducted by Experimental Pathology Laboratories (EPL), Sterling, VA, USA according to Wolf (2015).

This change is being made to clarify the fish being sampled for thyroid histopathology.

None of the above changes will have a negative impact on the study.

Approval Signatures:

Joseph Marini
Smithers Viscient Study Director

4 Oct 16
Effective Date

Vincent J. Brown
Study Sponsor Representative

4 OCT 2016
Date

Smithers Viscient LLC
www.smithersviscient.com
790 Main Street | Wareham, MA 02571 | p 508.295.2550
2900 Quakenbush Road | Snow Camp, NC 27349 | p 336.376.0141

SMITHERS VISCIENT

PROTOCOL AMENDMENT

Amendment No.: 3

Protocol Title: Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines

Study Sponsor: U.S. Environmental Protection Agency

Test Substance: 2-ethylhexyl paraben

Study No.: 13784.6120

Amendment:

Section 2.6.9 - Histopathology of Fish Gonads and Thyroids

The protocol states histopathology of fish thyroids will be performed on four males and four female fish from each concentration. One male and one female from each of the four replicate tanks will be examined for thyroid histopathology.

Based on a request in the Addendum to the Statement of Work For Task Order 14 (May, 2016), this is being revised to state histopathology of fish thyroids will follow a step-wise approach, reading, if possible, one male and one female fish from each replicate vessel from the high and control groups only. Then, if positive results are observed in the high or control groups, the pathologist will read one male and one female fish from each replicate of the remaining concentration groups (if possible, a total of four male and four female fish per concentration). All fish that have thyroid histopathology will be selected randomly within a replicate vessel.

Section 2.6.9 - Histopathology of Fish Gonads and Thyroids

The protocol states histopathological procedures and evaluations will be conducted by Experimental Pathology Laboratories (EPL), Sterling, VA, USA according to Wolf (2015).

This is being revised to state that histopathological procedures and evaluations will be conducted by Experimental Pathology Laboratories (EPL), Sterling, VA, USA. Gonad histopathology procedures will be conducted according to Appendix E of the OPPTS 850.1350 guideline (U.S. EPA, 2009). Thyroid histopathology procedures will be conducted according to the OECD Series on Testing and Assessment No. 82 (OECD, 2007).

This change is being made to remove the reference to Wolf (2015).

Section 5.0 – Sample Disposal
The protocol states all study specimens and /or samples collected during the study, and test materials and reference standards etc., provided by the sponsor, client or customer, will either be returned to the originator, shipped to a third party archival facility on behalf of the study sponsor who will incur the costs of shipping and archival, or disposed on according to SMV SOPs.

This section will be amended to:

Per Sponsor, following study finalization, specimens remaining at Smithers Viscient, Wareham, MA, and embedded tissues or specimens maintained by EPL will be disposed of in accordance with QMP, QAPP,

Smithers Viscient LLC
www.smithersviscient.com
790 Main Street | Wareham, MA 02571 | p 508.295.2550
2900 Quakenbush Road | Snow Camp, NC 27349 | p 336.376.0141

6120 3
13784.~~6126~~ amendment #~~1~~ ①
① Page 2 of 2

and respective facility SOPs. All slides produced during the histopathological analyses will be stored at EPL until study finalization. After study finalization, all slides will be shipped to the sponsor at the address below.

Sharlene R. Matten, Ph.D.
Senior Biologist, US EPA
Exposure Assessment Coordination and Policy Division
Office of Science Coordination and Policy
1200 Pennsylvania Ave., N.W., Mail Code 7203M
Washington, D.C. 20460 Tel: 202-564-0130
e-mail: matten.sharlene@epa.gov

This change is being made to provide specific archive location for slides produced by study no. 13784.6120 and disposal instructions following study finalization for specimens remaining at Smithers Viscient and embedded tissues or specimens maintained at EPL.

Section 9.0 – References
The following reference is being removed from this section:

Wolf, J. 12 Aug 2015. Draft Procedures for EDSP Studies. EPL (Experimental Pathology Laboratories, Inc.) internal document.

The following reference is being revised to state the following:

OECD. 2007a. Preliminary Draft Guideline – The Fish Screening Assay for Endocrine Active Substances. As of the OECD EDTA VMG-Eco (23-24 January 2007).

The following reference is being added to this section:

OECD. 2007. OECD Series on Testing and Assessment No. 82: Guidance Document of Amphibian Thyroid Histology. OECD, Paris, France.

None of the above changes will have a negative impact on the study.

Approval Signatures:

Joseph Marini
Smithers Viscient Study Director — 13 Sept 17 Effective Date

Study Sponsor Representative — 13 SEP 2017 Date

① Correction of typographical error for study number and amendment # JM 15Sep17

SMITHERS VISCIENT

PROTOCOL AMENDMENT

Amendment No.: 4

Protocol Title: Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OCSPP 890.1350

Study Sponsor: U.S. Environmental Protection Agency

Test Substance: 2-ethylhexyl paraben

Study No.: 13784.6120

Amendment:
Protocol Title:
The protocol title is being revised to the following:

Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OCSPP 890.1350

Section 1.0 – Objective
The protocol states the following:

This protocol describes the procedures to be used in the performance of a short-term assay with fathead minnow (*Pimephales promelas*) based on the protocol developed by Ankley et al. (2001) and meets the requirements of the OPPTS 890.1350 and OECD 229 guidelines for the fish short-term reproduction assay (FSTRA).

This is being revised to state the following:

This protocol describes the procedures to be used in the performance of a short-term assay with fathead minnow (*Pimephales promelas*) based on the protocol developed by Ankley et al. (2001) and meets the requirements of the OPPTS 890.1350 for the fish short-term reproduction assay (FSTRA).

Section 9.0 – References
The following reference is being removed from this section:

OECD. 2009. Fish Short Term Reproduction Assay. OECD Guideline for the Testing of Chemicals: Test No. 229. Paris, France. 40 pp.

All of the above changes are being made to remove the OECD 229 guideline from the protocol.

None of the above changes will have a negative impact on the study.

Approval Signatures:

Joseph Marini
Smithers Viscient Study Director — 16 Feb 18 (Effective Date)

Study Sponsor Representative — 16 FEB 2018 (Date)

Smithers Viscient LLC
www.smithersviscient.com
790 Main Street | Wareham, MA 02571 | p 508.295.2550
2900 Quakenbush Road | Snow Camp, NC 27349 | p 336.376.0141

SMITHERS VISCIENT

PROTOCOL AMENDMENT

Amendment No.: 5

Protocol Title: Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OCSPP 890.1350 and OECD 229 Guidelines

Study Sponsor: U.S. Environmental Protection Agency

Test Substance: 2-ethylhexyl paraben

Study No.: 13784.6120

Amendment:
Protocol Title:
The protocol title is being revised to the following:

Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OCSPP 890.1350 and OECD 229 Guidelines

Section 1.0 – Objective
The protocol states the following:

This protocol describes the procedures to be used in the performance of a short-term assay with fathead minnow (*Pimephales promelas*) based on the protocol developed by Ankley et al. (2001) and meets the requirements of the OPPTS 890.1350 for the fish short-term reproduction assay (FSTRA).

This is being revised to state the following:

This protocol describes the procedures to be used in the performance of a short-term assay with fathead minnow (*Pimephales promelas*) based on the protocol developed by Ankley et al. (2001) and meets the requirements of the OPPTS 890.1350 and OECD 229 guidelines for the fish short-term reproduction assay (FSTRA).

Section 9.0 – References
The following reference is being added to this section:

OECD. 2009. Fish Short Term Reproduction Assay. OECD Guideline for the Testing of Chemicals: Test No. 229. Paris, France. 40 pp.

All of the above changes are being made to add the OECD 229 guideline to the protocol.

None of the above changes will have a negative impact on the study.

Approval Signatures:

Joseph P. Marini
Smithers Viscient Study Director

2 Mar 18
Effective Date

Study Sponsor Representative

2 MAR 2018
Date

Smithers Viscient LLC
www.smithersviscient.com
790 Main Street | Wareham, MA 02571 | p 508.295.2550
2900 Quakenbush Road | Snow Camp, NC 27349 | p 336.376.0141

SMITHERS VISCIENT

PROTOCOL AMENDMENT

Amendment No.: 6

Protocol Title: Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines

Study Sponsor: U.S. Environmental Protection Agency

Test Substance: 2-ethylhexyl paraben

Study No.: 13784.6120

Amendment:
Protocol Title:
The protocol title is the following:

Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OCSPP 890.1350 and OECD 229 Guidelines

The protocol title is being revised to the following:

Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines

This change is being made to present the guideline followed as OPPTS rather than OCSPP.

None of the above changes will have a negative impact on the study.

Approval Signatures:

Joseph P. Marini
Smithers Viscient Study Director

8 Mar 18
Effective Date

Study Sponsor Representative

8 MAR 2018
Date

Smithers Viscient LLC
www.smithersviscient.com
790 Main Street | Wareham, MA 02571 | p 508.295.2550
2900 Quakenbush Road | Snow Camp, NC 27349 | p 336.376.0141

APPENDIX 2 – FOOD ANALYSIS

Frozen Brine (lot# 092116) approved for use. AB 3100776

ANALYTICAL REPORT

Reported Date: 19-Oct-16

CLIENT:	Smithers Viscient	**Client Sample ID:**	09.16-02 Frozen Brine
Lab Order:	1609129	**Collection Date:**	9/22/2016 9:15:00 AM
Project:	New Lot Analysis	**Date Received:**	9/22/2016
Lab ID:	1609129-002	**Matrix:**	OTHER

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
POLYCHLORINATED BIPHENYLS - SW8082						Analyst: **DM**
Prep Method: (SW3545A)			**Prep Date: 9/27/2016 12:27:57 PM**			
Aroclor 1016	ND	417		µg/Kg-dry	1	9/28/2016
Aroclor 1221	ND	833		µg/Kg-dry	1	9/28/2016
Aroclor 1232	ND	417		µg/Kg-dry	1	9/28/2016
Aroclor 1242	ND	417		µg/Kg-dry	1	9/28/2016
Aroclor 1248	ND	417		µg/Kg-dry	1	9/28/2016
Aroclor 1254	ND	417		µg/Kg-dry	1	9/28/2016
Aroclor 1260	ND	417		µg/Kg-dry	1	9/28/2016
Surr: Decachlorobiphenyl Sig 1	62.0	30-150		%REC	1	9/28/2016
Surr: Decachlorobiphenyl Sig 2	62.0	30-150		%REC	1	9/28/2016
Surr: Tetrachloro-m-Xylene Sig 1	84.0	30-150		%REC	1	9/28/2016
Surr: Tetrachloro-m-Xylene Sig 2	99.0	30-150		%REC	1	9/28/2016
TOTAL METALS BY ICP - SW6010B						Analyst: **QS**
Prep Method: (SW3050B)			**Prep Date: 9/28/2016 2:19:02 PM**			
Aluminum	*2730*	39.3		mg/Kg-dry	1	9/28/2016
Antimony	ND	39.3		mg/Kg-dry	1	9/28/2016
Arsenic	ND	39.3		mg/Kg-dry	1	9/28/2016
Barium	ND	39.3		mg/Kg-dry	1	9/28/2016
Beryllium	ND	11.8		mg/Kg-dry	1	9/28/2016
Boron	*44.5*	39.3		mg/Kg-dry	1	9/28/2016
Cadmium	ND	7.86		mg/Kg-dry	1	9/28/2016
Calcium	*2320*	39.3		mg/Kg-dry	1	9/28/2016
Chromium	ND	39.3		mg/Kg-dry	1	9/28/2016
Cobalt	ND	39.3		mg/Kg-dry	1	9/28/2016
Copper	ND	39.3		mg/Kg-dry	1	9/28/2016
Iron	*3480*	157		mg/Kg-dry	1	9/28/2016
Lead	ND	39.3		mg/Kg-dry	1	9/28/2016
Lithium	ND	39.3		mg/Kg-dry	1	9/28/2016
Magnesium	*3410*	39.3		mg/Kg-dry	1	9/28/2016
Manganese	*136*	39.3		mg/Kg-dry	1	9/28/2016
Molybdenum	ND	39.3		mg/Kg-dry	1	9/28/2016
Nickel	ND	39.3		mg/Kg-dry	1	9/28/2016
Potassium	*10400*	197		mg/Kg-dry	5	9/28/2016
Selenium	ND	39.3		mg/Kg-dry	1	9/28/2016

Qualifiers:			
B	Analyte detected in the associated Method Blank	BRL	Below Reporting Limit
E	Value above quantitation range	H	Holding times for preparation or analysis exceeded
J	Analyte detected below quantitation limits	ND	Not Detected at the Reporting Limit
RL	Reporting Limit	S	Spike Recovery outside recovery limits

GeoLabs, Inc.
45 Johnson Lane ~ Braintree MA 02184 ~ 781 848 7844 ~ 781 848 7811

DF = Dilution Factor

ANALYTICAL REPORT **Reported Date:** 19-Oct-16

CLIENT:	Smithers Viscient	**Client Sample ID:**	09.16-02 Frozen Brine
Lab Order:	1609129	**Collection Date:**	9/22/2016 9:15:00 AM
Project:	New Lot Analysis	**Date Received:**	9/22/2016
Lab ID:	1609129-002	**Matrix:**	OTHER

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
TOTAL METALS BY ICP - SW6010B						Analyst: **QS**
Prep Method: (SW3050B)			**Prep Date:**	**9/28/2016 2:19:02 PM**		
Silver	ND	39.3		mg/Kg-dry	1	9/28/2016
Strontium	ND	39.3		mg/Kg-dry	1	9/28/2016
Vanadium	ND	39.3		mg/Kg-dry	1	9/28/2016
Zinc	*115*	39.3		mg/Kg-dry	1	9/28/2016
MERCURY - SW7471A						Analyst: **EC**
Prep Method: (SW7471A)			**Prep Date:**	**9/28/2016 3:30:03 PM**		
Mercury	ND	0.692		mg/Kg-dry	1	9/28/2016
PESTICIDES - SW8081A						Analyst: **SUB**
Prep Method:			**Prep Date:**	**9/23/2016**		
4,4'-DDD	ND	458		µg/Kg-dry	2	9/29/2016
4,4'-DDE	ND	458		µg/Kg-dry	2	9/29/2016
4,4'-DDT	ND	458		µg/Kg-dry	2	9/29/2016
alpha-BHC	ND	458		µg/Kg-dry	2	9/29/2016
beta-BHC	ND	458		µg/Kg-dry	2	9/29/2016
Chlordane	ND	2250		µg/Kg-dry	2	9/29/2016
delta-BHC	ND	458		µg/Kg-dry	2	9/29/2016
Dieldrin	ND	225		µg/Kg-dry	2	9/29/2016
Endosulfan I	ND	458		µg/Kg-dry	2	9/29/2016
Endosulfan II	ND	458		µg/Kg-dry	2	9/29/2016
Endosulfan Sulfate	ND	458		µg/Kg-dry	2	9/29/2016
Endrin	ND	458		µg/Kg-dry	2	9/29/2016
Endrin Aldehyde	ND	458		µg/Kg-dry	2	9/29/2016
Endrin Ketone	ND	458		µg/Kg-dry	2	9/29/2016
gamma-BHC	ND	91.7		µg/Kg-dry	2	9/29/2016
Heptachlor	ND	458		µg/Kg-dry	2	9/29/2016
Heptachlor Epoxide	ND	458		µg/Kg-dry	2	9/29/2016
Methoxychlor	ND	2250		µg/Kg-dry	2	9/29/2016
Toxaphene	ND	9170		µg/Kg-dry	2	9/29/2016
Surr: Decachlorobiphenyl	62.0	41-142		%REC	2	9/29/2016
Surr: Tetrachloro-m-Xylene	111	42-126		%REC	2	9/29/2016

Qualifiers:

B	Analyte detected in the associated Method Blank	BRL	Below Reporting Limit
E	Value above quantitation range	H	Holding times for preparation or analysis exceeded
J	Analyte detected below quantitation limits	ND	Not Detected at the Reporting Limit
RL	Reporting Limit	S	Spike Recovery outside recovery limits

GeoLabs, Inc.
45 Johnson Lane ~ Braintree MA 02184 ~ 781 848 7844 ~ 781 848 7811

DF = Dilution Factor

ANALYTICAL REPORT

Reported Date: 19-Oct-16

CLIENT:	Smithers Viscient	**Client Sample ID:**	09.16-02 Frozen Brine
Lab Order:	1609129	**Collection Date:**	9/22/2016 9:15:00 AM
Project:	New Lot Analysis	**Date Received:**	9/22/2016
Lab ID:	1609129-002	**Matrix:**	OTHER

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
PESTICIDES - SW8081A						Analyst: **SUB**
Prep Method:			**Prep Date:**	**9/23/2016**		
NOTES: Analyzed by Phoenix Environmental Laboratories MA-CT-007						
ORGANOPHOSPHOROUS PESTICIDES - SW8141						Analyst: **SUB**
Prep Method:			**Prep Date:**	**9/23/2016**		
Alachlor	ND	2250		µg/Kg-dry	1	9/30/2016
Atrazine	ND	2250		µg/Kg-dry	1	9/30/2016
Azinphos Methyl	ND	2250		µg/Kg-dry	1	9/30/2016
Diazinon	ND	2250		µg/Kg-dry	1	9/30/2016
Disulfoton	ND	2250		µg/Kg-dry	1	9/30/2016
Malathion	ND	2250		µg/Kg-dry	1	9/30/2016
Simazine	ND	2250		µg/Kg-dry	1	9/30/2016
NOTES: Analyzed by Phoenix Environmental Laboratories MA-CT-007						

Qualifiers:				
B	Analyte detected in the associated Method Blank	BRL	Below Reporting Limit
E	Value above quantitation range	H	Holding times for preparation or analysis exceeded
J	Analyte detected below quantitation limits	ND	Not Detected at the Reporting Limit
RL	Reporting Limit	S	Spike Recovery outside recovery limits

GeoLabs, Inc.
45 Johnson Lane ~ Braintree MA 02184 ~ 781 848 7844 ~ 781 848 7811

DF = Dilution Factor

Flake fish food (Sera Vipan) lot# 081216 approved for use. AB 14Sept16

ANALYTICAL REPORT

Reported Date: 30-Aug-16

CLIENT:	Smithers Viscient	**Client Sample ID:**	08.16.03 FFF Sera Vipan
Lab Order:	1608097	**Collection Date:**	8/17/2016 11:30:00 AM
Project:	New Lot Analysis	**Date Received:**	8/17/2016
Lab ID:	1608097-001	**Matrix:**	OTHER

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
POLYCHLORINATED BIPHENYLS - SW8082						Analyst: **DM**
Prep Method: (SW3545A)			**Prep Date:**	**8/22/2016 11:54:08 AM**		
Aroclor 1016	ND	52.6		µg/Kg-dry	1	8/27/2016
Aroclor 1221	ND	105		µg/Kg-dry	1	8/27/2016
Aroclor 1232	ND	52.6		µg/Kg-dry	1	8/27/2016
Aroclor 1242	ND	52.6		µg/Kg-dry	1	8/27/2016
Aroclor 1248	ND	52.6		µg/Kg-dry	1	8/27/2016
Aroclor 1254	ND	52.6		µg/Kg-dry	1	8/27/2016
Aroclor 1260	ND	52.6		µg/Kg-dry	1	8/27/2016
Surr: Decachlorobiphenyl Sig 1	155	30-150	S	%REC	1	8/27/2016
Surr: Decachlorobiphenyl Sig 2	168	30-150	S	%REC	1	8/27/2016
Surr: Tetrachloro-m-Xylene Sig 1	157	30-150	S	%REC	1	8/27/2016
Surr: Tetrachloro-m-Xylene Sig 2	183	30-150	S	%REC	1	8/27/2016
TOTAL METALS BY ICP - SW6010B						Analyst: **QS**
Prep Method: (SW3050B)			**Prep Date:**	**8/19/2016 1:17:56 PM**		
Aluminum	***744***	5.09		mg/Kg-dry	1	8/26/2016
Antimony	ND	5.09		mg/Kg-dry	1	8/26/2016
Arsenic	ND	5.09		mg/Kg-dry	1	8/26/2016
Barium	***13.9***	5.09		mg/Kg-dry	1	8/26/2016
Beryllium	ND	1.53		mg/Kg-dry	1	8/26/2016
Boron	***12.6***	5.09		mg/Kg-dry	1	8/26/2016
Cadmium	ND	1.02		mg/Kg-dry	1	8/26/2016
Calcium	***28300***	509		mg/Kg-dry	100	8/26/2016
Chromium	ND	5.09		mg/Kg-dry	1	8/26/2016
Cobalt	ND	5.09		mg/Kg-dry	1	8/26/2016
Copper	***10.9***	5.09		mg/Kg-dry	1	8/26/2016
Iron	***1290***	204		mg/Kg-dry	10	8/26/2016
Lead	ND	5.09		mg/Kg-dry	1	8/26/2016
Lithium	ND	5.09		mg/Kg-dry	1	8/26/2016
Magnesium	***1750***	50.9		mg/Kg-dry	10	8/26/2016
Manganese	***14.0***	5.09		mg/Kg-dry	1	8/26/2016
Molybdenum	ND	5.09		mg/Kg-dry	1	8/26/2016
Nickel	ND	5.09		mg/Kg-dry	1	8/26/2016
Potassium	***12500***	509		mg/Kg-dry	100	8/26/2016
Selenium	ND	5.09		mg/Kg-dry	1	8/26/2016

Qualifiers:

B	Analyte detected in the associated Method Blank	BRL	Below Reporting Limit
E	Value above quantitation range	H	Holding times for preparation or analysis exceeded
J	Analyte detected below quantitation limits	ND	Not Detected at the Reporting Limit
RL	Reporting Limit	S	Spike Recovery outside recovery limits

GeoLabs, Inc.
45 Johnson Lane ~ Braintree MA 02184 ~ 781 848 7844 ~ 781 848 7811

DF = Dilution Factor

ANALYTICAL REPORT **Reported Date:** 30-Aug-16

CLIENT:	Smithers Viscient	**Client Sample ID:**	08.16.03 FFF Sera Vipan
Lab Order:	1608097	**Collection Date:**	8/17/2016 11:30:00 AM
Project:	New Lot Analysis	**Date Received:**	8/17/2016
Lab ID:	1608097-001	**Matrix:**	OTHER

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
TOTAL METALS BY ICP - SW6010B						Analyst: **QS**
Prep Method: (SW3050B)			**Prep Date:**	**8/19/2016 1:17:56 PM**		
Silver	ND	5.09		mg/Kg-dry	1	8/26/2016
Strontium	*134*	50.9		mg/Kg-dry	10	8/26/2016
Vanadium	ND	5.09		mg/Kg-dry	1	8/26/2016
Zinc	*71.9*	5.09		mg/Kg-dry	1	8/26/2016
MERCURY - SW7471A						Analyst: **EC**
Prep Method: (SW7471A)			**Prep Date:**	**8/23/2016 4:33:41 PM**		
Mercury	ND	0.0874		mg/Kg-dry	1	8/23/2016
PESTICIDES - SW8081A						Analyst: **SUB**
Prep Method:			**Prep Date:**	**8/18/2016**		
4,4'-DDD	ND	34.0		µg/Kg-dry	2	8/22/2016
4,4'-DDE	ND	34.0		µg/Kg-dry	2	8/22/2016
4,4'-DDT	ND	34.0		µg/Kg-dry	2	8/22/2016
alpha-BHC	ND	34.0		µg/Kg-dry	2	8/22/2016
beta-BHC	ND	34.0		µg/Kg-dry	2	8/22/2016
Chlordane	ND	170		µg/Kg-dry	2	8/22/2016
delta-BHC	ND	34.0		µg/Kg-dry	2	8/22/2016
Dieldrin	ND	17.0		µg/Kg-dry	2	8/22/2016
Endosulfan I	ND	34.0		µg/Kg-dry	2	8/22/2016
Endosulfan II	ND	34.0		µg/Kg-dry	2	8/22/2016
Endosulfan Sulfate	ND	34.0		µg/Kg-dry	2	8/22/2016
Endrin	ND	34.0		µg/Kg-dry	2	8/22/2016
Endrin Aldehyde	ND	34.0		µg/Kg-dry	2	8/22/2016
Endrin Ketone	ND	34.0		µg/Kg-dry	2	8/22/2016
gamma-BHC	ND	6.80		µg/Kg-dry	2	8/22/2016
Heptachlor	ND	34.0		µg/Kg-dry	2	8/22/2016
Heptachlor Epoxide	ND	34.0		µg/Kg-dry	2	8/22/2016
Methoxychlor	ND	170		µg/Kg-dry	2	8/22/2016
Toxaphene	ND	680		µg/Kg-dry	2	8/22/2016
Surr: Decachlorobiphenyl	62.0	41-142		%REC	2	8/22/2016
Surr: Tetrachloro-m-Xylene	75.0	42-126		%REC	2	8/22/2016

Qualifiers:

B	Analyte detected in the associated Method Blank	BRL	Below Reporting Limit
E	Value above quantitation range	H	Holding times for preparation or analysis exceeded
J	Analyte detected below quantitation limits	ND	Not Detected at the Reporting Limit
RL	Reporting Limit	S	Spike Recovery outside recovery limits

GeoLabs, Inc.
45 Johnson Lane ~ Braintree MA 02184 ~ 781 848 7844 ~ 781 848 7811

DF = Dilution Factor

ANALYTICAL REPORT **Reported Date:** 30-Aug-16

CLIENT:	Smithers Viscient	**Client Sample ID:**	08.16.03 FFF Sera Vipan
Lab Order:	1608097	**Collection Date:**	8/17/2016 11:30:00 AM
Project:	New Lot Analysis	**Date Received:**	8/17/2016
Lab ID:	1608097-001	**Matrix:**	OTHER

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
PESTICIDES - SW8081A						Analyst: **SUB**
Prep Method:			**Prep Date:**	**8/18/2016**		

NOTES:
Analyzed by Phoenix Environmental Laboratories M-CT007

ORGANOPHOSPHOROUS PESTICIDES - SW8141					Analyst: **SUB**
Prep Method:			**Prep Date:**	**8/18/2016**	
Alachlor	ND	1600	µg/Kg-dry	10	8/22/2016
Atrazine	ND	1600	µg/Kg-dry	10	8/22/2016
Azinphos Methyl	ND	1600	µg/Kg-dry	10	8/22/2016
Diazinon	ND	1600	µg/Kg-dry	10	8/22/2016
Disulfoton	ND	1600	µg/Kg-dry	10	8/22/2016
Malathion	ND	1600	µg/Kg-dry	10	8/22/2016
Simazine	ND	1600	µg/Kg-dry	10	8/22/2016

NOTES:
Analyzed by Phoenix Environmental Laboratories M-CT007

Qualifiers:			
B	Analyte detected in the associated Method Blank	BRL	Below Reporting Limit
E	Value above quantitation range	H	Holding times for preparation or analysis exceeded
J	Analyte detected below quantitation limits	ND	Not Detected at the Reporting Limit
RL	Reporting Limit	S	Spike Recovery outside recovery limits

GeoLabs, Inc.
45 Johnson Lane ~ Braintree MA 02184 ~ 781 848 7844 ~ 781 848 7811

DF = Dilution Factor

Flake fish food (Sera Vipan) lot# 081216 approved For use. AB 14Sept16

ANALYTICAL REPORT

Reported Date: 30-Aug-16

CLIENT:	Smithers Viscient	**Client Sample ID:**	08.16.03 FFF Sera Vipan
Lab Order:	1608097	**Collection Date:**	8/17/2016 11:30:00 AM
Project:	New Lot Analysis	**Date Received:**	8/17/2016
Lab ID:	1608097-001	**Matrix:**	OTHER

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
POLYCHLORINATED BIPHENYLS - SW8082						Analyst: **DM**
Prep Method: (SW3545A)		**Prep Date: 8/22/2016 11:54:08 AM**				
Aroclor 1016	ND	52.6		µg/Kg-dry	1	8/27/2016
Aroclor 1221	ND	105		µg/Kg-dry	1	8/27/2016
Aroclor 1232	ND	52.6		µg/Kg-dry	1	8/27/2016
Aroclor 1242	ND	52.6		µg/Kg-dry	1	8/27/2016
Aroclor 1248	ND	52.6		µg/Kg-dry	1	8/27/2016
Aroclor 1254	ND	52.6		µg/Kg-dry	1	8/27/2016
Aroclor 1260	ND	52.6		µg/Kg-dry	1	8/27/2016
Surr: Decachlorobiphenyl Sig 1	155	30-150	S	%REC	1	8/27/2016
Surr: Decachlorobiphenyl Sig 2	168	30-150	S	%REC	1	8/27/2016
Surr: Tetrachloro-m-Xylene Sig 1	157	30-150	S	%REC	1	8/27/2016
Surr: Tetrachloro-m-Xylene Sig 2	183	30-150	S	%REC	1	8/27/2016
TOTAL METALS BY ICP - SW6010B						Analyst: **QS**
Prep Method: (SW3050B)		**Prep Date: 8/19/2016 1:17:56 PM**				
Aluminum	***744***	5.09		mg/Kg-dry	1	8/26/2016
Antimony	ND	5.09		mg/Kg-dry	1	8/26/2016
Arsenic	ND	5.09		mg/Kg-dry	1	8/26/2016
Barium	***13.9***	5.09		mg/Kg-dry	1	8/26/2016
Beryllium	ND	1.53		mg/Kg-dry	1	8/26/2016
Boron	***12.6***	5.09		mg/Kg-dry	1	8/26/2016
Cadmium	ND	1.02		mg/Kg-dry	1	8/26/2016
Calcium	***28300***	509		mg/Kg-dry	100	8/26/2016
Chromium	ND	5.09		mg/Kg-dry	1	8/26/2016
Cobalt	ND	5.09		mg/Kg-dry	1	8/26/2016
Copper	***10.9***	5.09		mg/Kg-dry	1	8/26/2016
Iron	***1290***	204		mg/Kg-dry	10	8/26/2016
Lead	ND	5.09		mg/Kg-dry	1	8/26/2016
Lithium	ND	5.09		mg/Kg-dry	1	8/26/2016
Magnesium	***1750***	50.9		mg/Kg-dry	10	8/26/2016
Manganese	***14.0***	5.09		mg/Kg-dry	1	8/26/2016
Molybdenum	ND	5.09		mg/Kg-dry	1	8/26/2016
Nickel	ND	5.09		mg/Kg-dry	1	8/26/2016
Potassium	***12500***	509		mg/Kg-dry	100	8/26/2016
Selenium	ND	5.09		mg/Kg-dry	1	8/26/2016

Qualifiers:

B	Analyte detected in the associated Method Blank	BRL	Below Reporting Limit
E	Value above quantitation range	H	Holding times for preparation or analysis exceeded
J	Analyte detected below quantitation limits	ND	Not Detected at the Reporting Limit
RL	Reporting Limit	S	Spike Recovery outside recovery limits

GeoLabs, Inc.
45 Johnson Lane ~ Braintree MA 02184 ~ 781 848 7844 ~ 781 848 7811

DF = Dilution Factor

ANALYTICAL REPORT **Reported Date:** 30-Aug-16

CLIENT:	Smithers Viscient	**Client Sample ID:**	08.16.03 FFF Sera Vipan
Lab Order:	1608097	**Collection Date:**	8/17/2016 11:30:00 AM
Project:	New Lot Analysis	**Date Received:**	8/17/2016
Lab ID:	1608097-001	**Matrix:**	OTHER

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
TOTAL METALS BY ICP - SW6010B						Analyst: **QS**
Prep Method: (SW3050B)		**Prep Date: 8/19/2016 1:17:56 PM**				
Silver	ND	5.09		mg/Kg-dry	1	8/26/2016
Strontium	*134*	50.9		mg/Kg-dry	10	8/26/2016
Vanadium	ND	5.09		mg/Kg-dry	1	8/26/2016
Zinc	*71.9*	5.09		mg/Kg-dry	1	8/26/2016
MERCURY - SW7471A						Analyst: **EC**
Prep Method: (SW7471A)		**Prep Date: 8/23/2016 4:33:41 PM**				
Mercury	ND	0.0874		mg/Kg-dry	1	8/23/2016
PESTICIDES - SW8081A						Analyst: **SUB**
Prep Method:		**Prep Date: 8/18/2016**				
4,4'-DDD	ND	34.0		µg/Kg-dry	2	8/22/2016
4,4'-DDE	ND	34.0		µg/Kg-dry	2	8/22/2016
4,4'-DDT	ND	34.0		µg/Kg-dry	2	8/22/2016
alpha-BHC	ND	34.0		µg/Kg-dry	2	8/22/2016
beta-BHC	ND	34.0		µg/Kg-dry	2	8/22/2016
Chlordane	ND	170		µg/Kg-dry	2	8/22/2016
delta-BHC	ND	34.0		µg/Kg-dry	2	8/22/2016
Dieldrin	ND	17.0		µg/Kg-dry	2	8/22/2016
Endosulfan I	ND	34.0		µg/Kg-dry	2	8/22/2016
Endosulfan II	ND	34.0		µg/Kg-dry	2	8/22/2016
Endosulfan Sulfate	ND	34.0		µg/Kg-dry	2	8/22/2016
Endrin	ND	34.0		µg/Kg-dry	2	8/22/2016
Endrin Aldehyde	ND	34.0		µg/Kg-dry	2	8/22/2016
Endrin Ketone	ND	34.0		µg/Kg-dry	2	8/22/2016
gamma-BHC	ND	6.80		µg/Kg-dry	2	8/22/2016
Heptachlor	ND	34.0		µg/Kg-dry	2	8/22/2016
Heptachlor Epoxide	ND	34.0		µg/Kg-dry	2	8/22/2016
Methoxychlor	ND	170		µg/Kg-dry	2	8/22/2016
Toxaphene	ND	680		µg/Kg-dry	2	8/22/2016
Surr: Decachlorobiphenyl	62.0	41-142		%REC	2	8/22/2016
Surr: Tetrachloro-m-Xylene	75.0	42-126		%REC	2	8/22/2016

Qualifiers:

B	Analyte detected in the associated Method Blank	BRL	Below Reporting Limit
E	Value above quantitation range	H	Holding times for preparation or analysis exceeded
J	Analyte detected below quantitation limits	ND	Not Detected at the Reporting Limit
RL	Reporting Limit	S	Spike Recovery outside recovery limits

GeoLabs, Inc.
45 Johnson Lane ~ Braintree MA 02184 ~ 781 848 7844 ~ 781 848 7811

DF = Dilution Factor

ANALYTICAL REPORT **Reported Date:** 30-Aug-16

CLIENT:	Smithers Viscient	**Client Sample ID:**	08.16.03 FFF Sera Vipan
Lab Order:	1608097	**Collection Date:**	8/17/2016 11:30:00 AM
Project:	New Lot Analysis	**Date Received:**	8/17/2016
Lab ID:	1608097-001	**Matrix:**	OTHER

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
PESTICIDES - SW8081A						Analyst: **SUB**
Prep Method:			**Prep Date:**	**8/18/2016**		
NOTES: Analyzed by Phoenix Environmental Laboratories M-CT007						
ORGANOPHOSPHOROUS PESTICIDES - SW8141						Analyst: **SUB**
Prep Method:			**Prep Date:**	**8/18/2016**		
Alachlor	ND	1600		µg/Kg-dry	10	8/22/2016
Atrazine	ND	1600		µg/Kg-dry	10	8/22/2016
Azinphos Methyl	ND	1600		µg/Kg-dry	10	8/22/2016
Diazinon	ND	1600		µg/Kg-dry	10	8/22/2016
Disulfoton	ND	1600		µg/Kg-dry	10	8/22/2016
Malathion	ND	1600		µg/Kg-dry	10	8/22/2016
Simazine	ND	1600		µg/Kg-dry	10	8/22/2016
NOTES: Analyzed by Phoenix Environmental Laboratories M-CT007						

Qualifiers:				
	B	Analyte detected in the associated Method Blank	BRL	Below Reporting Limit
	E	Value above quantitation range	H	Holding times for preparation or analysis exceeded
	J	Analyte detected below quantitation limits	ND	Not Detected at the Reporting Limit
	RL	Reporting Limit	S	Spike Recovery outside recovery limits

GeoLabs, Inc.
45 Johnson Lane ~ Braintree MA 02184 ~ 781 848 7844 ~ 781 848 7811

DF = Dilution Factor

APPENDIX 3 – PRELIMINARY EXPOSURE AND STUDY DATA

Prior to initiating the definitive exposure, a 96-hour range-finding acute exposure was conducted at Smithers Viscient exposing fathead minnow under flow-through conditions to nominal 2-ethylhexyl paraben concentrations of 0.062, 0.19, 0.56, 1.7, and 5.0 mg/L, a negative control and solvent (triethylene glycol) control. These levels were chosen based on available medaka toxicity data provided by the Study Sponsor. Two replicate aquaria containing a total of six fathead minnows (two males, four females) each were established for each treatment level and control. Following 96 hours of exposure, cumulative percent mortality of 92 and 100% was observed among fathead minnows exposed to the 1.7 and 5.0 mg/L nominal treatment levels, respectively. No mortality or sub-lethal effects were observed for all remaining treatment levels (0.062, 0.19, and 0.56 mg/L) or controls. A non-linear regression analysis was performed to calculate an LC_{50} concentration which was determined to be approximately 1.1 mg/L. Applicable test guidelines recommend a high concentration of 1/3 of the LC_{50} value for definitive tests (U.S. EPA, 2009). However, based on the steep dose response in the range-finder test and lethal/sub-lethal responses at 1/3 of the LC_{50} value in other definitive studies with this test design, a high concentration of 1/5 of the 2-ethylhexyl paraben LC_{50} value (220 µg/L) was chosen for the definitive exposure. Based on these results and in consultation with the Study Sponsor, nominal concentrations of 14, 35, 88, and 220 µg/L and a control were selected for the definitive exposure.

Study #:13784.6120

Summary of mortality and sublethal observations of *P. promelas* to 2-ethyl paraben over 96 hours under flow-through conditions

Nominal Concentration (mg/L)	Replicate	Number of fish added	Cumulative Mortality and SubLethal Effects							
			24 Hour		48 Hour		72 Hour		96 Hour	
			Mortality	Sublethal[1]	Mortality	Sublethal[1]	Mortality	Sublethal[1]	Mortality	Sublethal[1]
Control	A	6	0	0	0	0	0	0	0	0
	B	6	0	0	0	0	0	0	0	0
Solvent Control	A	6	0	0	0	0	0	0	0	0
	B	6	0	0	0	0	0	0	0	0
0.062	A	6	0	0	0	0	0	0	0	0
	B	6	0	0	0	0	0	0	0	0
0.19	A	6	0	0	0	0	0	0	0	0
	B	6	0	0	0	0	0	0	0	0
0.56	A	6	0	0	0	0	0	0	0	0
	B	6	0	0	0	0	0	0	0	0
1.7	A	6	3	3	5	1	5	1	5	1
	B	6	6	0	6	0	6	0	6	0
5.0	A	6	6	0	6	0	6	0	6	0
	B	6	6	0	6	0	6	0	6	0

L = lethargic; **PL** = partial loss of equilibrium; **CL** = complete loss of equilibrium; **B** = at bottom of test vessel; **S** = at solution's surface; **D** = dark; **-** = none

1 = sublethal effects were lethargy and fish on bottom of test vessel.

Table 2. Summary of percent mortality of of *P. promelas* exposed to 2-ethyl paraben over 96 hours under flowthrough conditions

Nominal Concentration (mg/L)	Total Number of fish added	Cumulative Percent Mortality			
		24 Hour	48 Hour	72 Hour	96 Hour
Control	12	0%	0%	0%	0%
Solvent Control	12	0%	0%	0%	0%
0.062	12	0%	0%	0%	0%
0.19	12	0%	0%	0%	0%
0.56	12	0%	0%	0%	0%
1.7	12	75%	92%	92%	92%
5.0	12	100%	100%	100%	100%

Based on nominal concentrations:					
NOEC:	**0.56 mg/L**				
LC50:	**1.1 mg/L**				
lowest concentration producing 100% mortality:				**5.0 mg/L**	

APPENDIX 4 – ANALYTICAL METHODOLOGY

SUMMARY

Methodology was validated (17 March 2016) to quantify the amount of 2-ethyl paraben present in recovery samples prepared in freshwater. Recovery samples were initially diluted with acetonitrile to a concentration of 20/80 acetonitrile/freshwater (v/v). The mid- and high-level recovery samples were further diluted into the calibration standard range with 20/80 acetonitrile/freshwater (v/v). All recovery samples were analyzed by liquid chromatography with mass spectrometry detection (LC-MS/MS).

The method was validated by fortification of freshwater with 2-ethyl paraben at concentrations of 0.200, 125, and 10,000 µg/L. Recoveries averaged 111 ± 6.98%, with a limit of quantitation (LOQ) of 0.135 µg/L. Defined limits for acceptance of quality control sample performance in subsequent studies were set at 80 to 120%.

EXPERIMENTAL

Equipment

1. Instrument:	AB MDS Sciex 4000 mass spectrometer equipped with an AB MDS Sciex ESI Turbo V source Shimadzu LC-20AD binary pumps Shimadzu 20AD/DGU-20A3 vacuum degasser Shimadzu 20AD/DGU-20A5R vacuum degasser Shimadzu 20AD/SIL-20ACHT autosampler Shimadzu CTO-20AC column oven Shimadzu CBM-20A Communications bus Analyst version 1.4.2 software for data acquisition
2. Balance:	Mettler Toledo XS205
3. Laboratory equipment:	Volumetric flasks, graduated cylinders, disposable glass pipets, positive displacement pipets, autosampler vials with split caps, and amber glass bottles with Teflon®-lined caps

Reagents

1. Purified reagent water:	prepared from a Millipore MilliQ® Direct 8 water purification system (meets ASTM Type II requirements)
2. Acetonitrile:	EMD, reagent grade
3. Methanol:	EMD, reagent grade
4. Dimethyl sulfoxide:	Fisher, reagent grade

5. 0.1% Formic Acid in Water: Fisher, reagent grade
6. 0.1% Formic Acid in Acetonitrile: Fisher, reagent grade

Test Substance

The test substance, 2-ethyl paraben, was received on 20 October 2015 from Tokyo Chemical Industry America, Montgomeryville, Pennsylvania. The following information was provided:

Name:	2-ethyl paraben
Synonyms:	2-ethylhexyl paraben, 2-ethylhexyl 4-hydroxybenzoate
Lot No.:	VXGDC
CAS No.:	5153-25-3
Purity:	99.8%
Expiration Date:	Not Listed

Upon receipt at Smithers Viscient, the test substance (SMV No. 7928) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance.

PROCEDURES

Liquid Reagent and Mobile Phase Preparation

A 20/80 acetonitrile/freshwater (v/v) liquid reagent solution was typically prepared by combining 40 mL of acetonitrile and 160 mL of freshwater. The solution was mixed well using a stir bar and stir plate for five minutes.

A 30/30/40 acetonitrile/methanol/dimethyl sulfoxide (v/v/v) liquid reagent autosampler wash solution was typically prepared by combining 1500 mL of acetonitrile with 1500 mL of methanol and 2000 mL of dimethyl sulfoxide. The solution was mixed well prior to use.

Preparation of Stock Solutions

Primary stock solutions were typically prepared as described in the table below:

Primary Stock ID	Amount Weighed (g), Net Weight	Amount Weighed (g), as Active Ingredient	Stock Solvent	Final Volume (mL)	Primary Stock Concentration (mg/L)	Primary Stock Use
7928C	0.0501	0.0500	Acetonitrile	50.0	1000	Secondary stock solutions
7928D	0.0503	0.0502		50.0	1000	High-level recovery samples and secondary stock solutions

Secondary stock solutions were typically prepared as described in the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (mg/L)	Stock Use
7928C	1000	5.00	50.0	Acetonitrile	7928C-2	100	Sub-stock solution
7928D	1000	5.00	50.0		7928D-2	100	Mid-level recovery samples and sub-stock solution

Sub-stock solutions were typically prepared as described in the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (µg/L)	Stock Use
7928C-2	100	0.0500	50.0	Acetonitrile	Ana Stk 1	100	Calibration standards
7928D-2	100	0.0500	50.0		Tech Stk 1	100	Low-level recovery samples

All primary and secondary stock solutions were stored refrigerated (2 to 8 ºC) in amber glass bottles fitted with Teflon®-lined caps. Sub-stock solutions were prepared fresh on the day of use and discarded after use.

Preparation of Calibration Standards

Calibration standards were prepared in 20/80 acetonitrile/freshwater (v/v) by fortifying with the 100 µg/L sub-stock solution to yield concentrations of 0.100, 0.250, 0.500, 1.00, 1.50, and 2.00 µg/L.

Sample Fortification and Preparation

Preparation of Recovery Samples:

Recovery samples were prepared by fortifying freshwater with the test substance to obtain concentrations of 0.200, 125, and 10,000 µg/L. Each concentration level was produced in triplicate. In addition, three samples were left unfortified to serve as controls and were diluted in the same fashion as the low-level recovery samples. The preparation procedure is outlined in the table below.

Sample ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)	Fortified Concentration (µg/L)
Control A, B & C	NA[a]	NA	16.0	0.00
Low A, B & C	0.100	0.0320	16.0	0.200
Mid A, B & C	100	0.0200	16.0	125
High A, B & C	1000	0.160	16.0	10,000

[a] NA = Not Applicable

Dilution of Samples:

To minimize the potential for losses of the test substance during processing, the aqueous test samples were not sub-sampled prior to dilution. The first dilution with acetonitrile was performed by the addition of acetonitrile to the entire volume of the aqueous sample in the container in which it was fortified to a final composition of 20/80 acetonitrile/freshwater (v/v). The mid- and high-level recovery samples were subsequently diluted into the calibration standard range with 20/80 acetonitrile/freshwater (v/v). The dilution procedure is outlined in the table below.

Sample ID	Fortified Concentration (µg/L)	Sample Volume (mL)	Final Volume[a] (mL)	Sample Volume (mL)	Final Volume[b] (mL)	Sample Volume (mL)	Final Volume[b] (mL)	Dilution Factor
Control A, B & C	0.00	16.0	20.0	NA[c]	NA	NA	NA	1.25
Low A, B & C	0.200	16.0	20.0	NA	NA	NA	NA	1.25
Mid A, B & C	125	16.0	20.0	0.125	10.0	NA	NA	100
High A, B & C	10,000	16.0	20.0	0.300	10.0	0.0500	10.0	8330

[a] Diluted with 100% acetonitrile.
[b] Diluted with 20/80 acetonitrile/freshwater (v/v).
[c] NA = Not Applicable

ANALYSIS

Instrumental Conditions

The LC-MS/MS analysis was conducted using the following instrumental conditions:

LC parameters:

Column: XBridge C18, 2.5 µm, 2.1 mm × 50 mm
Mobile Phase 1: 0.1% formic acid in water
Mobile Phase 2: 0.1% formic acid in acetonitrile
Gradient:

Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)
0.01	0.350	60.0	40.0
0.50	0.350	60.0	40.0
4.00	0.350	0.0	100
5.00	0.350	0.0	100
5.10	0.350	60.0	40.0
6.10	0.350	60.0	40.0

Run time: 6.10 minutes
Injector Rinse solvent: 30/30/40 acetonitrile/methanol/dimethyl sulfoxide
Column temperature: 40˚C
Sample temperature: 5˚C
Injection volume: 100 µL
Retention Time: Approximately 3.5 minutes

MS parameters:

Instrument: AB MDS Sciex 4000 mass spectrometer
Ionization Mode: Positive (+) ESI
Ion Spray Voltage: 5000 V
Scan type: MRM
Q1/Q3 Masses: 251.30/139.20 amu
Dwell Time: 250 milliseconds

Source Temperature:	500ºC
Curtain Gas:	20.00
Ion Source – Gas 1 / Gas 2:	30.0 / 80.0
Collision Gas:	8.00
Collision Energy:	9.00
Collision Cell Entrance Potential:	4.00
Collision Cell Exit Potential:	11.00
Declustering Potential:	35.00

Preparation of Standard Curve

Two sets of calibration standards were analyzed with each recovery sample set; one set prior to analysis of the recovery samples and the second set immediately following the analysis of the recovery samples. Injection of recovery samples and calibration standards onto the chromatographic system was performed by programmed automated injection.

CALCULATIONS

A calibration curve was constructed by plotting the analyte concentration (µg/L) in the calibration standards against the peak area of the calibration standards. The equation of the line (equation 1) was algebraically manipulated to give equation 2. The concentration of the test substance within each recovery sample was determined using the regression coefficients from the quadratic equation, the peak area of the recovery sample, and the dilution factor. Equations 2 and 3 were then used to calculate measured concentrations and analytical results.

$$(1) \qquad y = ax^2 + bx + c$$

$$(2) \qquad DC(x) = \frac{-b + \sqrt{b^2 - 4aC}}{2a}$$

$$(3) \qquad A = DC \text{ x } DF$$

where:

y	=	detector response (peak area) for analyte
a, b, and c	=	regression constants
DC (x)	=	detected concentration (µg/L) in the sample

C = constant c minus the peak area; C = (c - y)
DF = dilution factor (the final sample volume divided by the original sample volume)
A = concentration of the analyte in the original sample

The limit of quantitation (LOQ) was calculated using the following equation:

$$(4)\ LOQ_{INST} = \frac{-b + \sqrt{b^2 - 4aC}}{2a}$$

$$(5)\ LOQ = LOQ_{INST} \times DF_{CTRL}$$

where:

$Area_{LS}$ = mean detector response (peak area) of the low concentration calibration standard (two injections)
a, b, c = regression constants
C = regression constant; C = (c - $Area_{LS}$)
LOQ_{INST} = limit of quantitation on the instrument
DF_{CTRL} = dilution factor of the control samples (smallest dilution factor used)
LOQ = limit of quantitation reported for the analysis

RESULTS AND DISCUSSION

The mean recovery was 111% with a standard deviation of 6.98%. The limit of quantitation was 0.135 µg/L. The LOQ will vary from one analysis interval to another, since it is dependent upon the regression of the calibration standards, the peak area of the low standards, and dilution factor of the controls. These parameters, while relatively constant, do vary somewhat among runs and produce small variations in the LOQ. Defined limits for acceptance of quality control sample performance in subsequent studies were set at 80 to 120%.

Analytical results for the recovery of 2-ethyl paraben from freshwater are presented in Table 1A. Representative chromatograms from the analysis of a calibration standard, recovery sample, and a control sample are presented in Figure 1A through Figure 3A, respectively. A typical regression analysis for 2-ethyl paraben is presented in Figure 4A.

Table 1A. Analytical results for the recovery of 2-ethyl paraben in freshwater during the method validation.

Sample ID	Fortified Concentration (µg/L)	Dilution Factor		Analytical Result (µg/L)	Percent of Fortified (%)
Control A	0.00	1.25		< 0.135[a]	NA[b]
Control B	0.00	1.25		< 0.135	NA
Control C	0.00	1.25		< 0.135	NA
Low A	0.200	1.25		0.231	116
Low B	0.200	1.25		0.238	119
Low C	0.200	1.25		0.232	116
			Mean:	**0.234**	**117**
			SD:	**0.00400**	**2.00**
			% CV:	**1.71**	**1.71**
Mid A	125	100		126	101
Mid B	125	100		127	102
Mid C	125	100		137	110
			Mean:	**130**	**104**
			SD:	**6.12**	**4.90**
			% CV:	**4.71**	**4.71**
High A	10,000	8330		11,800	118
High B	10,000	8330		11,000	110
High C	10,000	8330		10,600	106
			Mean:	**11,100**	**111**
			SD:	**627**	**6.27**
			% CV:	**5.63**	**5.63**
				Overall Mean:	**111**
				Overall SD:	**6.98**
				% CV:	**6.30**
				N:	**9**

[a] Concentrations expressed as less than values were below the limit of quantitation (LOQ). The LOQ for each analysis is dependent upon the regression, the area of the low standards and the dilution factor of the controls. The limit of quantitation for the method validation was 0.135 µg/L.

[b] NA = Not Applicable

NOTE: Results were calculated using the actual analytical (unrounded) results and not the rounded values presented in this table.

Figure 1A. Representative chromatogram of a 1.50 µg/L calibration standard during the method validation with 2-ethyl paraben.

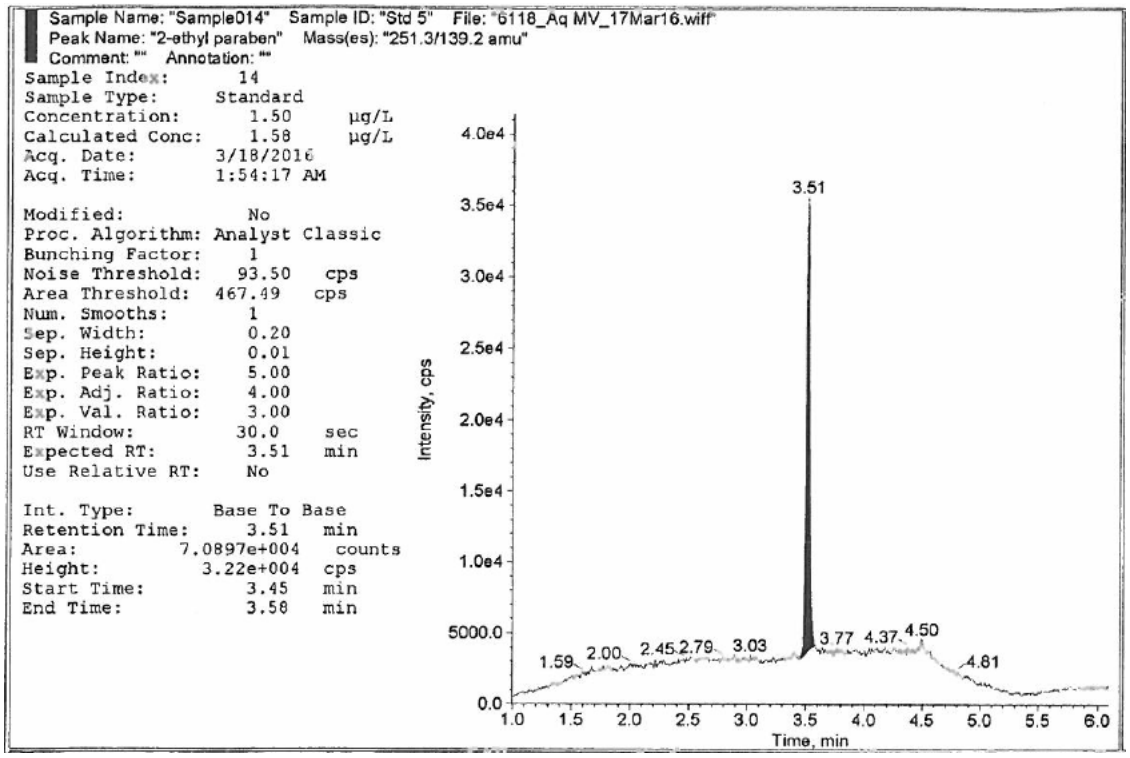


Figure 2A. Representative chromatogram of 125 µg/L recovery sample during the method validation with 2-ethyl paraben.

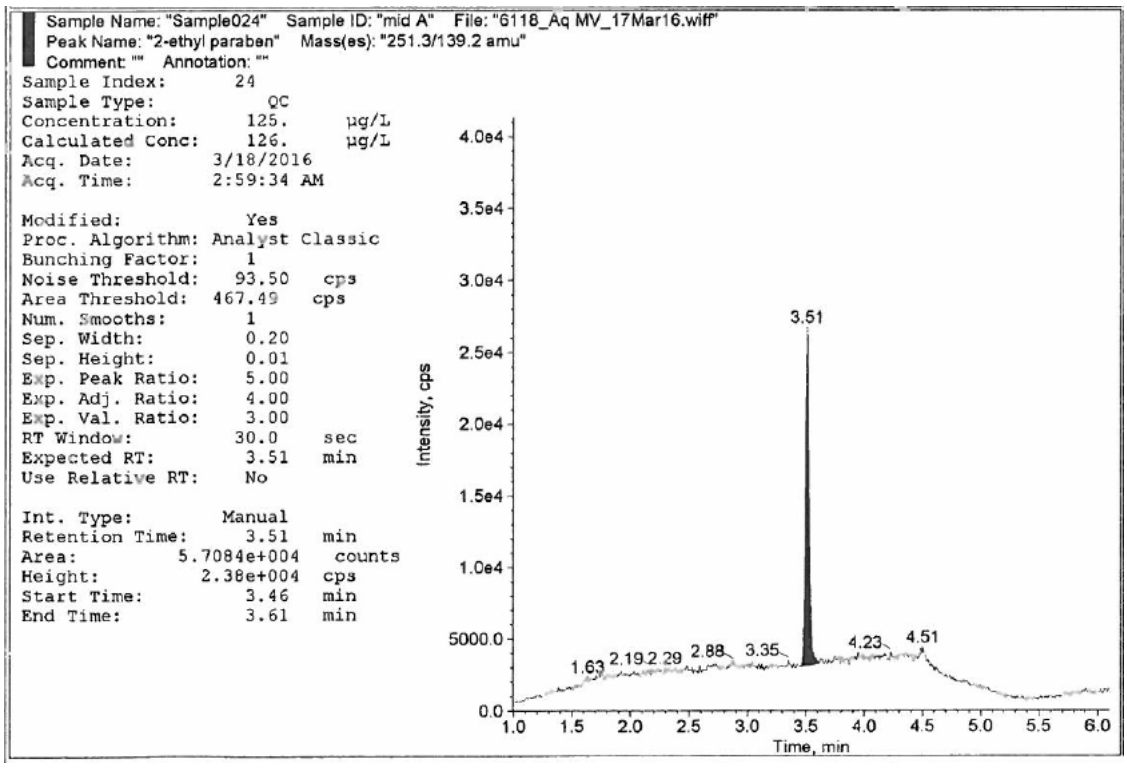
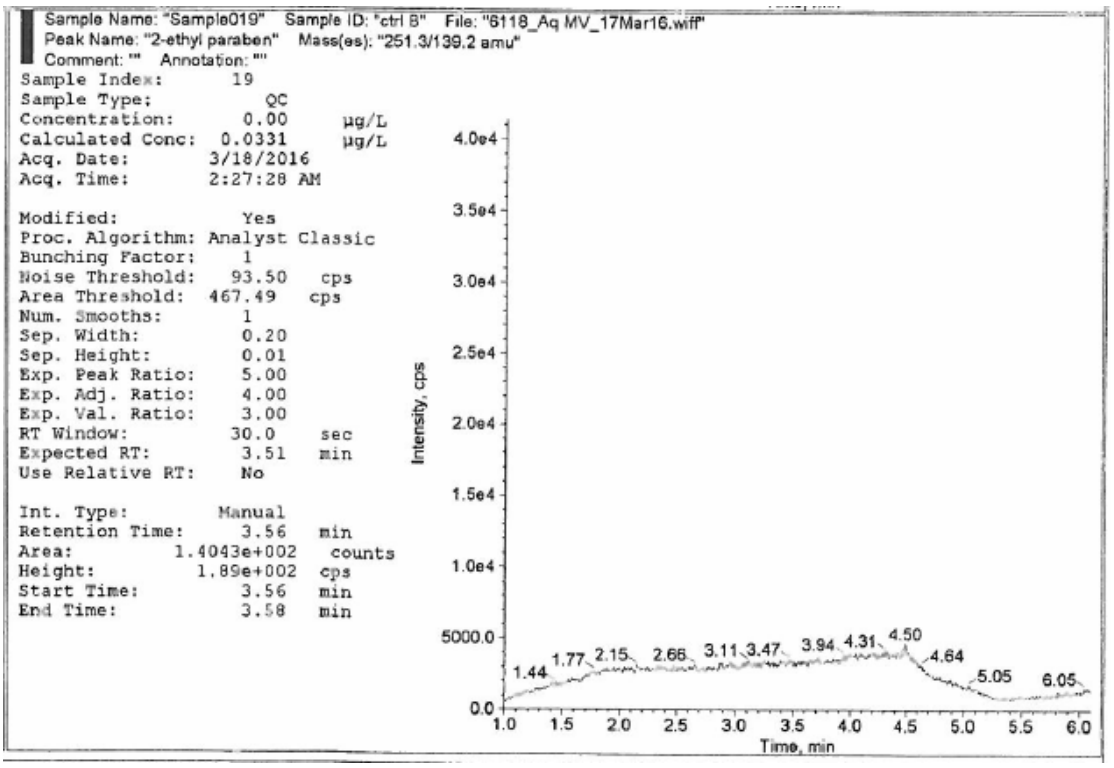
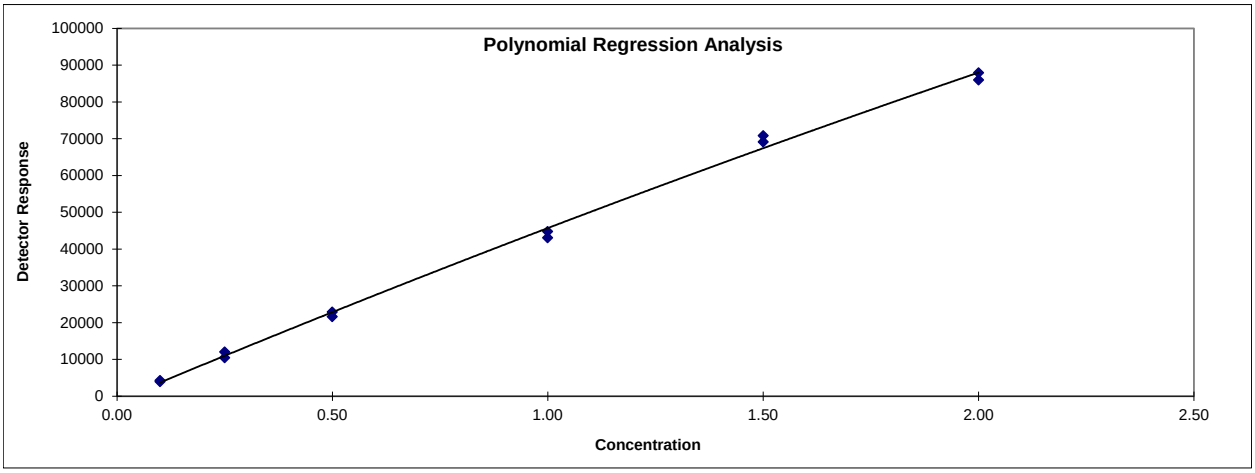


Figure 3A. Representative chromatogram of a control sample during the method validation with 2-ethyl paraben.



NOTE: There is no peak present at the retention time of 2-ethyl paraben. 2-Ethyl paraben is ordinarily detected at a retention time of approximately 3.5 minutes.

Figure 4A. A typical regression analysis for the calibration standards used to quantitate 2-ethyl paraben in the recovery samples.



Polynomial Regression Analysis
$R^2 = 0.99732$
$y = -2292.5x^2 + 49{,}167x + 1161.3$

Standard Concentration µg/L	Standard Response Area
0.100	3995.0
0.250	12087
0.500	21639
1.00	44832
1.50	70897
2.00	86013
0.100	4281.0
0.250	10464
0.500	22935
1.00	43130
1.50	69167
2.00	87992

APPENDIX 5 – 2-ETHYL PARABEN TESTING SUMMARY

ANALYTICAL SUMMARY

This appendix is a summary of the exact analytical procedures used during this testing. The procedures detailed here are those that are used for a specific interval (day 14 for this summary) of this study and are considered representative of those that were used for all intervals during the testing. These procedures are based on the methodology determined during the method validation (Appendix 4).

PROCEDURES

Preparation of Liquid Reagents and Mobile Phases

The volumes of reagents and mobile phases are representative of those prepared during testing but may not reflect the exact quantities at each interval. Volumes may be changed; however the proportions must remain the same.

A 20/80 acetonitrile/laboratory well water (v/v) liquid reagent solution was typically prepared by combining 100 mL of acetonitrile and 400 mL of laboratory well water. The solution was mixed well using a stir bar and stir plate for five minutes.

A 30/30/40 acetonitrile/methanol/purified reagent water (v/v/v) autosampler needle wash solution was typically prepared by combining 1500 mL of acetonitrile, 1500 mL of methanol, and 2000 mL of purified reagent water.

Preparation of Stock Solutions

Primary stock solutions were typically prepared as described in the table below:

Primary Stock ID	Amount Weighed (g), Net Weight	Amount Weighed (g), as Active Ingredient	Stock Solvent	Final Volume (mL)	Primary Stock Concentration (mg/L)	Primary Stock Use
7928AC	0.0503	0.0502	Acetonitrile	50.0	1000	Secondary stock solutions
7928AB	0.0503	0.0502	Acetonitrile	50.0	1000	Secondary stock solutions

Secondary stock solutions were typically prepared as described in the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (mg/L)	Stock Use
7928AC	1000	0.500	50.0	Acetonitrile	7928AC-1	10.0	Quality control samples
7928AC	1000	5.00	50.0	Acetonitrile	7928AC-2	100	Sub-stock solutions
7928AB	1000	5.00	50.0	Acetonitrile	7928AB-2	100	Sub-stock solutions

Sub-stock solutions were prepared as per the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (mg/L)	Stock Use
7928AC-2	100	0.100	10.0	Acetonitrile	Tech Stk 1	1.00	Quality control samples
7928AB-2	100	0.0500	50.0	Acetonitrile	Ana Stk 1	0.100	Calibration standards

All primary and secondary stock solutions were stored refrigerated (2 to 8 ºC) in amber glass bottles fitted with Teflon-lined caps. Sub-stock solutions were prepared fresh daily and discarded after use.

Preparation of Calibration Standards

Calibration standards were prepared in 20/80 acetonitrile/laboratory well water (v/v) by fortifying with the 100 µg/L primary stock solution to yield concentrations of 0.100, 0.250, 0.500, 1.00, 1.50, 2.00 µg/L.

QC Sample Preparation

The QC preparation listed below is representative of those prepared during testing but may not reflect the exact dosing volume and stock concentration at each interval. These may be changed; however, the proportions must remain the same.

The QC samples were prepared by fortifying laboratory well water with 2-ehtylhexyl paraben to obtain concentrations of 6.25, 37.5, and 225 µg/L. The preparation procedure is outlined in the table below.

Sample ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)	Fortified Concentration (µg/L)
QC 1	1.00	0.100	16.0	6.25
QC 2	10.0	0.0600	16.0	37.5
QC 3	10.0	0.360	16.0	225

Exposure Solution and QC Sample Processing

To minimize the potential for losses of the test substance during processing, the aqueous test samples were taken from the exposure solutions and directly transferred to disposable glass vials containing 100% acetonitrile (to a final composition of 20/80 acetonitrile/laboratory well water, v/v) according to the table below. QC samples were diluted in a similar fashion as the test samples by the addition of an exact volume of 100% acetonitrile to the aqueous QC samples. Samples were subsequently diluted into the calibration standard range as needed with 20/80 acetonitrile/laboratory well water (v/v) prior to analysis. The dilution procedure is outlined in the table below:

Nominal Concentration (mg/L)	Sample Volume (mL)	Final Volume (mL)[a]	Sample Volume (mL)	Final Volume (mL)[b]	Sample Volume (mL)	Final Volume (mL)[b]	Dilution Factor
0.00	16.0	20.0	1.00	10.0	NA[c]	NA	12.5
14	16.0	20.0	1.00	10.0	NA	NA	12.5
35	16.0	20.0	0.400	10.0	NA	NA	31.3
88	16.0	20.0	0.160	10.0	NA	NA	78.1
220	16.0	20.0	0.0650	10.0	NA	NA	192
0.00	16.0	20.0	1.00	10.0	NA	NA	12.5
14	16.0	20.0	1.00	10.0	NA	NA	12.5
35	16.0	20.0	0.400	10.0	NA	NA	31.3
88	16.0	20.0	0.160	10.0	NA	NA	78.1
220	16.0	20.0	0.0650	10.0	NA	NA	192
6000	16.0	20.0	0.150	10.0	0.175	10.0	4760
6.25	16.0	20.0	1.00	10.0	NA	NA	12.5
37.5	16.0	20.0	0.400	10.0	NA	NA	31.3
225	16.0	20.0	0.0650	10.0	NA	NA	192

[a] Diluted with acetonitrile
[b] Diluted with 20/80 acetonitrile/laboratory well water (v/v)
[c] NA = Not Applicable

ANALYSIS
Instrumental Conditions

LC parameters:

Column: Waters XBridge C18, 2.5 µm, 2.1 mm × 50 mm
Mobile Phase A: 0.1% formic acid in water
Mobile Phase B: 0.1% formic acid in acetonitrile
Gradient:

Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)
0.01	0.350	60.0	40.0
0.50	0.350	60.0	40.0
4.00	0.350	0.0	100
5.00	0.350	0.0	100
5.10	0.350	60.0	40.0
6.10	0.350	60.0	40.0

Run time: 6.1 minutes
Injector Rinse solvent: 30/30/40 acetonitrile/methanol/purified reagent water (v/v/v)
Column temperature: 40 °C
Sample temperature: 5 °C
Injection volume: 100 µL
Retention Time: Approximately 3.5 minutes

MS parameters:

Instrument:	Sciex API 4000 mass spectrometer
Ionization Mode:	Negative (-) ESI
Scan type:	MRM

	Primary Transition
Q1/Q3 Masses:	251.3/139.2 amu

APPENDIX 6 – TESTING SUMMARY – SEX STEROID ANALYSIS

ANALYTICAL SUMMARY

This appendix is a summary of the analytical procedures used during this testing for the sex steroid analysis. The procedures detailed here are those that are used for a specific interval of this study and are considered representative of those that were used for all intervals during the testing. These procedures follow the methodology determined during the method validation (DeVellis, 2017).

PROCEDURES

Preparation of Liquid Reagents and Mobile Phases

The volumes of reagents and mobile phases are representative of those prepared during testing but may not reflect the exact quantities at each interval. Volumes may be changed; however the proportions must remain the same.

A 10 mM sodium carbonate in purified reagent water solution was prepared by dissolving 0.00529 g of sodium carbonate in 50.0 mL of purified reagent water. An aliquot of 100 µL of 0.1 N sodium hydroxide was added to the solution to ensure that it was sufficiently basic. The solution was then mixed thoroughly using a vortex mixer.

A dansyl chloride solution was prepared by dissolving 0.0598 g of dansyl chloride in 20.0 mL of acetonitrile. The solution was then mixed thoroughly using a vortex mixer.

A 30/30/40 acetonitrile/methanol/purified reagent water (v/v/v) autosampler wash solution was prepared by combining 1500 mL of acetonitrile, 1500 mL of methanol, and 2000 mL of purified reagent water. The solution was mixed well before use.

Test Substances and Internal Standards

Test Substances

The test substance, testosterone, was received on 16 May 2016 from Sigma-Aldrich, St. Louis, Missouri. The following information was provided:

Name:	testosterone
Batch No.:	SLBJ9044V
CAS No.:	58-22-0
Purity:	100%
Retest Date:	March 2017

Upon receipt at Smithers Viscient, the test substance (SMV No. 8266) was stored at room temperature in the original container in a locked safe. Concentrations were not adjusted for the purity of the test substance. This sample was used to prepare calibration standards and recovery samples during the sex steroid analysis.

The test substance, estradiol, was received on 18 May 2016 from Sigma-Aldrich Inc., Allentown, Pennsylvania. The following information was provided:

Name:	estradiol
Batch No.:	SLBP6339V
CAS No.:	50-28-2
Purity:	99.2%
Retest Date:	October 2018

Upon receipt at Smithers Viscient, the test substance (SMV No. 8283) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the test substance. This sample was used to prepare calibration standards and recovery samples during the sex steroid analysis.

Determination of stability and characterization, verification of the test substance identity, maintenance of records on the test substances, and archival of a sample of the test substances are the responsibility of the Study Sponsor.

Internal Standards

The internal standard, testosterone-d_3, was received on 16 May 2016 from Sigma-Aldrich, St. Louis, Missouri. The following information was provided:

Name:	testosterone-d_3
Batch No.:	103M4039V
CAS No.:	77546-39-5
Purity:	100%
Retest Date:	June 2017

Upon receipt at Smithers Viscient, the internal standard (SMV No. 8267) was stored at room temperature in the original container in a locked safe. Concentrations were not adjusted for the purity of the internal standard.

The internal standard, estradiol-d_3, was received on 25 May 2016 from Sigma-Aldrich, Belfonte, Pennsylvania. The following information was provided:

Name:	estradiol-d3
Lot No.:	LC20990V
CAS No.:	79037-37-9
Purity:	99%
Expiration Date:	May 2019

Upon receipt at Smithers Viscient, the internal standard (SMV No. 8292) was stored at room temperature in a dark, ventilated cabinet in the original container. Concentrations were adjusted for the purity of the internal standard.

Preparation of Stock Solutions

Primary stock solutions were typically prepared as described in the table below:

Primary Stock ID	Amount Weighed (g), Net Weight	Amount Weighed (g), as Active Ingredient	Stock Solvent	Final Volume (mL)	Primary Stock Concentration (mg/L)	Primary Stock Use
Test Substance						
8266D	0.0502	0.0502	Acetonitrile	50.0	1000	Secondary Stock Solution
8283D	0.0252	0.0250	Acetonitrile	25.0	1000	Secondary Stock Solution
Internal Standard						
8267A	0.0100	0.0100	Acetonitrile	10.0	1000	Secondary Stock Solution
8292A	0.01010	0.01000	Acetonitrile	10.0	1000	Secondary Stock Solution

Secondary stock solutions were typically prepared as described in the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (mg/L)	Stock Use
Test Substance							
8266D	1000	0.500	50.0	Acetonitrile	8266D-1	10.0	Sub-stock solution
8283D	1000	0.500	50.0	Acetonitrile	8283D-1	10.0	Sub-stock solution
Internal Standard							
8267A	1000	0.500	50.0	Acetonitrile	8267A-1	10.0	Sub-stock solution
8292A	1000	0.500	50.0	Acetonitrile	8292A-1	10.0	Sub-stock solution

Sub-stock solutions were typically prepared as described in the table below:

Fortifying Stock ID	Fortifying Stock Concentration (mg/L)	Volume of Fortification (mL)	Final Volume (mL)	Stock Solvent	Stock ID	Stock Concentration (mg/L)	Stock Use
Test Substance							
8266D-1	10.0	1.00	10.0	Acetonitrile	Mixed Stk 1	1.00	Sub-stock solution, calibration standards, and QC samples
8283D-1	10.0	1.00					
Mixed Stk 1	1.00	0.100	10.0	Acetonitrile	Mixed Stk 2	0.0100	Calibration standards and QC samples
Internal Standard							
8267A-1	10.0	0.0100	100	Acetonitrile	IS Stk	0.00100	Diluent for recovery samples and calibration standards during derivatization
8292A-1	10.0	0.0100					

All primary and secondary stock solutions were stored refrigerated (2 to 8 °C) in amber glass bottles fitted with Teflon-lined caps. Sub-stock solutions were prepared fresh daily and discarded after use.

Preparation of Calibration Standards

Calibration standards were prepared in charcoal:dextran stripped fetal bovine serum by fortifying with the 10.0 µg/L sub-stock solution to yield concentrations of 0.100, 0.125, 0.200, 0.350, 0.500, 0.750, 1.00, and 2.50 µg/L, and by fortifying with the 1000 µg/L sub-stock solution to yield concentrations of 5.00, 10.0, 20.0, and 50.0 µg/L.

QC Sample Preparation

The QC samples were prepared in charcoal:dextran stripped fetal bovine serum at concentrations of 0.150, 3.00, and 12.5 µg/L for testosterone and at concentrations of 0.300, 3.00, and 12.5 µg/L for estradiol. The preparation procedure is outlined in the table below.

Sample ID	Stock Concentration (mg/L)	Fortification Volume (mL)	Final Volume (mL)	Fortified Concentration (µg/L)
QC T1[a]	0.0100	0.0150	1.00	0.150
QC E1[b]	0.0100	0.0300	1.00	0.300
QC 2[c]	0.0100	0.300	1.00	3.00
QC 3[c]	1.00	0.0125	1.00	12.5

[a] For quantitation of testosterone.
[b] For quantitation of estradiol.
[c] For quantitation of both testosterone and estradiol.

Derivatization of Samples

The calibration standards, plasma samples from the exposure, and QC samples were loaded into individual micro-centrifuge tubes. The reagents listed in the table below were added sequentially from left to right as they appear on the table. The sodium carbonate solution was added last, which marked the beginning of derivatization reaction. The solutions were heated at 60 °C for approximately 20 minutes. Following heating, the solutions were allowed to cool to room temperature. The entire contents of each centrifuge tube was transferred to a 350-µL, 0.2-µm Bio-inert 96-well filter plate with an attached 340-µL polypropylene V-shaped 96-well microtiter plate. The filter plate and the 96-well microtiter plate was centrifuged at 4400 rpm for 20 minutes in order to filter the derivitized samples prior to transfer to the mass spectrometer for analysis. After centrifugation, the filter plate was discarded, and the 96-well microtiter plate containing the test samples was sealed with a silicone/PTFE preslit injectable cap mat. Reagents were added to each micro-centrifuge tube as outlined in the table below.

Sample ID	Nominal Concentration (µg/L)	Dansyl Chloride (mL)	Aprotinin (mL)	IS Stk (mL)	Sample Volume (mL)	Na_2CO_3 (mL)
Reagent Water Blank	0.00	NA[a]	NA	NA	0.200	NA
Std 1	0.100	0.150	NA	0.0600	0.0300	0.0600
Std 2	0.125	0.150	NA	0.0600	0.0300	0.0600
Std 3	0.200	0.150	NA	0.0600	0.0300	0.0600
Std 4	0.350	0.150	NA	0.0600	0.0300	0.0600
Std 5	0.500	0.150	NA	0.0600	0.0300	0.0600
Std 6	0.750	0.150	NA	0.0600	0.0300	0.0600
Std 7	1.00	0.150	NA	0.0600	0.0300	0.0600
Std 8	2.50	0.150	NA	0.0600	0.0300	0.0600
Std 9	5.00	0.150	NA	0.0600	0.0300	0.0600
Std 10	10.0	0.150	NA	0.0600	0.0300	0.0600
Std 11	20.0	0.150	NA	0.0600	0.0300	0.0600
Std 12	50.0	0.150	NA	0.0600	0.0300	0.0600
QC T1	0.150	0.150	0.0300	0.0600	0.0300	0.0600
QC E1	0.300	0.150	0.0300	0.0600	0.0300	0.0600
QC 2	3.00	0.150	0.0300	0.0600	0.0300	0.0600
QC 3	12.5	0.150	0.0300	0.0600	0.0300	0.0600
Samples[b]	Unknown	0.0500	NA	0.0200	Entire Sample	0.0200

[a] NA = Not Applicable

[b] All plasma samples from the exposure were processed in the same manner as detailed in the table above.

ANALYSIS

The exact instrumentation and injection volume listed below are representative of the analytical instrument used during testing (e.g., MDS Sciex API 5000 mass spectrometer, etc.) but may not reflect the exact instrument and injection volume at each interval. All other instrumental conditions listed below remained consistent over the duration of the exposure.

Instrumental Conditions

LC parameters:

Column: Agilent Poroshell 120 EC-C8, 2.7 µm, 3.0 × 50 mm

Mobile Phase A: 0.1% formic acid in water

Mobile Phase B: 0.1% formic acid in acetonitrile

Gradient:

Time (min.)	Flow rate (mL/min.)	Solvent A (%)	Solvent B (%)
0.01	0.650	80.0	20.0
1.00	0.650	80.0	20.0
5.50	0.650	0.00	100
8.00	0.650	0.00	100
8.50	0.650	80.0	20.0

Run time: 10 minutes

Autosampler Wash Solvent: 30/30/40 acetonitrile/methanol/purified reagent water (v/v/v)

Column temperature: 30 °C

Sample temperature: 5 °C

Injection volume: 45 µL

Retention Times: approximately 3.5 minutes (testosterone)
approximately 5.1 minutes (estradiol)

MS parameters:

Instrument: MDS Sciex API 5000 mass spectrometer

Ionization Mode: Positive (+) ESI

Scan type: MRM

Test Substance:	**testosterone**	**testosterone-d_3**	**estradiol**	**estradiol-d_3**
Q1/Q3 Masses (amu):	289.50/97.00	292.50/97.20	506.20/171.30	509.20/171.00

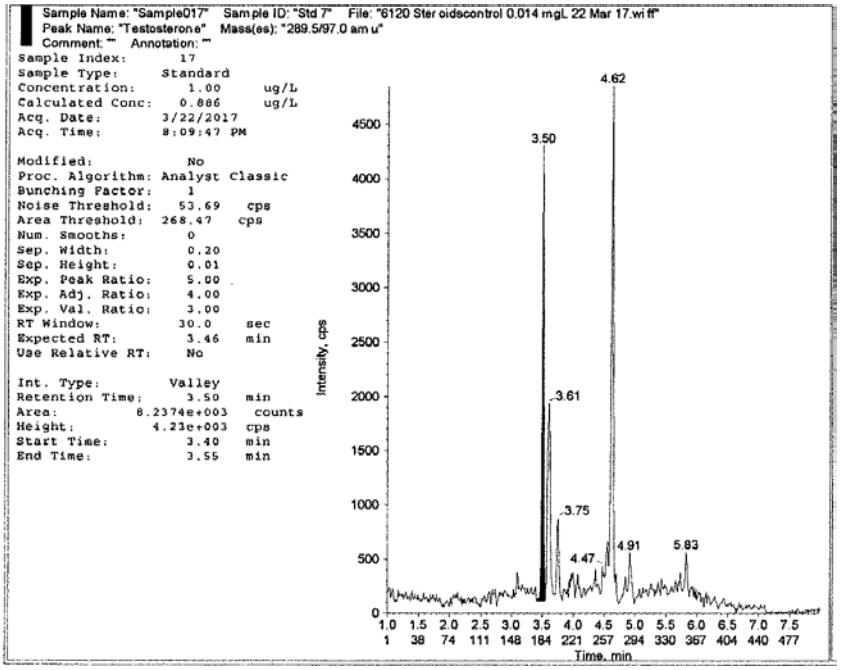
Representative Raw Data

Representative chromatograms from the analysis of a calibration standard, a control sample, and an exposure sample can be found in the following figures. It should be noted that the peak area and the retention time are the only raw data that are retrieved from the chromatograms. Some of the titles included in the chromatogram are specific to the software and may not have the same definition as those in the study. The unique sample identification serves to identify the sample type.

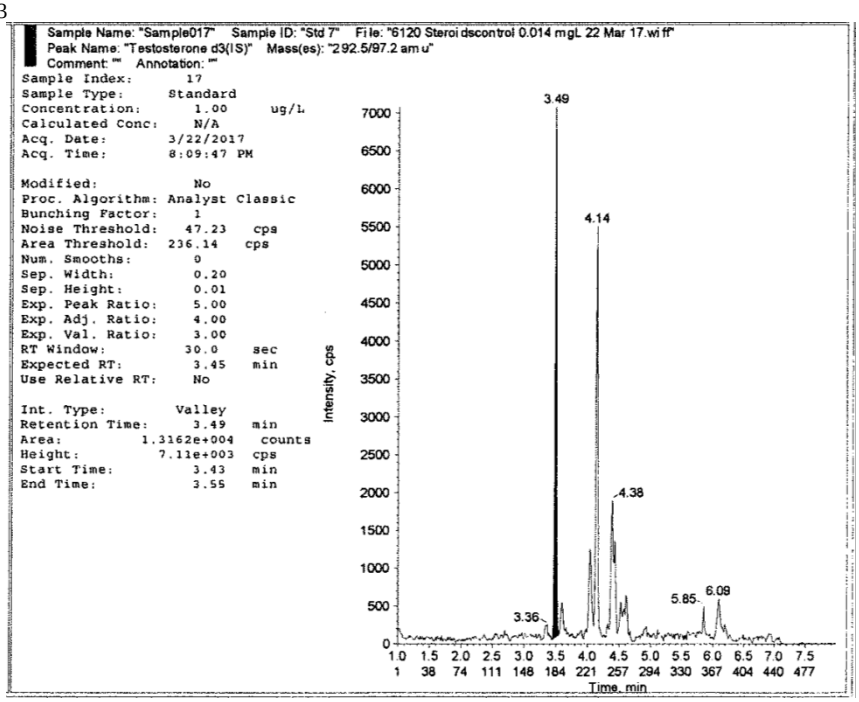
Due to the complex matrix, an internal standard was required for this analysis. The ratio of the analyte peak area and the deuterated internal standard was plotted in the calibration curve and used to quantify the exposure samples. Using this procedure, any impact to the analyte of interest (e.g., degradation, metabolism, complexing, catabolism, analyte suppression/enhancement, chelating, etc.) would be reflected in the performance of the internal standard. In some cases, the peak of the internal standard is not as prominent as would be expected, likely due to suppression by the matrix. Due to the use of the ratio of the analyte peak to the peak of the internal standard, this does not impact the quantification of the analyte of interest.

Calibration standard, 1.00 µg/L

Testosterone

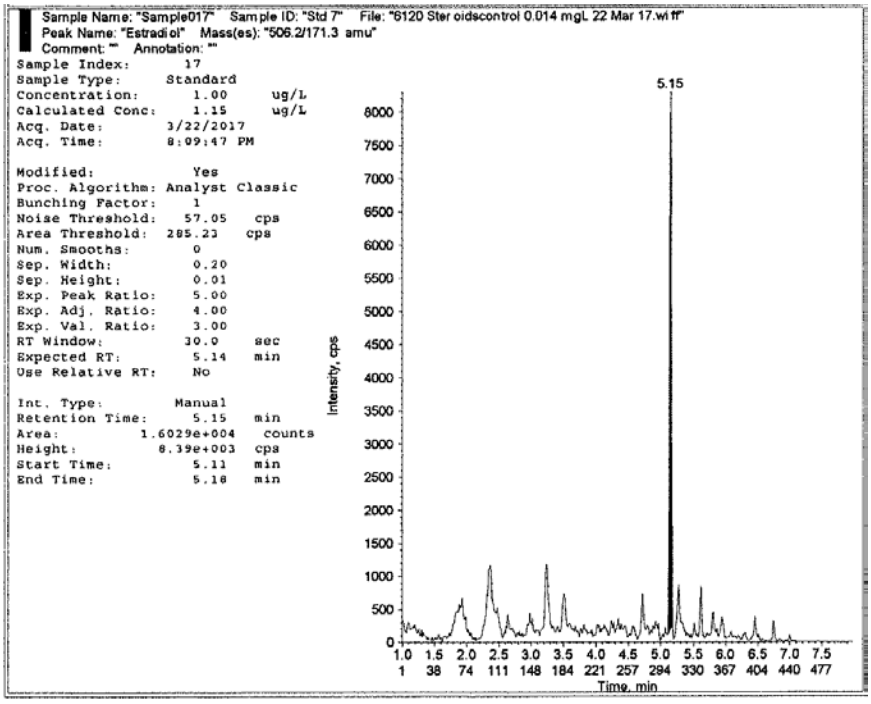


Testosterone-d_3

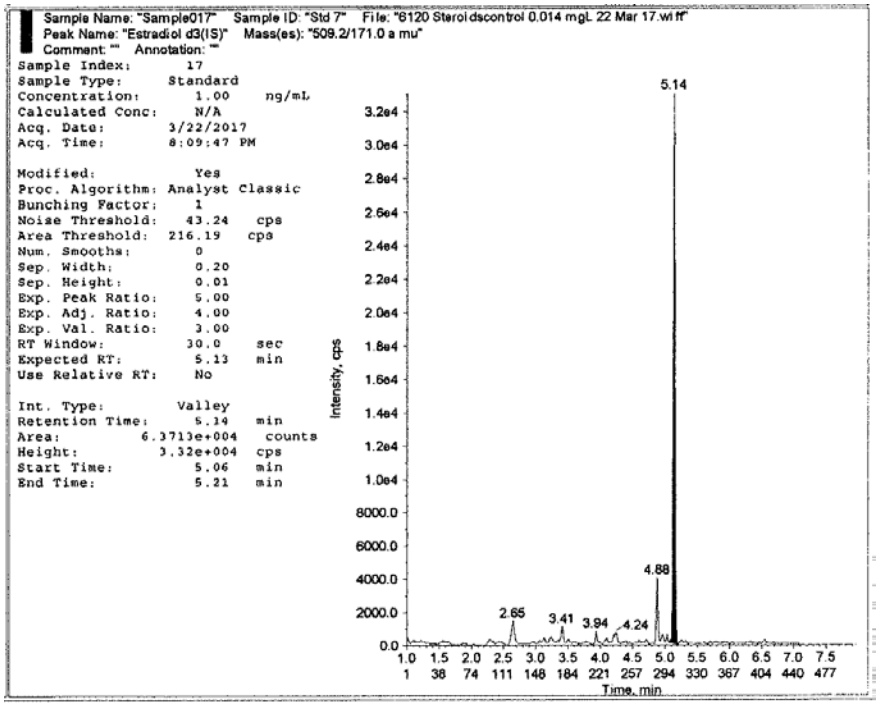


Calibration standard, 1.00 µg/L

Estradiol

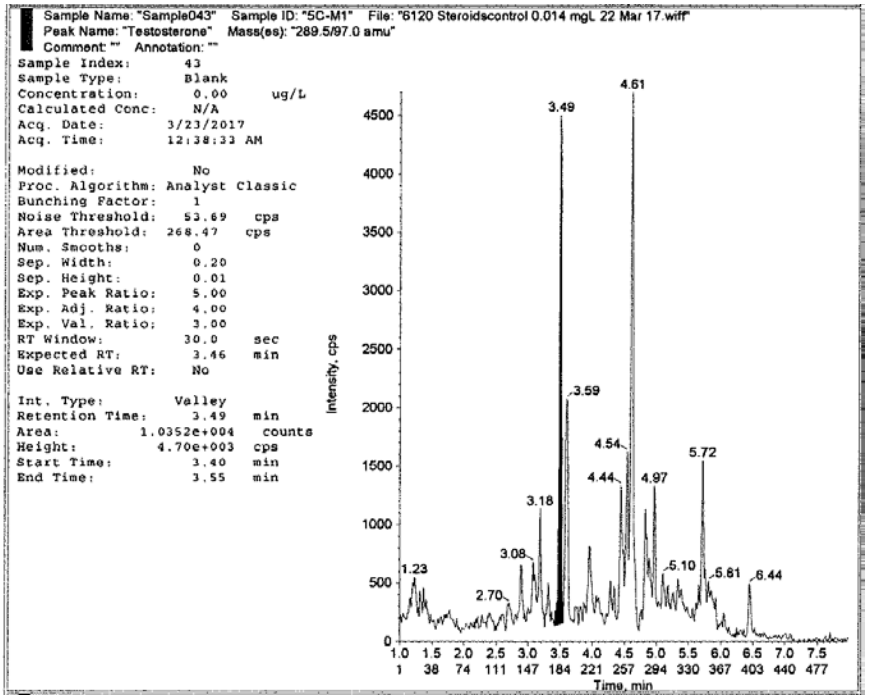


Estradiol-d_3

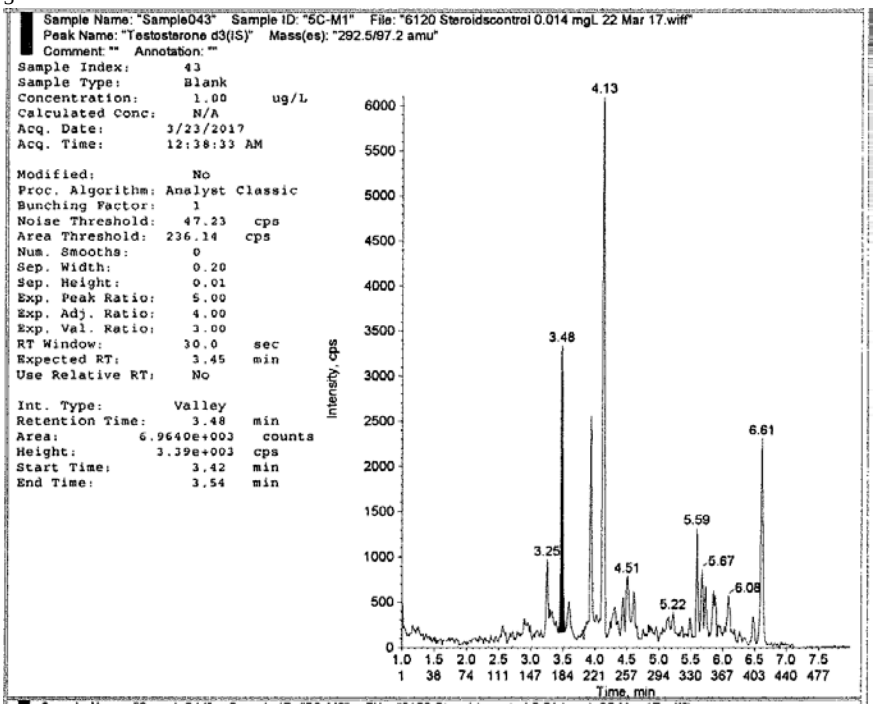


Control exposure sample (male fish)

Testosterone

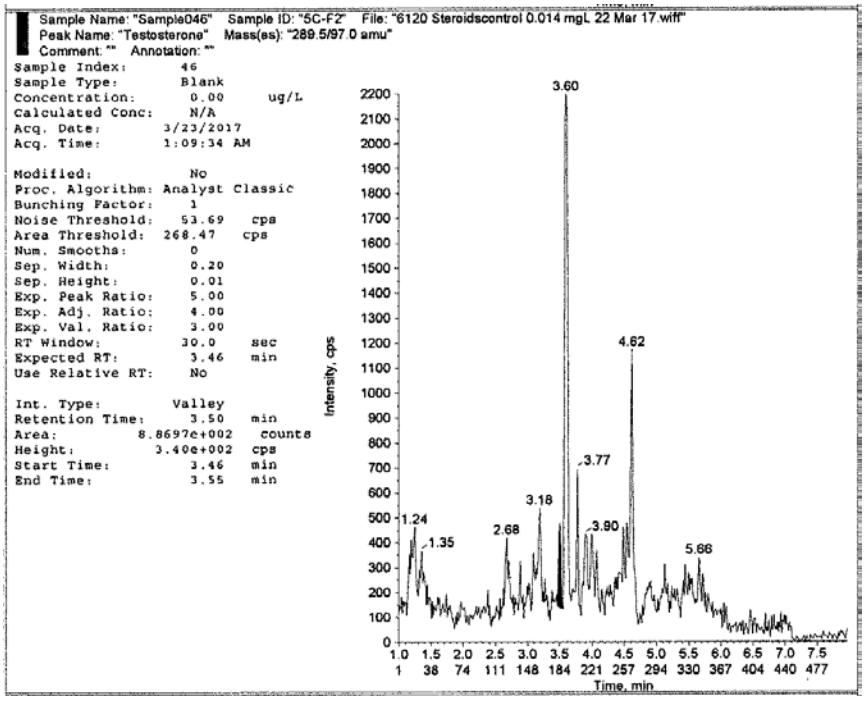


Testosterone-d_3

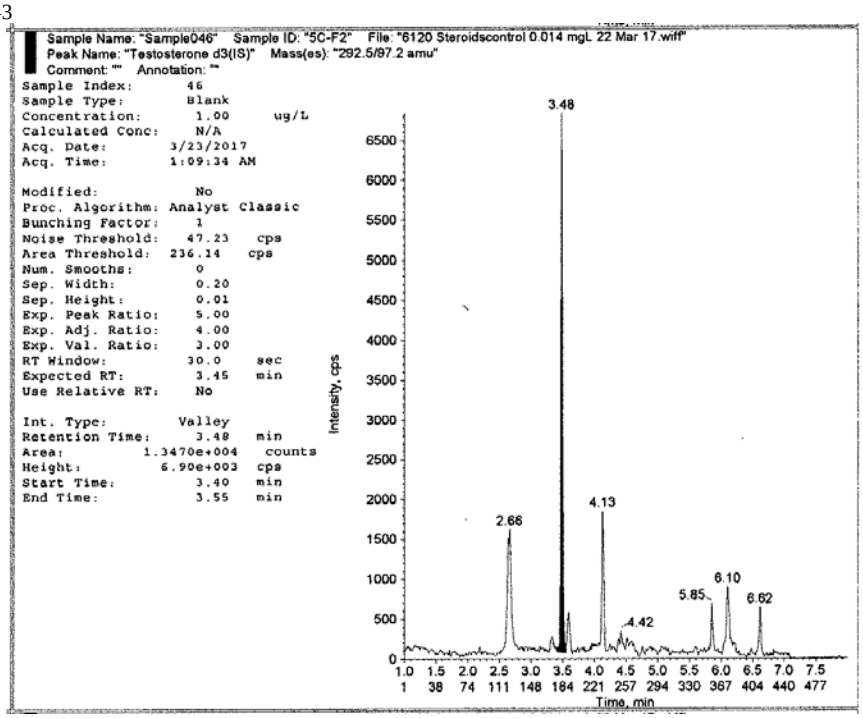


Control exposure sample (female fish)

Testosterone

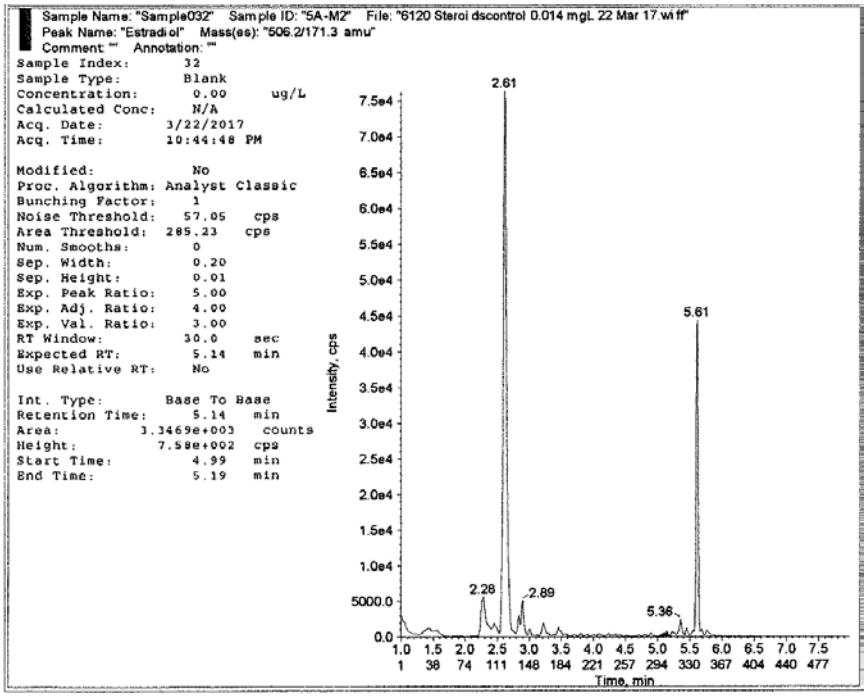


Testosterone-d_3

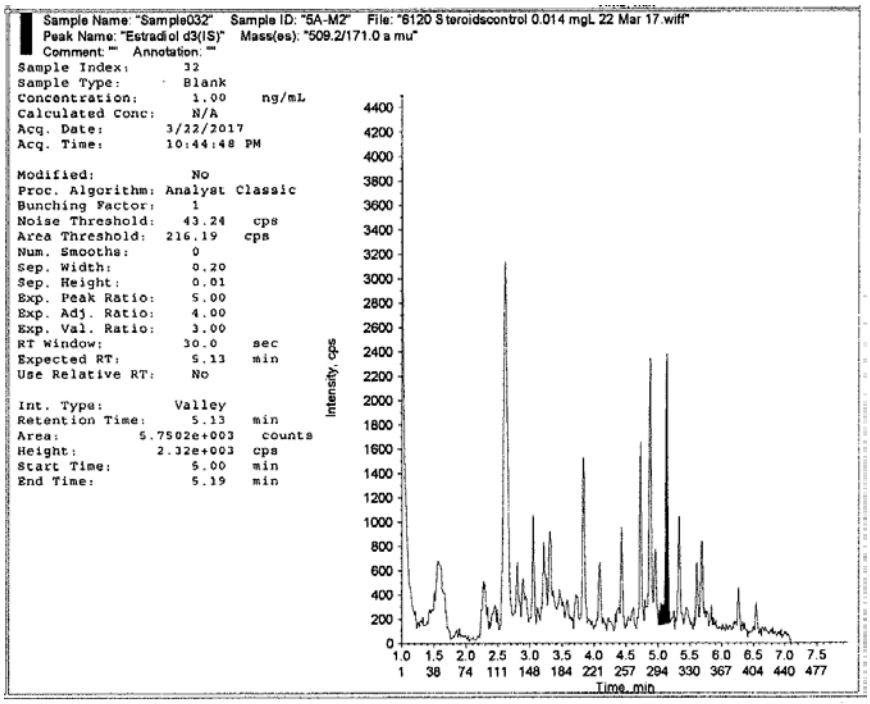


Control exposure sample (male fish)

Estradiol

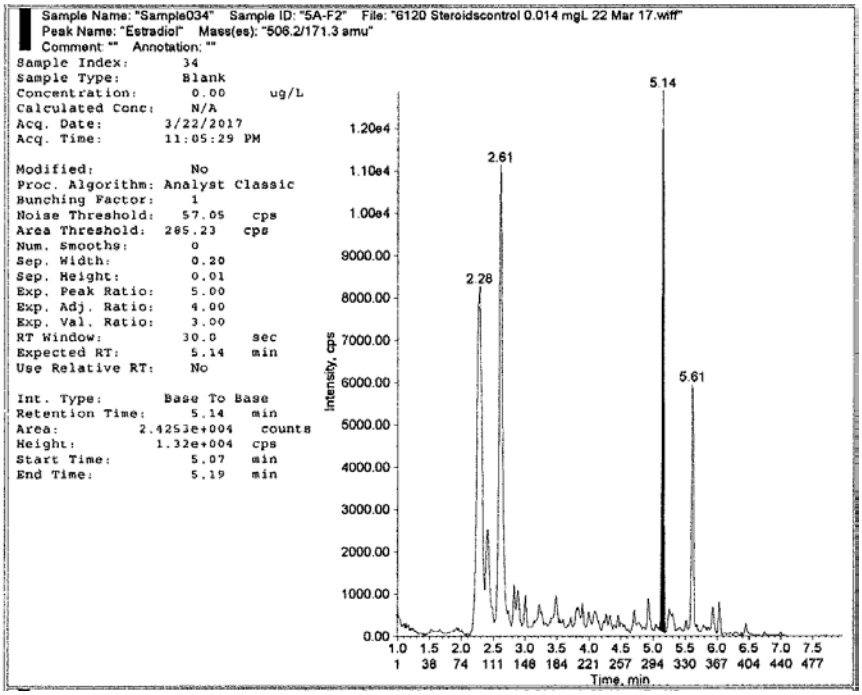


Estradiol-d_3

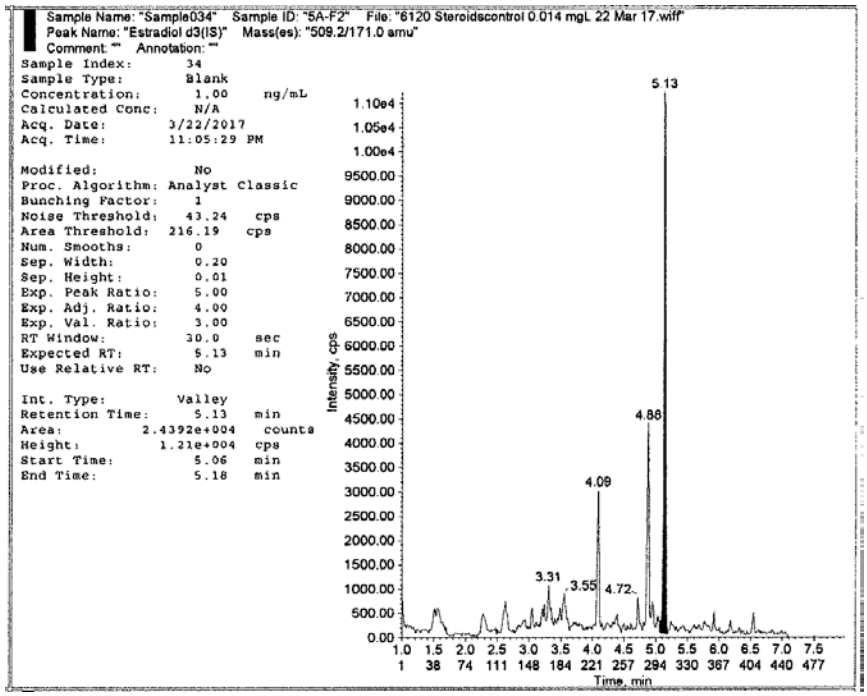


Control exposure sample (female fish)

Estradiol

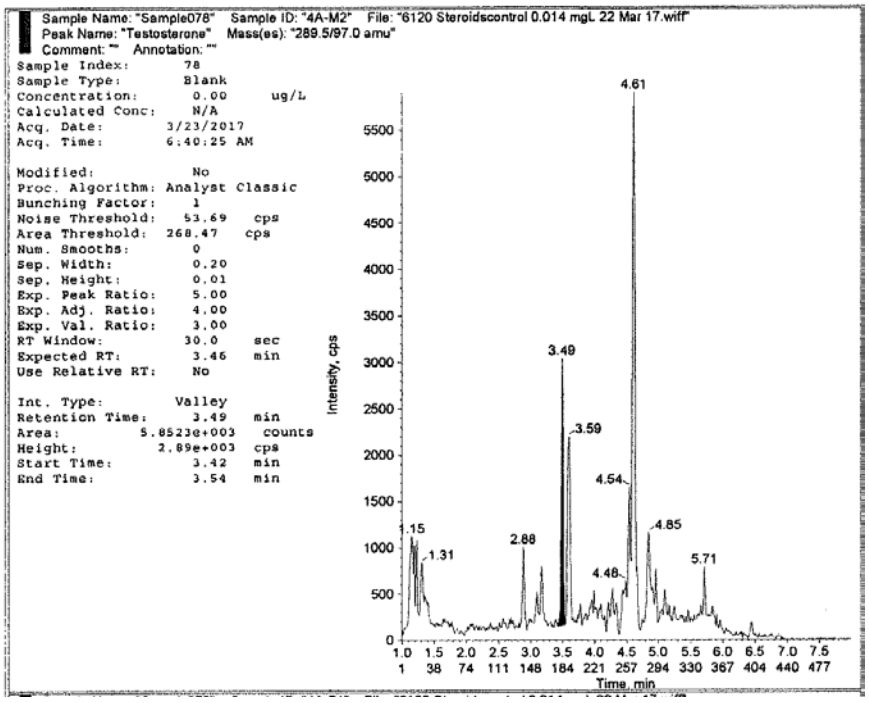


Estradiol-d_3

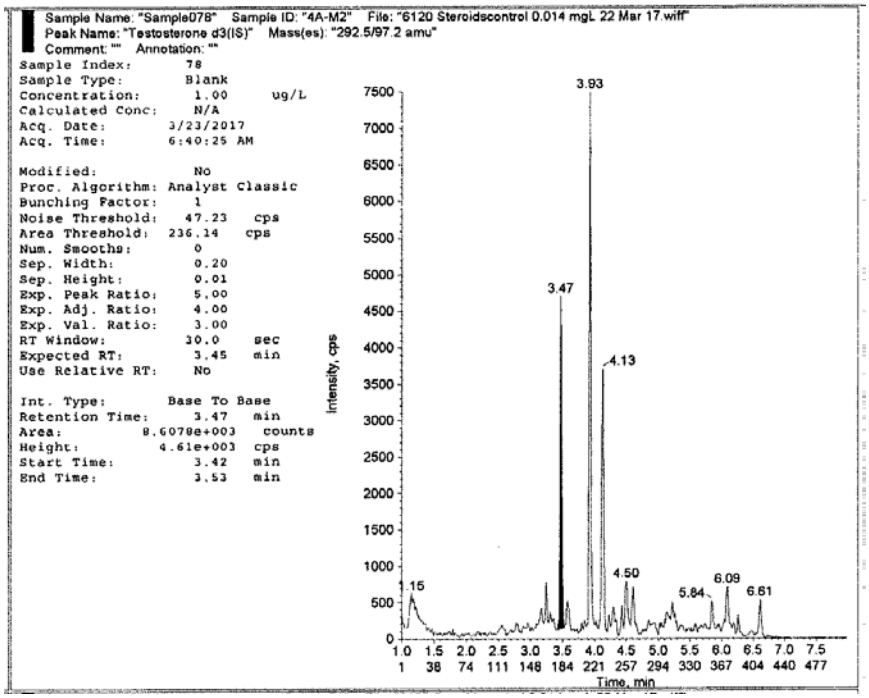


0.014 µg/L (nominal) sample (male fish)

Testosterone

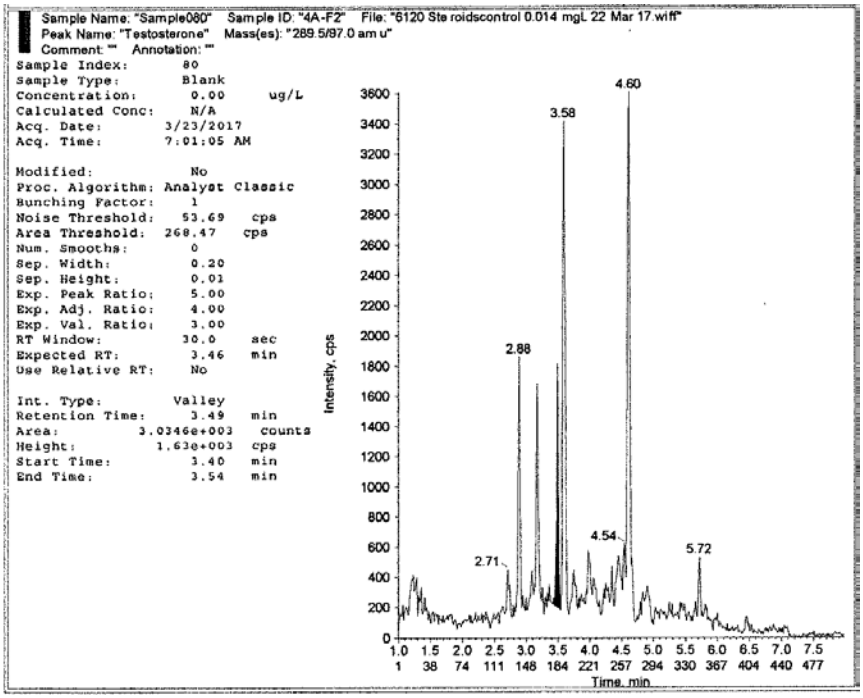


Testosterone-d_3

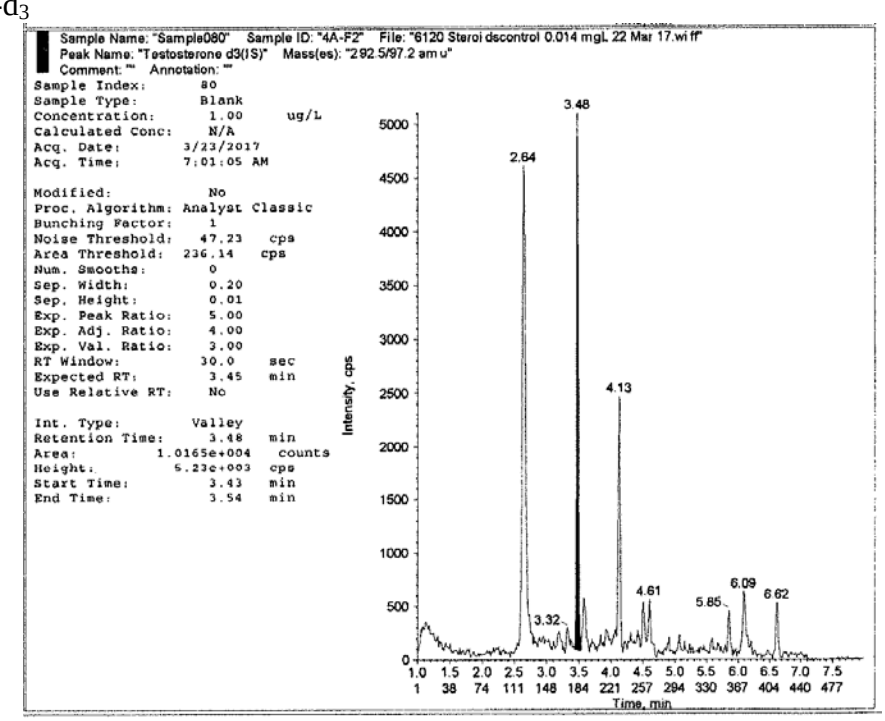


0.014 µg/L (nominal) sample (female fish)

Testosterone

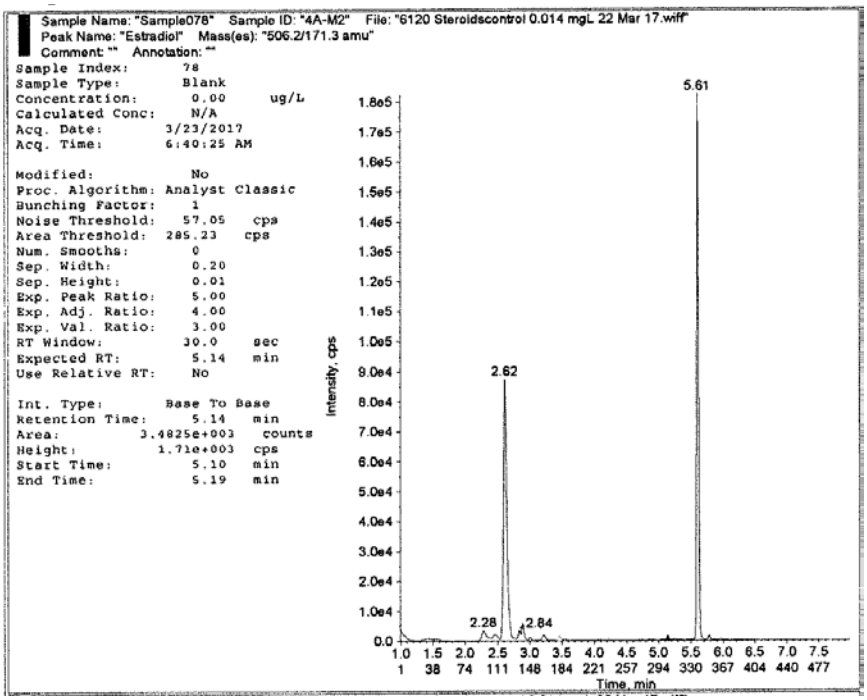


Testosterone-d_3

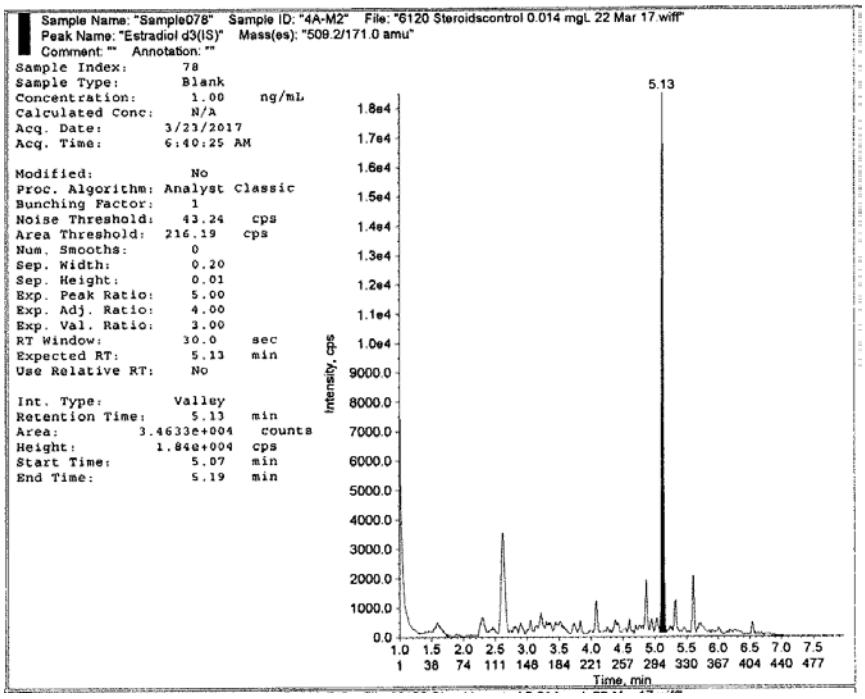


0.014 µg/L (nominal) sample (male fish)

Estradiol

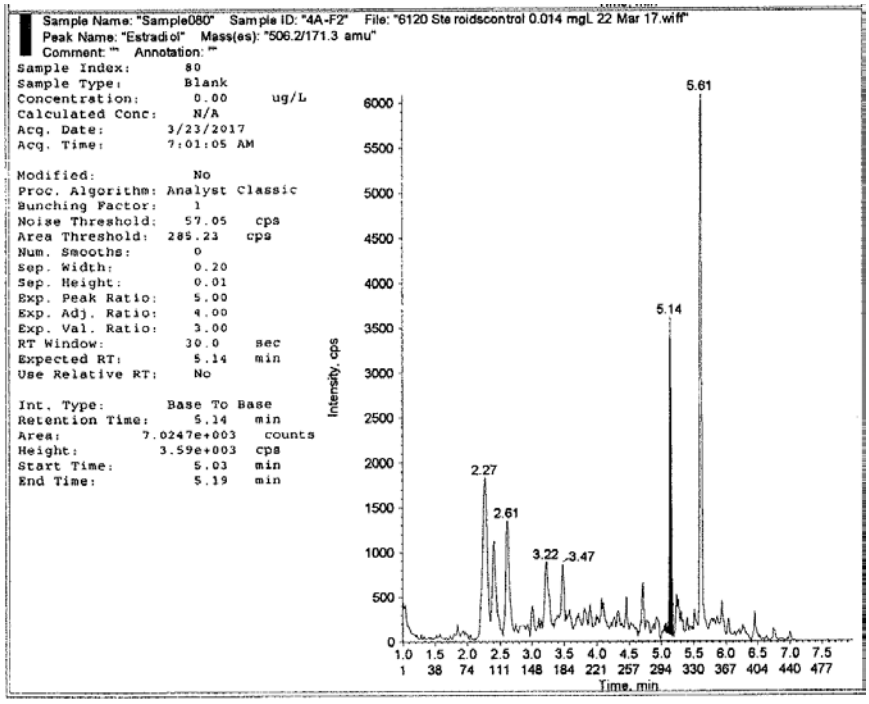


Estradiol-d_3

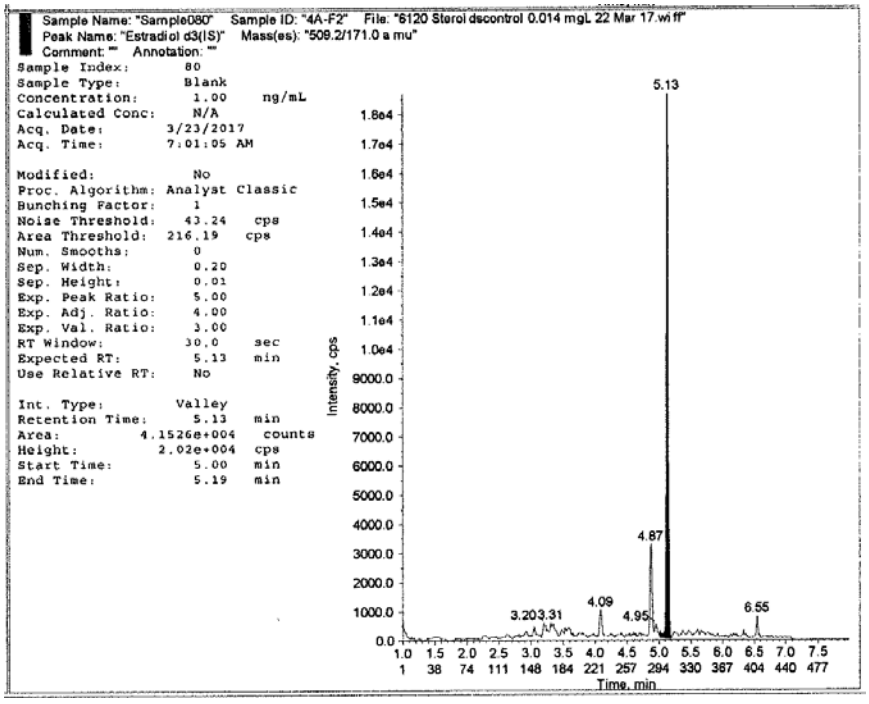


0.014 µg/L (nominal) sample (female fish)

Estradiol



Estradiol-d_3



APPENDIX 7 – STATISTICAL ANALYSIS

BATTELLE
It can be done

Date April 16, 2018

To Joseph Marini

From Ying-Liang Chou YC 4/16/18

Subject Final Statistical Analysis Report for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines. Smithers Viscient Study No. 13784.6120 (FSTRA #6120)

The attached final statistical analysis report summarizes the results of statistical analysis of the data collected under the Smithers Viscient Study Number 13784.6120, "Conducting a Short-Term Reproduction Assay with Fathead Minnow (*pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines" (FSTRA #6120).

Please call Ying-Liang Chou at 614-424-3538 or Vince Brown at 614-424-5928 if you have any questions.

Endocrine Disruptor Screening Program

USEPA Contract No: EP-W-11-063

Task Order No: 14

Final Statistical Analysis Report for

Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines

Smithers Viscient Study No. 13784.6120

(FSTRA #6120)

April 16, 2018

BATTELLE

505 King Avenue

Columbus, Ohio 43201

COMPLIANCE STATEMENT

Smithers Viscient Study No. 13784.6120

Study Title: Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) following OPPTS 890.1350 and OECD 229 Guidelines

Battelle Statistician / Report Originator: Po-Hsu Chen
Battelle Statistician / Technical Reviewer: Ying-Liang Chou

The statistical analysis portion of the above-referenced study was conducted in compliance with the Good Laboratory Practice regulations of the Environmental Protection Agency as stipulated by 40 CFR Part 160 (FIFRA), the study protocol, and all applicable amendments. Deviations, if present, can be found in study data file.

Ying-Liang Chou 4/16/18
Battelle Study Statistician Date

SIGNATURES

Name (Role)	Signature	Date
Po-Hsu Chen (Battelle Statistician / Report Originator)		4/16/18
Ying-Liang Chou (Battelle Statistician/ Technical Reviewer)		4/16/18
Vince Brown (Program Manager)		4/16/2018

Quality Assurance Statement
Battelle

USEPA Contract No.: EP-W-11-063, TO 14
Study No. 13784.6120

This study was inspected by the Quality Assurance Unit. Reports were submitted to the Study Director and Management as follows:

Audit	Date of Audit	Date Reported to Study Director and Management
Audit Study Data	June 21, 2017	June 22, 2017
Audit Draft Statistical Report	June 21, 2017 July 13, 2017 August 7, 2017	June 22, 2017 July 13, 2017 August 7, 2017
Audit Study File Addendum	July 13, 2017 August 7, 2017	July 13, 2017 August 7, 2017
Audit Final Statistical Report	April 10, 2018	April 10, 2018

Quality Assurance Unit, Battelle — Date: 4/16/2018

INTRODUCTION

This report summarizes the statistical analysis of the data collected under the Smithers Viscient Study Number 13784.6120, Fish Short-Term Reproduction Assay (FSTRA) of 2-ethylhexyl paraben with fathead minnow (*Pimephales promelas*) (FSTRA #6120). The purpose of this test was to assess the potential for the test substance to interact with the endocrine system in fathead minnow exposed under flow-through conditions.

A fish short-term reproduction assay was performed in which fathead minnows were exposed to different concentrations of the test substance (2-ethylhexyl paraben) for 21 days. Fish were exposed to one of four different concentrations of the test substance (14, 35, 88, and 220 µg/L) and dilution water control. Four replicates were included for each treatment group and the control group. Each exposure vessel (tank) contained a total of six fish, two male fish and four female fish, for a total of eight males and 16 females per treatment. All fish were impartially assigned to tanks prior to pre-exposure and then were randomly assigned to the exposure treatments within a block after successful spawning had been established. The experimental design is presented in Table 1.

Table 1. Experimental Design

2-ethylhexyl paraben Treatment Group (µg/L)	Number of Replicates (tanks)	Number of Male Fish per Replicate	Number of Female Fish per Replicate	Total Number of Fish per Treatment Group
0.0 (control)	4	2	4	24
14	4	2	4	24
35	4	2	4	24
88	4	2	4	24
220	4	2	4	24
	TOTAL	40	80	120

The primary endpoints were fecundity (the number of eggs per female per reproductive test day), fertilization success (the number of embryos divided by the total number of eggs × 100), nuptial tubercle score, female/male blood plasma vitellogenin (VTG) concentration, female/male gonadal somatic index (GSI), female/male body weight, and survival (yes/no).

STATISTICAL METHODS

Statistical analyses were performed consistent with OPPTS 890.1350 (1), OECD 229 guidelines (2), and the TO 14 QAPP (3). Preliminary analyses (i.e. descriptive statistics such as means, standard errors, and percent coefficient of variation) were performed separately on continuous quantitative endpoints: fecundity, fertilization success, nuptial tubercle score, female/male VTG, female/male GSI, and female/male body weight. Concentration-response monotonicity was assessed visually via plots of replicate means and the group medians of the replicate means.

To determine if significant differences in mortality existed between treatment groups, survival data were evaluated with the Cochran-Armitage test with survival results pooled across replicates within treatment or control group if data were considered to be monotonic. If monotonicity was not observed, Fisher's Exact test with a Bonferroni-Holm adjustment was performed.

The treatment effect assessment was performed using data from the control group and the four treatment groups. The treatment effect for nuptial tubercle score was determined using the Jonckheere-Terpstra test. For other continuous quantitative endpoints that followed a monotonic concentration-response, the Jonckheere-Terpstra test was applied in a step-down manner. For endpoints that were not consistent with a monotonic concentration-response, the data were evaluated for normality (Shapiro-Wilk's test) and homogeneity of variance (Levene's test). If a data set was found to have a non-normal distribution or a heterogeneous distribution of variance, a normalizing, variance stabilizing transformation was used. If data sets were normally distributed with homogeneous variance following transformation, the data set was evaluated using Dunnett's test. If the data set was normally distributed with heterogeneous variance following data transformation, the Mann-Whitney-Wilcoxon test (with Bonferroni-Holm adjustment) was used to evaluate the data. Where no normalizing transformation was found, the Mann-Whitney-Wilcoxon test with a Bonferroni-Holm adjustment to the p-values was used to evaluate the data sets. The Jonckheere-Terpstra test and Mann-Whitney-Wilcoxon test with a Bonferroni-Holm adjustment to the p-values were performed on replicate medians, and Dunnett's test was performed on replicate means.

Potential statistical outliers were assessed for all treatment and control data for all continuous quantitative endpoints. An analysis of variance model was fit to the data separately for each endpoint. Model residuals were estimated for each observation. If the residual value was greater than the median residual plus three times the residual interquartile range (i.e., the difference between the 75^{th} and 25^{th} percentiles), then the observed value was flagged as a potential statistical outlier. Analyses were performed with and without the potential outliers. Analyses in this report present results using all data, while Appendix B contains the results when potential outliers were removed.

The statistical analysis software package SAS® (version 9.4) was used in all statistical analyses, with statistical significance assessed at the two-sided 0.05 level.

RESULTS

Table 2 summarizes the survival rate for each of the four replicates within the five treatment groups. Also included in Table 2 is the mean survival rate by group across the four replicates along with the standard error of the mean and percent coefficient of variation. Four deaths (all female fish) were reported during the exposure period: two in the 14 µg/L treatment group, and one in each of the 35 µg/L and 88 µg/L treatment groups. Survival data were considered to be non-monotonic because three of the four fish that died were in the two lowest treatment groups (14 and 35 µg/L) while no fish died in the highest treatment group (220 µg/L). Fisher's Exact test with a Bonferroni-Holm adjustment was performed and no significant pairwise comparison results was seen between treatment groups and control.

Tables 3 through 7 present descriptive statistics (replicate means, number of samples, overall treatment mean, overall treatment median, standard error of the mean, and percent coefficient of variation) by treatment group for fecundity and fertilization success, nuptial tubercle score, female and male VTG, female and male GSI, and female and male body weight, respectively.

Table 8 presents results from the statistical analysis to assess the significance of treatment effects. Male VTG, female GSI, male GSI, and female body weight each followed a monotonic concentration-response trend based on a visual assessment using medians of replicate and treatment means. Significant increasing trends were seen for male VTG at the highest two treatment groups (88 and 220 µg/L), and for both female and male GSI at the highest treatment group (220 µg/L), while the other endpoint (female body weight) did not have a significant increasing trend. Note that male VTG data were converted from ng/mL to µg/mL in the statistical analysis so that a mixed model could be fitted.

For the four endpoints (not including survival rate) without a monotonic-concentration response (Table 8), fecundity and male body weight were normally distributed with homogeneous variance, fertilization success was normally distributed but with heterogeneous variance, and female VTG was not normally distributed. Thus, a log transformation was applied to the fertilization success and female VTG endpoints to remedy the heterogeneous variance and non-normality issues, respectively. Note that female VTG data were converted from ng/mL to µg/mL in the statistical analysis so that a mixed model could be fitted. For each of these four endpoints, the pairwise comparison results showed no significant difference between treatment groups and control.

Per OPPTS 890.1350 guidelines (1), a test for monotonicity was not performed for nuptial tubercle score, while a test for significant treatment effect was performed using the Jonckheere-Terpstra step-down test. No significant trend was seen for nuptial tubercle score.

Figures A-1 to A-9 in Appendix A present plots showing replicate means as well as the median of the replicate means from each group for the visual assessment of concentration-response monotonicity. Upon performing the analysis of variance to identify the presence of statistical outliers, seven statistical outliers were identified for female VTG, and five statistical outliers were identified for male VTG. As noted earlier, the above statistical analyses were

repeated for endpoints having statistical outliers identified, with those outliers removed from analysis. Tables and figures in Appendix B present statistical analyses performed on the data with the potential statistical outliers removed for these two endpoints. Table B-1 lists the potential statistical outliers for these two endpoints. Note that both female and male VTG data were converted from ng/mL to µg/mL in the statistical analysis so that a mixed model could be fitted. Table B-2 displays results from the statistical analysis of these two endpoints to assess the significance of treatment effects upon excluding the outliers. There was no change in the statistical significance results upon removing the potential outliers. Figure B-1 and B-2 present the plot of the replicate means as well as the median of the replicate means for the visual assessment of concentration-response monotonicity.

STUDY ARCHIVAL

Supporting data and the final report were archived at Battelle.

REFERENCES

1. OPPTS. 890.1350 Fish Short-Term Reproduction Assay, United States Environmental Protection Agency, Washington DC, EPA 740-C-09-007, October 2009.

2. OECD/OCDE 229. OECD Guideline for the Testing of Chemicals. Fish Short Term Reproduction Assay. 2 October 2012.

3. QAPP, Endocrine Disruptor Screening Program (EDSP) Tier 1 and Tier 2 In Vivo Testing of Selected Chemicals for Potential Endocrine Effects in Non-Mammals. EPA Contract No. EP-W-11-063 Task Order 14, version 1.2, August 28, 2015.

Table 2. Summary of 21-Day Survival Rate.

		Female Survival (%)					Male Survival (%)					Overall Survival (%)				
Treatment (µg/L)	Replicate	N	Percent	Mean	SEM	CV (%)	N	Percent	Mean	SEM	CV (%)	N	Percent	Mean	SEM	CV (%)
0.0	A	4	100.0	100	0.0	0.0	2	100	100	0.0	0.0	6	100.0	100	0.0	0.0
	B	4	100.0				2	100				6	100.0			
	C	4	100.0				2	100				6	100.0			
	D	4	100.0				2	100				6	100.0			
14	A	4	75.0	87.50	7.22	16.5	2	100	100	0.0	0.0	6	83.3	91.67	4.81	10.5
	B	4	75.0				2	100				6	83.3			
	C	4	100.0				2	100				6	100.0			
	D	4	100.0				2	100				6	100.0			
35	A	4	75.0	93.75	6.25	13.3	2	100	100	0.0	0.0	6	83.3	95.83	4.17	8.7
	B	4	100.0				2	100				6	100.0			
	C	4	100.0				2	100				6	100.0			
	D	4	100.0				2	100				6	100.0			
88	A	4	75.0	93.75	6.25	13.3	2	100	100	0.0	0.0	6	83.3	95.83	4.17	8.7
	B	4	100.0				2	100				6	100.0			
	C	4	100.0				2	100				6	100.0			
	D	4	100.0				2	100				6	100.0			
220	A	4	100.0	100	0.0	0.0	2	100	100	0.0	0.0	6	100.0	100	0.0	0.0
	B	4	100.0				2	100				6	100.0			
	C	4	100.0				2	100				6	100.0			
	D	4	100.0				2	100				6	100.0			

SEM Standard error of the mean.
CV(%) Coefficient of variation = (standard deviation / mean) × 100.
Mean, SEM, and CV (%) calculated from the percent survival estimate for each replicate within treatment (i.e., N=4).

Table 3. Descriptive Statistics for Fecundity and Fertilization Success.

		Fecundity (Number of Eggs per Female per Day)						Fertilization Success (%)					
Treatment (µg/L)	Replicate	N	Replicate Mean	Mean	Median	SEM	CV (%)	N	Replicate Mean	Mean	Median	SEM	CV (%)
0	A	4	45.51	47.88	46.52	4.28	17.88	4	99.61	99.40	99.62	0.24	0.48
	B	4	59.49					4	99.70				
	C	4	47.52					4	99.62				
	D	4	38.98					4	98.69				
14	A	4	28.64	35.33	34.63	3.32	18.78	4	98.88	99.50	99.62	0.22	0.43
	B	4	31.42					4	99.59				
	C	4	43.44					4	99.64				
	D	4	37.83					4	99.87				
35	A	4	43.17	41.40	43.38	3.33	16.10	4	97.90	98.64	98.70	0.56	1.14
	B	4	47.07					4	99.70				
	C	4	31.75					4	99.51				
	D	4	43.60					4	97.46				
88	A	4	57.50	42.99	44.08	6.40	29.79	4	99.43	99.51	99.66	0.29	0.58
	B	4	44.29					4	98.74				
	C	4	26.29					4	100.00				
	D	4	43.87					4	99.89				
220	A	4	38.49	37.02	38.45	2.79	15.05	4	99.60	99.44	99.46	0.09	0.18
	B	4	42.11					4	99.58				
	C	4	29.07					4	99.22				
	D	4	38.42					4	99.35				

SEM Standard error of the mean.
CV(%) Coefficient of variation = (standard deviation / mean) × 100.
Mean, SEM, and CV (%) calculated from the replicate means for each treatment (i.e., N=4).
For fish that died, data collected prior to death was included in the analysis of Fecundity and Fertilization Success endpoints.

Table 4. Descriptive Statistics for Male Nuptial Tubercle Score.

		Male Nuptial Tubercle Score					
Treatment (µg/L)	Replicate	N	Replicate Mean	Mean	Median	SEM	CV (%)
0	A	2	23.0	21.9	21.8	1.1	10.5
	B	2	20.5				
	C	2	24.5				
	D	2	19.5				
14	A	2	18.0	20.1	21.0	2.5	25.0
	B	2	24.0				
	C	2	14.0				
	D	2	24.5				
35	A	2	16.5	16.1	16.5	0.9	11.7
	B	2	16.5				
	C	2	13.5				
	D	2	18.0				
88	A	2	17.5	17.8	18.0	2.3	25.5
	B	2	23.0				
	C	2	18.5				
	D	2	12.0				
220	A	2	21.5	18.9	18.5	1.0	10.9
	B	2	17.0				
	C	2	19.5				
	D	2	17.5				

SEM Standard error of the mean.
CV(%) Coefficient of variation = (standard deviation / mean) × 100.
Mean, SEM, and CV (%) calculated from the replicate means for each treatment (i.e., N=4).

Table 5. Descriptive Statistics Blood Plasma Vitellogenin (VTG) by Sex.

		Female VTG (ng/mL)						Male VTG (ng/mL)					
Treatment (µg/L)	Replicate	N	Replicate Mean	Mean	Median	SEM	CV (%)	N	Replicate Mean	Mean	Median	SEM	CV (%)
0	A	4	1225000	1518858	1399462	296300	39	2	85	164	92	76	92
	B	4	1573924					2	390				
	C	4	954284					2	100				
	D	4	2322223					2	80				
14	A	3	3287348	2885458	3452430	686915	48	2	104060	28392	4709	25288	178
	B	2*	848469					2	1155				
	C	4	3788503					2	8263				
	D	4	3617513					2	90				
35	A	3	918261	4186704	2505213	2253011	108	2	1753	12896	5414	9474	147
	B	4	1957802					2	40704				
	C	4	10818131					2	9075				
	D	4	3052624					2	52				
88	A	3	3390670	1336833	918692	712244	107	2	11045548	8479752	9367774	3045043	72
	B	4	1054948					2	7690001				
	C	3*	782436					2	14750057				
	D	4	119277					2	433403				
220	A	4	595388	1938782	1930735	659979	68	1*	24186650	18732401	18732401	5454249	41
	B	3*	1043657					0*	--				
	C	2*	3298271					2	13278152				
	D	4	2817814					0*	--				

SEM Standard error of the mean.
CV(%) Coefficient of variation = (standard deviation / mean) × 100.
-- No measurements available.
* Measurements not available for all fish because: 1) fish were found dead at termination and thus no VTG measurement was completed; 2) assay results fall outside the readable range; or 3) data was not reportable.

Mean, SEM, and CV (%) calculated from the replicate means for each treatment (i.e., N=4 or 2).

Table 6. Descriptive Statistics for Gonadal Somatic Index (GSI%) by Sex.

		Female GSI (%)						Male GSI (%)					
Treatment (µg/L)	Replicate	N	Replicate Mean	Mean	Median	SEM	CV (%)	N	Replicate Mean	Mean	Median	SEM	CV (%)
0	A	4	13.7793	15.1757	15.1283	0.7860	10.3582	2	1.1621	1.1668	1.1022	0.1716	29.4081
	B	4	16.6668					2	0.8256				
	C	4	13.8565					2	1.0422				
	D	4	16.4001					2	1.6372				
14	A	3	10.9498	13.3346	13.6836	0.9664	14.4943	2	1.6792	1.5698	1.5320	0.1431	18.2339
	B	3	12.5814					2	1.3848				
	C	4	14.7858					2	1.2940				
	D	4	15.0215					2	1.9213				
35	A	3	14.5630	14.7592	14.8485	0.3270	4.4305	2	1.1363	1.3401	1.2881	0.1508	22.5033
	B	4	15.4075					2	1.7207				
	C	4	15.1340					2	1.4400				
	D	4	13.9325					2	1.0636				
88	A	3	16.2892	16.8589	17.2764	1.8450	21.8877	2	1.3215	1.5093	1.5472	0.0658	8.7188
	B	4	20.8120					2	1.5721				
	C	4	12.0709					2	1.5223				
	D	4	18.2637					2	1.6214				
220	A	4	17.0872	17.8280	17.8408	0.9064	10.1683	2	1.8618	2.0586	2.0547	0.1152	11.1908
	B	4	19.9036					2	2.2683				
	C	4	18.5943					2	2.2476				
	D	4	15.7269					2	1.8566				

SEM Standard error of the mean.
CV(%) Coefficient of variation = (standard deviation / mean) × 100.
Mean, SEM, and CV (%) calculated from the replicate means for each treatment (i.e., N=4).

Table 7. Descriptive Statistics for Body Weight by Sex.

		Female Body Weight (g)						Male Body Weight (g)					
Treatment (µg/L)	Replicate	N	Replicate Mean	Mean	Median	SEM	CV (%)	N	Replicate Mean	Mean	Median	SEM	CV (%)
0	A	4	2.3213	2.2539	2.2555	0.0607	5.3839	2	4.3649	4.3577	4.4693	0.3592	16.4861
	B	4	2.3857					2	5.1055				
	C	4	2.1192					2	4.5737				
	D	4	2.1897					2	3.3869				
14	A	3	1.9247	2.2986	2.3188	0.1602	13.9402	2	4.0615	4.2063	4.1696	0.0779	3.7055
	B	3	2.1512					2	4.4244				
	C	4	2.6321					2	4.1407				
	D	4	2.4864					2	4.1985				
35	A	3	2.5605	2.4256	2.4108	0.1019	8.3992	2	4.6283	4.6273	4.7597	0.1880	8.1264
	B	4	2.2418					2	4.8946				
	C	4	2.2612					2	4.8912				
	D	4	2.6390					2	4.0952				
88	A	3	2.3974	2.2726	2.3209	0.0762	6.7053	2	4.0019	4.1175	4.0988	0.1396	6.7788
	B	4	2.3069					2	4.1957				
	C	4	2.0512					2	4.4630				
	D	4	2.3348					2	3.8095				
220	A	4	2.2431	2.3553	2.3185	0.0677	5.7505	2	4.0433	4.0573	4.0132	0.1692	8.3407
	B	4	2.2678					2	3.6927				
	C	4	2.3692					2	4.5100				
	D	4	2.5413					2	3.9832				

SEM Standard error of the mean.
CV(%) Coefficient of variation = (standard deviation / mean) × 100.
Mean, SEM, and CV (%) calculated from the replicate means for each treatment (i.e., N=4).

Table 8. Statistical Analysis Results

Endpoints	Monotonicity Assessment[1]	Normality Test[2]	Homogeneity of Variance Test[3]	Jonckheere-Terpstra Test[4] (p-value)	Significant Pairwise Comparisons to Control[5] (p-value)
Fecundity	Non-Monotonic	Normal	Homogeneity	NP	NS
Fertilization Success	Non-Monotonic	Normal	Heterogeneity	NP	NS
Nuptial Tubercle Score[6]	NP	NP	NP	NS	NP
Female VTG[7]	Non-Monotonic	Non-Normal	Homogeneity	NP	NS
Male VTG[7]	Monotonic	NP	NP	Group 5 (0.0001) Group 4 (0.0015)	NP
Female GSI	Monotonic	NP	NP	Group 5 (0.0463)	NP
Male GSI	Monotonic	NP	NP	Group 5 (0.0053)	NP
Female Body Weight (g)	Monotonic	NP	NP	NS	NP
Male Body Weight (g)	Non-Monotonic	Normal	Homogeneity	NP	NS
Survival	Non-Monotonic	NP	NP	NP	NS[8]

1. Monotonicity was assessed visually from the replicate and treatment means.
2. Shapiro-Wilk test for normality.
3. Levene's Test for homogeneity of variance.
4. Jonckheere-Terpstra step-down trend test was performed on monotonic concentration-response data. Only statistically significant treatment trends were listed.
5. Control is group 1 and test concentration groups are groups 2 to 5 (ordering from 14 to 220 µg/L). Only statistically significant pairwise comparisons were listed.
   - For non-monotonic, normally distributed, and homogeneous variance data, Dunnett's test was used in the pairwise comparisons to control.
   - For other types of data (e.g., non-monotonic, normally distributed, and heterogeneous variance data), Mann-Whitney-Wilcoxon test was used in the pairwise comparisons to control with Bonferroni-Holm multiple comparison adjustment.
6. The treatment effect for tubercle score is determined using the Jonckheere-Terpstra step-down trend test on replicate medians.
7. Both male/Female VTG data were converted from ng/mL to µg/mL so that a mixed model could be fitted.
8. Fisher's Exact test with a Bonferroni-Holm adjustment was performed.

NP Statistical test or comparison was not performed.

NS No statistically significant differences were found for Jonckheere-Terpstra step-down trend test, Dunnett's test, or Mann-Whitney-Wilcoxon test with a Bonferroni-Holm adjustment.

Appendix A

Plots Used for the Visual Assessment of Concentration-Response Monotonicity

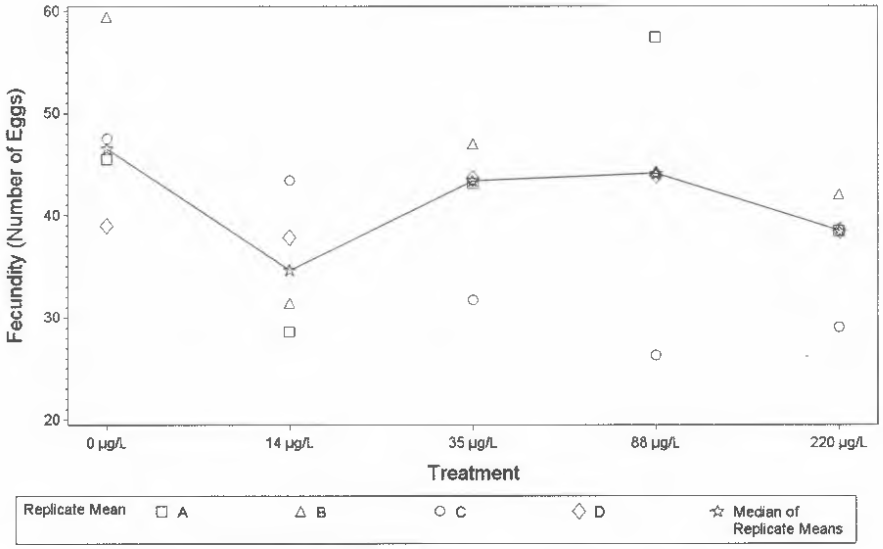


Figure A-1. Replicate Means and Median of Replicate Means for Fecundity.

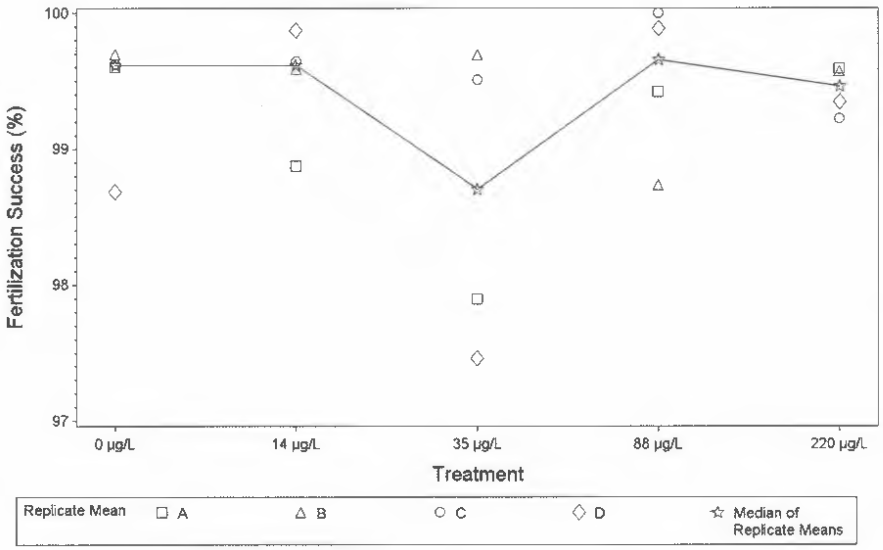


Figure A-2. Replicate Means and Median of Replicate Means for Fertilization Success (%).

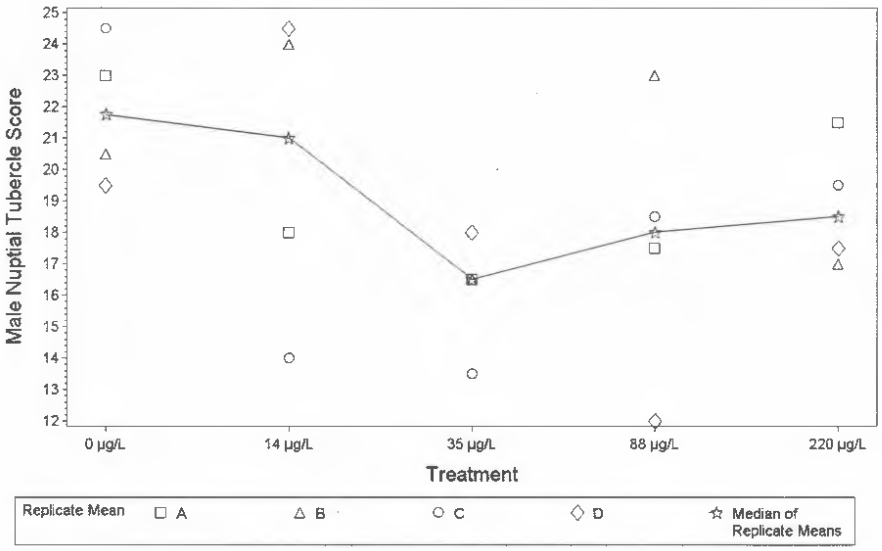


Figure A-3. Replicate Means and Median of Replicate Means for Male Nuptial Tubercle Score.

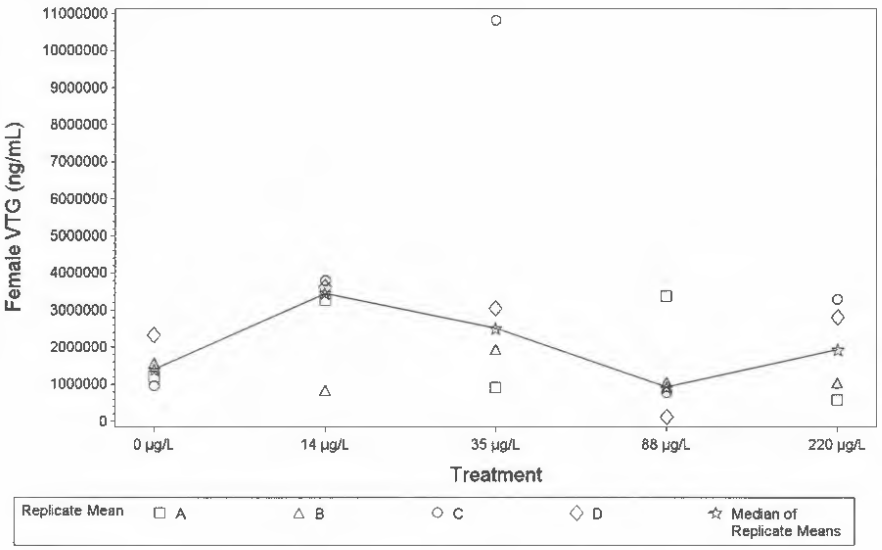


Figure A-4. Replicate Means and Median of Replicate Means for Female VTG (ng/mL).

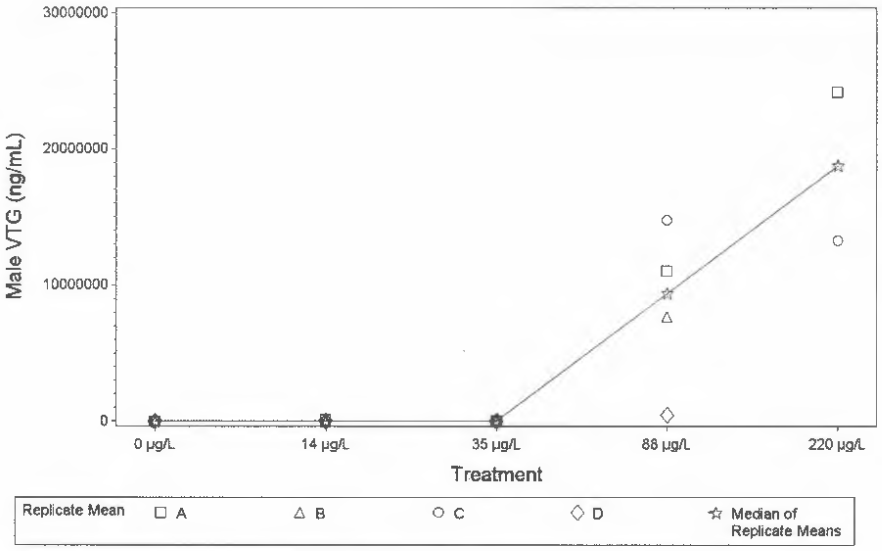


Figure A-5. Replicate Means and Median of Replicate Means for Male VTG (ng/mL).

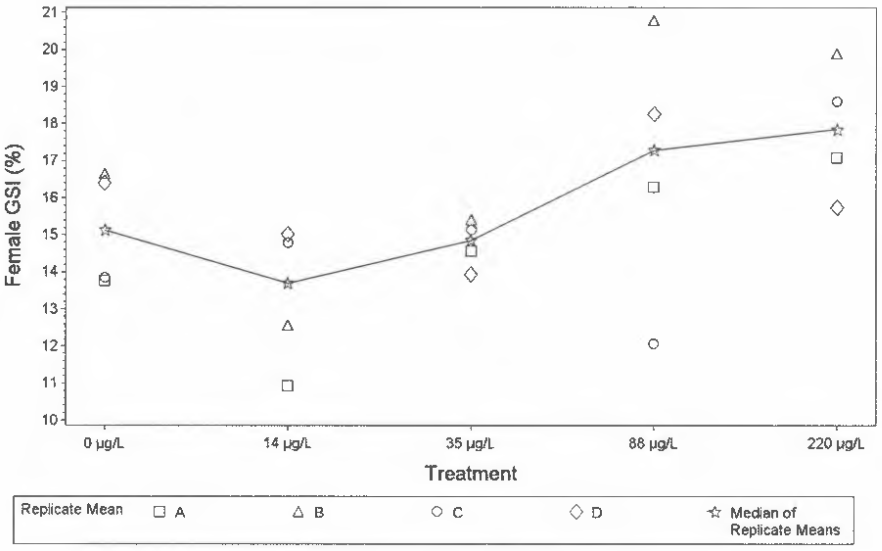


Figure A-6. Replicate Means and Median of Replicate Means for Female GSI (%).

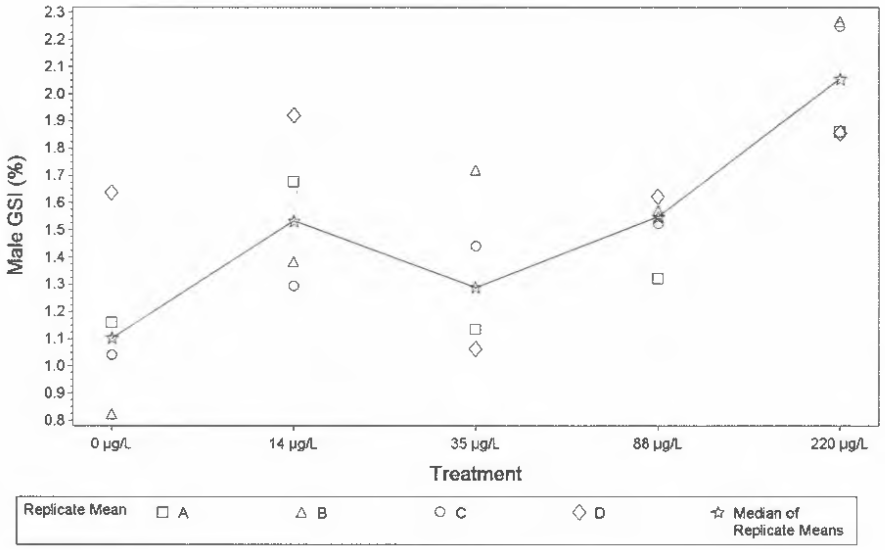


Figure A-7. Replicate Means and Median of Replicate Means for Male GSI (%).

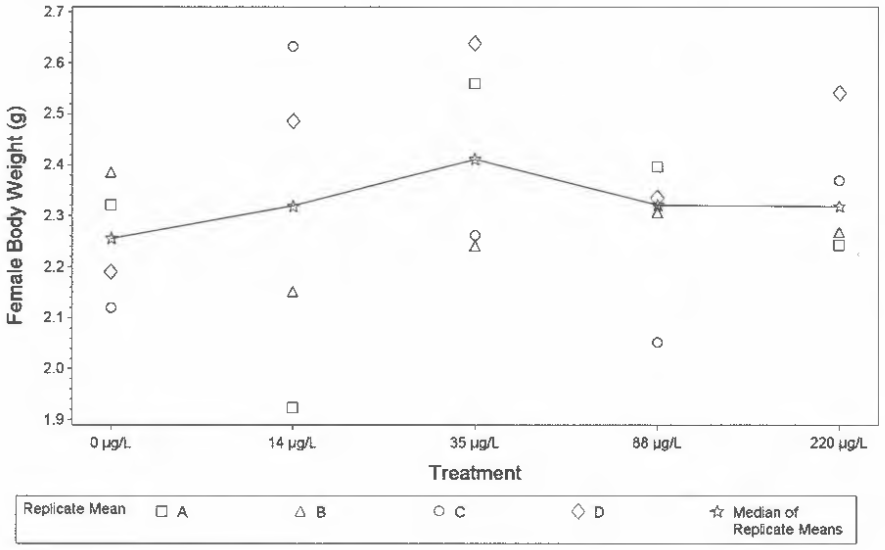


Figure A-8. Replicate Means and Median of Replicate Means for Female Body Weight (g).

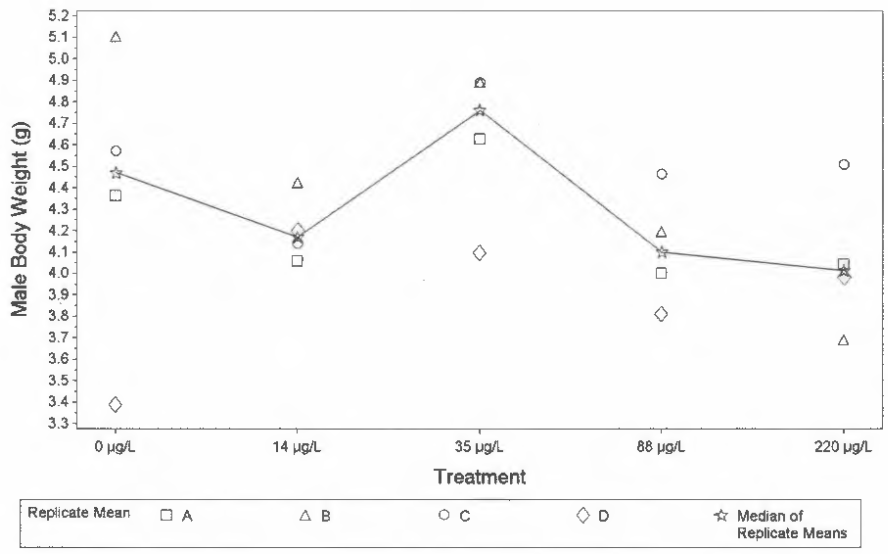


Figure A-9. Replicate Means and Median of replicate means for Male Body Weight (g).

Appendix B

Statistical Analyses Performed on Data with Potential Statistical Outliers Removed

Table B-1. Potential Statistical Outliers.

Endpoints	Treatment (µg/L)	Replicate	Observed Value	Predicted Value	Residual
Female VTG (ng/mL)	0	D	7,112,278	1,518,858	5,593,420
Female VTG (ng/mL)	14	D	7,965,098	3,167,926	4,797,172
Female VTG (ng/mL)	35	C	42,295,354	4,404,600	37,890,754
Female VTG (ng/mL)	35	D	8,993,854	4,404,600	4,589,254
Female VTG (ng/mL)	88	A	7,809,050	1,229,730	6,579,320
Female VTG (ng/mL)	220	C	6,482,298	1,798,486	4,683,812
Female VTG (ng/mL)	220	D	6,951,133	1,798,486	5,152,647
Male VTG (ng/mL)	14	A	200663	28392	172271
Male VTG (ng/mL)	88	A	16,311,566	8,479,752	7,831,814
Male VTG (ng/mL)	88	B	10,716,174	8,479,752	2,236,422
Male VTG (ng/mL)	88	C	23,557,855	8,479,752	15,078,103
Male VTG (ng/mL)	220	A	24,186,650	16,914,351	7,272,299

Note that in the calculation of potential statistical outliers both female and male VTG data were converted from ng/mL to µg/mL so that mixed models could be fitted.

Table B-2. Statistical Analysis Results, Potential Statistical Outliers Removed.

Endpoints	Monotonicity Assessment[1]	Normality Test[2]	Homogeneity of Variance Test[3]	Jonckheere-Terpstra Test[4] (p-value)	Significant Pairwise Comparisons to Control[5] (p-value)
Female VTG	Non-Monotonic	Non-Normal	Log-Homogeneity	NP	NS
Male VTG	Monotonic	NP	NP	Group 5 (0.0002) Group 4 (0.0008)	NP

1. Monotonicity was assessed visually from the replicate and treatment means.
2. Shapiro-Wilk test for normality.
3. Levene's Test for homogeneity of variance.
4. Jonckheere-Terpstra step-down trend test was performed on monotonic concentration-response data. Only statistically significant treatment trends were listed.
5. Control is group 1 and test concentration groups are groups 2 to 5 (ordering from 14 to 220 µg/L). Only statistically significant pairwise comparisons were listed.
   - For non-monotonic, normally distributed, and homogeneous variance data, Dunnett's test was used in the pairwise comparisons to control.
   - For other types of data (e.g., non-monotonic, normally distributed, and heterogeneous variance data), Mann-Whitney-Wilcoxon test was used in the pairwise comparisons to control with Bonferroni-Holm multiple comparison adjustment.
6. Both male and Female VTG data were converted from ng/mL to µg/mL so that a mixed model could be fitted.

NP Statistical test or comparison was not performed.

NS No statistically significant differences were found for Jonckheere-Terpstra step-down trend test or Mann-Whitney-Wilcoxon test with a Bonferroni-Holm adjustment.

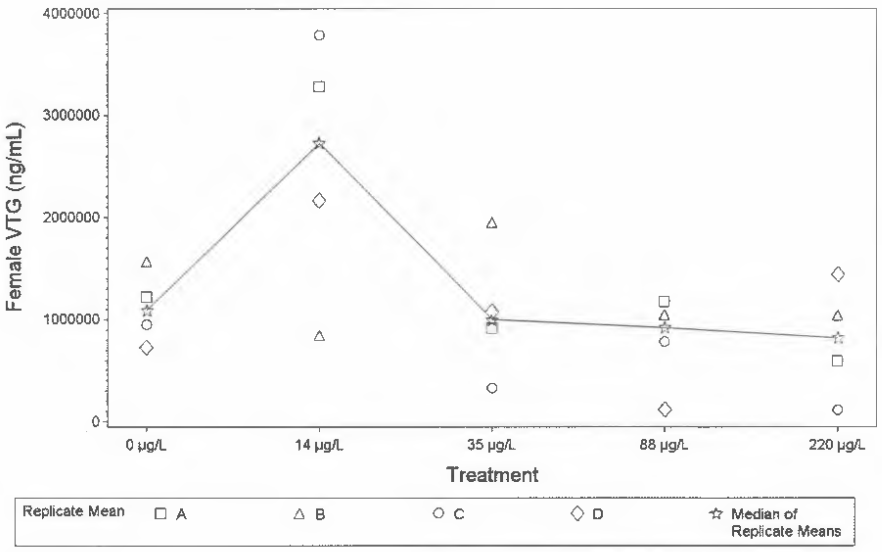


Figure B-1. Replicate Means and Median of Replicate Means for Female VTG (ng/mL), Potential Statistical Outliers Removed.

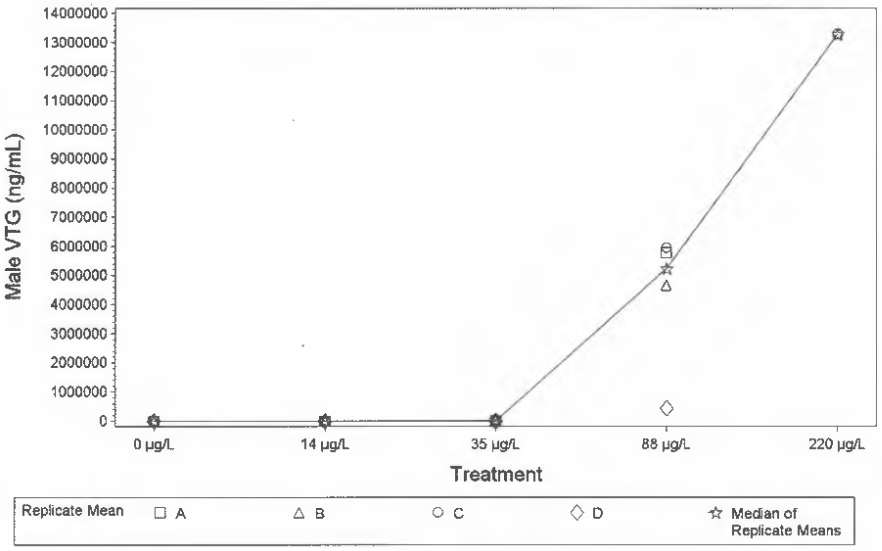


Figure B-2. Replicate Means and Median of Replicate Means for Male VTG (ng/mL), Potential Statistical Outliers Removed.

APPENDIX 8 – STUDY DATA

Water quality measurements during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration (µg/L)	Ranges						
	Dissolved Oxygen		Temperature (°C)	pH	Total Hardness (mg/L as $CaCO_3$)	Total Alkalinity (mg/L as CaCO3)	Conductivity (µS/cm)
	mg/L	% of Saturation					
Control	5.89 - 8.34	71.4 - 101	25 - 26	7.0 - 7.5	68 - 88	20 - 26	490 - 710
14	5.56 - 8.25	67.5 - 99.9	24 - 26	7.0 - 7.5	72	24	460
35	4.63 - 8.47	56.1 - 103	25 - 26	6.8 - 7.5	72	24	530
88	6.64 - 8.14	79.9 - 99.3	25 - 26	7.0 - 7.5	68	20	490
220	6.76 - 8.43	82.0 - 102	25 - 27	7.1 - 7.5	88	20	460

Continuous temperature monitoring of both the upper and lower water bath established a temperature range of 24.4 to 26 °C throughout the exposure period.

Pre-exposure spawning summary for the exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Day	1		2		3		4		5		6	
Spawning Group ID	# of Eggs	# of Spawns	# of Eggs	# of Spawns	# of Eggs	# of Spawns	# of Eggs	# of Spawns	# of Eggs	# of Spawns	# of Eggs	# of Spawns
1	160	1	270	1	145	1	156	1	66	3	52	1
2	41	2	541	2	0	0	404	2	92	2	0	0
3	103	1	231	2	206	2	86	1	116	1	99	1
4	0	0	89	1	0	0	127	2	86	2	0	0
5	209	1	92	1	0	0	585	2	32	1	0	0
6	287	2	140	2	194	1	436	2	111	2	128	1
7	0	0	250	2	0	0	111	1	161	1	67	1
8	0	0	39	2	0	0	98	1	97	2	23	1
9	0	0	366	2	159	1	76	1	230	1	38	1
10	0	0	408	1	0	0	213	1	301	1	0	0
11	0	0	46	1	198	1	220	1	73	1	130	1
12	225	2	235	1	174	1	0	0	365	2	0	0
13	225	1	246	1	0	0	622	2	0	0	451	2
14	0	0	21	1	462	2	149	2	84	2	0	0
15	0	0	16	1	59	3	0	0	127	1	0	0
16	0	0	178	1	181	1	162	1	97	1	141	1
17	96	1	227	2	0	0	90	1	113	2	105	1
18	42	1	262	2	278	2	46	1	201	2	36	1
19	0	0	0	0	54	1	0	0	30	1	44	2
20	0	0	103	2	331	2	0	0	167	2	49	1
21	0	0	323	1	160	2	88	1	382	2	0	0
22	212	1	340	2	225	2	232	2	57	1	77	1
23	136	2	344	2	208	1	75	1	198	2	31	1
24	0	0	396	3	42	2	336	1	269	2	0	0
25	0	0	408	2	0	0	0	0	202	2	118	1
26	0	0	336	2	0	0	22	1	130	2	156	1
27	0	0	260	2	262	2	132	1	244	1	0	0
28	157	2	0	0	319	2	61	1	50	1	202	2
29	298	1	362	2	167	2	161	2	106	1	0	0
30	0	0	305	1	0	0	103	1	71	1	226	1
31	312	1	444	2	0	0	289	1	392	2	59	2
32	0	0	36	1	395	2	64	2	201	2	0	0
33	0	0	72	1	169	2	303	2	17	1	92	1
34	78	1	104	2	222	2	35	1	243	2	17	1
35	199	2	40	1	38	1	207	1	83	1	0	0
36	0	0	643	3	105	2	74	1	115	2	20	1

Pre-exposure spawning summary for the exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben (continued)

Day	7		8		9		10		11		12	
Spawning Group ID	# of Eggs	# of Spawns	# of Eggs	# of Spawns	# of Eggs	# of Spawns	# of Eggs	# of Spawns	# of Eggs	# of Spawns	# of Eggs	# of Spawns
1	136	1	74	1	73	1	268	2	247	1	578	1
2	388	2	0	0	98	1	149	2	198	2	614	2
3	0	0	0	0	121	1	158	1	360	2	444	2
4	271	1	260	1	0	0	0	0	0	0	192	1
5	308	1	213	1	0	0	225	1	0	0	296	3
6	189	1	197	1	0	0	504	1	260	1	664	2
7	72	1	39	1	155	1	108	1	0	0	118	1
8	121	1	56	1	67	1	162	1	0	0	317	2
9	92	1	91	2	138	1	0	0	150	2	289	2
10	197	2	319	1	0	0	341	2	211	2	15	1
11	159	2	107	1	243	2	171	1	0	0	555	1
12	0	0	48	1	357	2	0	0	244	2	352	1
13	239	1	103	1	0	0	336	2	62	2	160	1
14	115	1	68	1	158	2	144	1	128	2	309	1
15	22	1	135	1	218	3	0	0	0	0	571	2
16	59	1	0	0	191	1	92	1	78	1	244	1
17	107	1	85	1	0	0	225	2	0	0	0	0
18	112	1	0	0	323	1	0	0	84	1	193	1
19	28	1	0	0	142	1	63	1	32	2	311	2
20	211	1	44	1	161	1	117	1	19	1	607	2
21	49	1	213	1	237	3	274	2	0	0	307	1
22	160	1	0	0	286	2	298	1	0	0	638	2
23	76	1	56	1	175	1	209	2	0	0	585	2
24	0	0	149	1	93	1	166	1	0	0	521	2
25	0	0	102	1	108	1	0	0	94	1	347	2
26	131	1	0	0	249	1	126	1	0	0	713	2
27	150	2	188	1	167	2	66	2	218	1	313	1
28	0	0	65	1	76	1	319	1	0	0	357	1
29	73	1	204	2	35	1	107	1	0	0	70	1
30	118	1	114	1	0	0	68	1	38	1	429	2
31	149	1	302	1	211	1	93	1	115	1	696	1
32	107	1	112	1	63	1	162	1	310	2	4	1
33	0	0	19	1	154	1	124	1	145	1	0	0
34	38	1	234	1	58	2	180	2	206	1	335	1
35	0	0	96	1	59	2	0	0	0	0	828	2
36	88	2	105	2	290	1	261	2	0	0	217	1

Pre-exposure spawning summary for the exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben (continued)

Day	13		14		15		16		Pre Exposure Totals			
Spawning Group ID	# of Eggs	# of Spawns	# of Eggs	# of Spawns	# of Eggs	# of Spawns	# of Eggs	# of Spawns	Eggs/Female/Day	Total # of Spawns	# Spawns in Last 7 Days	Criteria Met
1	389	1	41	1	197	1	278	1	48.9	19	8	**YES**
2	275	1	256	2	158	1	215	1	53.6	22	11	**YES**
3	0	0	132	1	365	1	0	0	37.8	16	7	**YES**
4	0	0	107	1	142	1	166	1	22.5	11	4	**YES**
5	697	2	0	0	174	2	477	2	51.7	17	10	**YES**
6	215	1	119	1	271	1	109	1	59.8	20	8	**YES**
7	0	0	0	0	320	2	182	1	24.7	13	5	**YES**
8	178	1	148	2	198	1	72	1	24.6	17	8	**YES**
9	0	0	92	1	235	2	0	0	30.6	17	7	**YES**
10	305	1	181	1	0	0	361	1	44.6	14	8	**YES**
11	378	2	151	2	130	2	325	1	45.1	19	9	**YES**
12	402	1	278	1	179	1	271	1	48.9	16	7	**YES**
13	511	1	0	0	234	2	32	1	50.3	17	9	**YES**
14	107	1	306	2	185	1	228	2	38.5	21	10	**YES**
15	152	1	0	0	333	3	0	0	25.5	16	6	**YES**
16	219	2	0	0	270	2	55	1	30.7	15	8	**YES**
17	115	1	134	1	0	0	347	2	25.7	15	6	**YES**
18	0	0	0	0	0	0	40	1	25.3	14	3	**YES**
19	192	2	0	0	215	1	78	1	18.6	15	9	**YES**
20	271	1	0	0	410	2	0	0	38.9	17	7	**YES**
21	482	2	0	0	138	1	437	2	48.3	19	8	**YES**
22	351	2	0	0	363	1	197	2	53.7	20	8	**YES**
23	109	1	246	1	452	2	28	1	45.8	21	9	**YES**
24	78	1	0	0	297	2	0	0	36.7	16	6	**YES**
25	92	1	171	1	63	1	0	0	26.6	13	6	**YES**
26	197	1	0	0	0	0	340	2	37.5	14	6	**YES**
27	230	1	266	2	251	1	276	2	47.2	21	10	**YES**
28	381	1	143	1	173	1	23	1	36.3	16	6	**YES**
29	297	1	0	0	161	2	218	1	35.3	18	6	**YES**
30	214	1	177	2	246	1	203	2	36.1	16	10	**YES**
31	319	1	190	1	184	1	16	1	58.9	18	7	**YES**
32	478	1	217	1	0	0	0	0	33.6	16	6	**YES**
33	0	0	143	1	0	0	0	0	19.3	12	3	**YES**
34	501	1	176	2	228	2	142	1	43.7	23	10	**YES**
35	0	0	134	1	126	2	0	0	28.3	14	5	**YES**
36	108	1	251	2	349	2	197	1	44.1	23	9	**YES**

Biological Observation Key for the Following Observation Tables

Fish Appearance Observation Key								
Def = Deformed Fish	BL = Bloated Fish	DF = Dead Fish	DRK = Dark Fish	FP = Fatpad Present	LF = Live Fish	CB= Color Bars Present	PP = Papillae Present	TP = Tubercles Present

Fish Behavior Observation Key						
AS = Fish At Surface	CLE = Fish With Complete Loss Of Equilibrium	ERR = Erratic Movement	TA = Territorial Aggression	LETH = Lethargic	LTA = Loss Of Territorial Aggressiveness	N = None
PLE = Fish With Partial Loss Of Equilibrium	RA = Rapid Respiration	OB = On Bottom	NA = Not Applicable			

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Observation	Exposure Day										
			0	1	2	3	4	5	6	7	8	9	10
Control	A	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	239	216	93	0	123	62	26	113	527	37
		# of Infertile Eggs	NA	0	0	0	0	2	0	2	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	B	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	137	258	179	221	161	88	234	167	626	590
		# of Infertile Eggs	NA	0	0	0	0	0	0	0	2	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	C	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	115	303	206	447	191	0	360	165	0	75
		# of Infertile Eggs	NA	0	0	0	3	0	0	0	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	D	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	158	66	217	84	180	201	0	16	476	0
		# of Infertile Eggs	NA	0	0	0	0	0	0	0	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Observation	Exposure Day										
			11	12	13	14	15	16	17	18	19	20	21
Control	A	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	331	209	0	110	360	0	115	286	223	463	290
		# of Infertile Eggs	0	2	0	0	0	0	0	5	1	3	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	B	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	0	308	394	0	322	602	0	109	404	0	197
		# of Infertile Eggs	0	0	2	0	0	3	0	0	6	0	2
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	C	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	376	0	173	196	202	31	210	98	250	376	218
		# of Infertile Eggs	8	0	2	0	0	0	1	0	0	1	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	D	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	608	0	0	291	150	87	133	53	248	0	306
		# of Infertile Eggs	3	0	0	0	0	0	0	0	0	0	3
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Observation	Exposure Day										
			0	1	2	3	4	5	6	7	8	9	10
14 µg/L	A	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	343	133	176	0	0	0	97	270	151	0
		# of Infertile Eggs	NA	7	1	0	0	0	0	0	0	15	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	B	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	71	190	182	105	0	0	142	0	71	128
		# of Infertile Eggs	NA	5	1	1	1	0	0	0	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	C	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	159	348	0	278	357	0	0	88	26	402
		# of Infertile Eggs	NA	0	5	0	1	0	0	0	0	0	1
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	D	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	0	311	0	53	215	135	197	153	332	0
		# of Infertile Eggs	NA	0	0	0	0	0	0	2	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Observation	Exposure Day										
			11	12	13	14	15	16	17	18	19	20	21
14 µg/L	A	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	3
		# of Eggs	116	0	27	46	0	112	25	207	336	4	272
		# of Infertile Eggs	0	0	0	0	0	0	0	0	0	0	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	B	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	3	3	3	3	3	3	3	3	3	3	3
		# of Eggs	0	327	0	0	242	79	0	413	145	0	107
		# of Infertile Eggs	0	0	0	0	0	0	0	0	0	0	1
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	C	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	402	215	300	82	98	12	442	0	179	221	263
		# of Infertile Eggs	1	0	0	0	0	0	1	0	0	2	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	D	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	400	0	284	74	0	0	351	160	0	436	77
		# of Infertile Eggs	0	0	0	0	0	0	0	2	0	0	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Observation	Exposure Day										
			0	1	2	3	4	5	6	7	8	9	10
35 µg/L	A	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	3	3	3	3	3	3	3
		# of Eggs	NA	154	0	0	503	0	0	329	0	210	268
		# of Infertile Eggs	NA	0	0	0	52	0	0	3	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	B	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	106	142	289	418	0	0	239	200	0	687
		# of Infertile Eggs	NA	0	0	1	3	0	0	0	2	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	C	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	89	135	169	452	203	0	0	67	125	209
		# of Infertile Eggs	NA	0	0	4	5	0	0	0	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	D	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	0	232	220	147	213	278	167	29	343	0
		# of Infertile Eggs	NA	0	4	42	14	0	12	6	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Observation	Exposure Day										
			11	12	13	14	15	16	17	18	19	20	21
35 µg/L	A	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	3	3	3	3	3	3	3	3	3	3	3
		# of Eggs	168	0	245	435	0	0	0	0	0	272	174
		# of Infertile Eggs	0	0	0	0	0	0	0	0	0	1	2
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	B	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	200	0	0	0	121	42	337	320	201	251	401
		# of Infertile Eggs	0	0	0	0	0	0	2	2	0	0	2
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	C	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	307	0	0	0	0	216	0	273	343	0	79
		# of Infertile Eggs	0	0	0	0	0	2	0	2	0	0	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	D	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	147	497	127	37	305	59	283	140	0	67	371
		# of Infertile Eggs	0	0	0	0	0	0	0	0	0	0	5
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Observation	Exposure Day										
			0	1	2	3	4	5	6	7	8	9	10
88 µg/L	A	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	3	3	3	3	3	3	3
		# of Eggs	NA	0	0	126	157	0	97	291	25	345	338
		# of Infertile Eggs	NA	0	0	0	0	0	0	0	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	B	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	0	208	0	350	0	178	288	28	434	256
		# of Infertile Eggs	NA	0	0	0	0	0	0	0	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	C	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	224	359	0	0	0	66	23	0	457	0
		# of Infertile Eggs	NA	0	0	0	0	0	0	0	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	D	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	0	306	169	170	226	191	69	178	276	307
		# of Infertile Eggs	NA	0	0	1	0	0	0	0	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Observation	Exposure Day										
			11	12	13	14	15	16	17	18	19	20	21
88 µg/L	A	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	3	3	3	3	3	3	3	3	3	3	3
		# of Eggs	151	292	0	0	108	81	225	203	179	635	401
		# of Infertile Eggs	0	0	0	0	0	1	2	3	0	1	6
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	B	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	210	256	109	0	245	0	48	246	629	164	71
		# of Infertile Eggs	0	12	0	0	19	0	0	0	7	0	4
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	C	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	0	509	0	0	299	0	27	186	0	0	58
		# of Infertile Eggs	0	0	0	0	0	0	0	0	0	0	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	D	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	0	411	407	97	225	197	0	259	197	0	0
		# of Infertile Eggs	0	0	0	0	0	1	0	0	0	0	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Observation	Exposure Day										
			0	1	2	3	4	5	6	7	8	9	10
220 µg/L	A	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	50	62	271	0	77	270	0	129	227	0
		# of Infertile Eggs	NA	0	2	0	0	0	0	0	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	B	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	0	0	0	337	143	150	208	256	207	452
		# of Infertile Eggs	NA	0	0	0	0	0	0	2	5	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	C	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	0	196	188	405	309	0	71	218	0	0
		# of Infertile Eggs	NA	0	2	0	5	12	0	0	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0
	D	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	NA	0	251	324	10	0	178	210	311	481	0
		# of Infertile Eggs	NA	0	0	9	3	0	0	0	0	0	0
		# of Undiscernible Eggs	NA	0	0	0	0	0	0	0	0	0	0

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Observation	Exposure Day										
			11	12	13	14	15	16	17	18	19	20	21
220 µg/L	A	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	396	357	0	191	156	169	317	114	42	213	192
		# of Infertile Eggs	1	0	0	5	0	3	2	0	0	0	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	B	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	318	359	121	392	0	71	115	0	107	301	0
		# of Infertile Eggs	0	0	0	0	0	0	0	0	2	6	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	C	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	321	0	0	151	207	42	196	0	0	138	0
		# of Infertile Eggs	0	0	0	0	0	0	0	0	0	0	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0
	D	# of Surviving Males	2	2	2	2	2	2	2	2	2	2	2
		# of Surviving Females	4	4	4	4	4	4	4	4	4	4	4
		# of Eggs	702	0	341	0	0	0	102	122	0	107	88
		# of Infertile Eggs	0	0	0	0	0	0	3	0	0	2	0
		# of Undiscernible Eggs	0	0	0	0	0	0	0	0	0	0	0

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Rep	Observation		Exposure Day																				
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	A	CB PRESENT	Males	2	2	2	2	0	0	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	B	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Rep	Observation		Exposure Day																				
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	C	CB PRESENT	Males	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	D	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Rep	Observation		Exposure Day																				
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
14 µg/L	A	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	B	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Rep	Observation		Exposure Day																				
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
14 µg/L	C	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	D	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Rep	Observation		Exposure Day																				
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
35 µg/L	A	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	B	CB PRESENT	Males	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Rep	Observation		Exposure Day																				
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
35 µg/L	C	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	D	CB PRESENT	Males	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Rep	Observation		Exposure Day																				
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
88 µg/L	A	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	B	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Rep	Observation		Exposure Day																				
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
88 µg/L	C	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	D	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Rep	Observation		Exposure Day																				
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
220 µg/L	A	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	B	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Daily exposure observations during the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Rep	Observation		Exposure Day																				
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
220 µg/L	C	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	D	CB PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		FP PRESENT	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		TA OBSERVED	Males	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
			Females	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		BEHAVIOR OBS	Males	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			Females	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Day 21 Termination Measurements for the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Gender	Sample ID	Total Length (mm)	Wet Weight (g)	Gonad Weight (g)	Tubercle Score	GSI (%)	Gonad Type (O or T)	Ovipositor Present? (X = present)
Control	A	M	5AM1	51.41	4.5377	0.0744	21	1.6396	T	
		M	5AM2	48.63	4.1920	0.0287	25	0.6846	T	
		F	5AF1	45.46	2.1604	0.2044	0	9.4612	O	X
		F	5AF2	47.37	2.4371	0.4160	0	17.0695	O	X
		F	5AF3	50.26	2.8440	0.4466	0	15.7032	O	X
		F	5AF4	41.57	1.8435	0.2375	0	12.8831	O	X
	B	M	5BM1	58.88	4.9410	0.0288	20	0.5829	T	
		M	5BM2	57.93	5.2699	0.0563	21	1.0683	T	
		F	5BF1	43.83	2.4587	0.3195	0	12.9947	O	X
		F	5BF2	40.92	2.2568	0.5403	0	23.9410	O	X
		F	5BF3	47.17	2.2036	0.2892	0	13.1240	O	X
		F	5BF4	49.71	2.6235	0.4357	0	16.6076	O	X
	C	M	5CM1	59.76	5.0419	0.0373	20	0.7398	T	
		M	5CM2	53.11	4.1054	0.0552	29	1.3446	T	
		F	5CF1	40.37	1.9731	0.3614	0	18.3164	O	X
		F	5CF2	42.34	1.7432	0.1892	0	10.8536	O	X
		F	5CF3	47.65	2.4997	0.2222	0	8.8891	O	X
		F	5CF4	45.01	2.2606	0.3926	0	17.3671	O	X
	D	M	5DM1	47.65	3.4066	0.0561	23	1.6468	T	
		M	5DM2	49.77	3.3671	0.0548	16	1.6275	T	
		F	5DF1	47.81	2.3741	0.2838	0	11.9540	O	X
		F	5DF2	43.16	1.9845	0.3862	0	19.4608	O	X
		F	5DF3	45.15	2.5674	0.5241	0	20.4136	O	X
		F	5DF4	43.83	1.8327	0.2524	0	13.7720	O	X

Day 21 Termination Measurements for the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Gender	Sample ID	Total Length (mm)	Wet Weight (g)	Gonad Weight (g)	Tubercle Score	GSI (%)	Gonad Type (O or T)	Ovipositor Present? (X = present)
14 µg/L	A	M	4AM1	55.76	4.1621	0.0554	17	1.3311	T	
		M	4AM2	53.74	3.9609	0.0803	19	2.0273	T	
		F	4AF1	49.99	2.3406	0.2171	0	9.2754	O	X
		F	4AF2	41.19	1.7958	0.2197	0	12.2341	O	X
		F	4AF3	44.11	1.6376	0.1857	0	11.3398	O	X
		F	4AF4	NA	NA	NA	0	NA	O	
	B	M	4BM1	49.56	3.9205	0.0521	23	1.3289	T	
		M	4BM2	54.29	4.9282	0.0710	25	1.4407	T	
		F	4BF1	44.95	2.1113	0.2690	0	12.7410	O	X
		F	4BF2	43.80	1.9412	0.3299	0	16.9946	O	X
		F	4BF3	49.51	2.4012	0.1923	0	8.0085	O	X
		F	4BF4	NA	NA	NA	0	NA	O	
	C	M	4CM1	58.23	4.3830	0.0634	14	1.4465	T	
		M	4CM2	52.70	3.8984	0.0445	14	1.1415	T	
		F	4CF1	46.09	2.5041	0.3809	0	15.2111	O	X
		F	4CF2	48.01	2.5120	0.3582	0	14.2596	O	X
		F	4CF3	46.99	2.6631	0.3497	0	13.1313	O	X
		F	4CF4	48.38	2.8492	0.4713	0	16.5415	O	X
	D	M	4DM1	55.44	4.7729	0.0974	27	2.0407	T	
		M	4DM2	46.48	3.6240	0.0653	20	1.8019	T	
		F	4DF1	45.77	2.1854	0.2632	0	12.0436	O	X
		F	4DF2	44.89	2.5644	0.4231	0	16.4990	O	X
		F	4DF3	45.68	2.4140	0.3706	0	15.3521	O	X
		F	4DF4	47.82	2.7817	0.4504	0	16.1915	O	X

Day 21 Termination Measurements for the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Gender	Sample ID	Total Length (mm)	Wet Weight (g)	Gonad Weight (g)	Tubercle Score	GSI (%)	Gonad Type (O or T)	Ovipositor Present? (X = present)
35 µg/L	A	M	3AM1	54.90	4.5971	0.0360	19	0.7831	T	
		M	3AM2	57.78	4.6594	0.0694	15	1.4895	T	
		F	3AF1	47.83	2.4145	0.2368	0	9.8074	O	X
		F	3AF2	48.53	2.8164	0.3012	0	10.6945	O	X
		F	3AF3	44.86	2.4505	0.5682	0	23.1871	O	X
		F	3AF4	NA	NA	NA	0	NA	O	
	B	M	3BM1	61.33	5.6141	0.1019	20	1.8151	T	
		M	3BM2	54.23	4.1750	0.0679	14	1.6263	T	
		F	3BF1	45.81	2.6944	0.4758	0	17.6588	O	X
		F	3BF2	44.86	2.2435	0.3663	0	16.3272	O	X
		F	3BF3	45.58	2.0859	0.2935	0	14.0707	O	X
		F	3BF4	43.76	1.9435	0.2638	0	13.5734	O	X
	C	M	3CM1	57.00	5.3589	0.0722	8	1.3473	T	
		M	3CM2	52.68	4.4235	0.0678	19	1.5327	T	
		F	3CF1	47.04	2.3914	0.4469	0	18.6878	O	X
		F	3CF2	48.31	1.9548	0.2342	0	11.9808	O	X
		F	3CF3	46.96	2.5294	0.4614	0	18.2415	O	X
		F	3CF4	46.06	2.1693	0.2522	0	11.6259	O	X
	D	M	3DM1	54.06	4.0024	0.0342	16	0.8545	T	
		M	3DM2	52.67	4.1880	0.0533	20	1.2727	T	
		F	3DF1	51.11	2.9943	0.5077	0	16.9555	O	X
		F	3DF2	46.33	2.5638	0.3415	0	13.3201	O	X
		F	3DF3	47.96	2.6335	0.3666	0	13.9206	O	X
		F	3DF4	45.49	2.3644	0.2727	0	11.5336	O	X

Day 21 Termination Measurements for the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Gender	Sample ID	Total Length (mm)	Wet Weight (g)	Gonad Weight (g)	Tubercle Score	GSI (%)	Gonad Type (O or T)	Ovipositor Present? (X = present)
88 µg/L	A	M	2AM1	50.79	4.1689	0.0380	11	0.9115	T	
		M	2AM2	49.36	3.8348	0.0664	24	1.7315	T	
		F	2AF1	46.16	2.3670	0.2696	0	11.3899	O	X
		F	2AF2	47.11	2.4651	0.4406	0	17.8735	O	X
		F	2AF3	45.17	2.3602	0.4627	0	19.6043	O	X
		F	2AF4	NA	NA	NA	0	NA	O	
	B	M	2BM1	48.31	3.7593	0.0480	23	1.2768	T	
		M	2BM2	51.11	4.6320	0.0865	23	1.8674	T	
		F	2BF1	49.02	2.5697	0.4313	0	16.7841	O	X
		F	2BF2	46.83	2.3529	0.5533	0	23.5157	O	X
		F	2BF3	44.70	2.1494	0.4544	0	21.1408	O	X
		NA	2BF4	44.50	2.1557	0.4701	0	21.8073	O	X
	C	M	2CM1	54.89	4.6935	0.0686	23	1.4616	T	
		M	2CM2	56.41	4.2325	0.0670	14	1.5830	T	
		F	2CF1	45.20	1.8338	0.0398	0	2.1704	O	X
		F	2CF2	47.81	2.3384	0.3467	0	14.8264	O	X
		F	2CF3	43.77	1.8674	0.1938	0	10.3781	O	X
		F	2CF4	45.13	2.1651	0.4527	0	20.9090	O	X
	D	M	2DM1	54.74	3.7342	0.0416	18	1.1140	T	
		M	2DM2	50.64	3.8848	0.0827	6	2.1288	T	
		F	2DF1	43.42	2.1732	0.2671	0	12.2906	O	X
		F	2DF2	45.43	2.4197	0.4156	0	17.1757	O	X
		F	2DF3	46.93	2.4475	0.6135	0	25.0664	O	X
		F	2DF4	46.22	2.2989	0.4258	0	18.5219	O	X

Day 21 Termination Measurements for the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Nominal Concentration	Replicate	Gender	Sample ID	Total Length (mm)	Wet Weight (g)	Gonad Weight (g)	Tubercle Score	GSI (%)	Gonad Type (O or T)	Ovipositor Present? (X = present)
220 µg/L	A	M	1AM1	53.51	4.3592	0.0918	22	2.1059	T	
		M	1AM2	49.78	3.7273	0.0603	21	1.6178	T	
		F	1AF1	46.26	2.1602	0.3427	0	15.8643	O	X
		F	1AF2	46.42	2.4051	0.3526	0	14.6605	O	X
		F	1AF3	44.55	2.0396	0.2865	0	14.0469	O	X
		F	1AF4	46.23	2.3674	0.5629	0	23.7771	O	X
	B	M	1BM1	50.17	3.6827	0.0694	17	1.8845	T	
		M	1BM2	50.12	3.7027	0.0982	17	2.6521	T	
		F	1BF1	45.60	2.4362	0.4380	0	17.9788	O	X
		F	1BF2	44.22	2.0507	0.4246	0	20.7051	O	X
		F	1BF3	46.78	2.5791	0.5437	0	21.0810	O	X
		F	1BF4	44.39	2.0051	0.3980	0	19.8494	O	X
	C	M	1CM1	57.02	4.7427	0.1022	23	2.1549	T	
		M	1CM2	51.11	4.2773	0.1001	16	2.3403	T	
		F	1CF1	47.08	2.6389	0.4931	0	18.6858	O	X
		F	1CF2	47.36	2.2358	0.3416	0	15.2786	O	X
		F	1CF3	45.99	2.6148	0.5432	0	20.7741	O	X
		F	1CF4	44.47	1.9874	0.3903	0	19.6387	O	X
	D	M	1DM1	51.10	4.0646	0.0704	18	1.7320	T	
		M	1DM2	52.33	3.9018	0.0773	17	1.9811	T	
		F	1DF1	47.57	2.3165	0.2271	0	9.8036	O	X
		F	1DF2	43.91	2.1282	0.3902	0	18.3347	O	X
		F	1DF3	46.55	2.7065	0.5565	0	20.5616	O	X
		F	1DF4	51.94	3.0139	0.4282	0	14.2075	O	X

VTG Averages by Sex and Concentration All Samples								
		MALES	%			FEMALES	%	
		ng/mL	reduction	SD		ng/mL	reduction	SD
Control	5A	85				1225000		
	5B	390				1573924		
	5C	99				954283		
	5D	80				2322224		
	TOTAL AVERAGE:	164	NA	151		1518858	NA	592600
0.0012	4A	104060				3287348		
	4B	1155				848469		
	4C	8263				3788502		
	4D	90				3617512		
	TOTAL AVERAGE:	28392	-17262.43%	50576		2885458	-89.98%	1373831
0.0029	3A	1753				918261		
	3B	40704				1957802		
	3C	9075				10818131		
	3D	52				3052624		
	TOTAL AVERAGE:	12896	-7786.09%	18947		4186704	-175.65%	4506021
0.0072	2A	11045548				3390670		
	2B	7690001				1054948		
	2C	14750057				782436		
	2D	433403				119277		
	TOTAL AVERAGE:	8479752	-5185487.92%	6090087		1336833	11.98%	1424489
0.018	1A	24186650				595388		
	1B	ADL				1043657		
	1C	13278152				3298270		
	1D	ADL				2817815		
	TOTAL AVERAGE:	18732401	-11455248.21%	7713473		1938782	-27.65%	1319958

VTG Averages by Sex and Concentration Excluding Outliers								
		MALES	%			FEMALES	%	
		ng/mL	reduction	SD		ng/mL	reduction	SD
Control	5A	85				1225000		
	5B	239				1573924		
	5C	99				954283		
	5D	80				2322224		
	TOTAL AVERAGE:	126	NA	76		1518858	NA	592600
0.0012	4A	7457				3287348		
	4B	1155				848469		
	4C	8263				3863420		
	4D	90				3617512		
	TOTAL AVERAGE:	4241	-3272.53%	4214		2904187	-91.21%	1390654
0.0029	3A	1753				1376675		
	3B	10686				1957802		
	3C	9075				10818131		
	3D	52				3052624		
	TOTAL AVERAGE:	5391	-4187.12%	5271		4301308	-183.19%	4399761
0.0072	2A	11045548				3390670		
	2B	7690001				1054948		
	2C	14750057				782436		
	2D	433403				119277		
	TOTAL AVERAGE:	8479752	-6742846.16%	6090087		1336833	11.98%	1424489
0.018	1A	24186650				595388		
	1B	ADL				1043657		
	1C	13278152				3298270		
	1D	ADL				3757084		
	TOTAL AVERAGE:	18732401	-14895568.04%	7713473		2173600	-43.11%	1585332

QC's									
Applicable Plate	**Un/Fortified**	**Conc Reading**		**Dilution Factor**	**Conc x Dil Factor (ng/mL)**		**Mean Result**	**SD**	**CV**
		Replicate A	**Replicate B**		**Replicate A**	**Replicate B**			
03-010417	Unfortified	5.753	5.96	50	288	298	293	7	2.5%
		BDL	BDL	5000	BDL	BDL	NA	NA	NA
		BDL	BDL	500000	BDL	BDL	NA	NA	NA
	Fortified	ADL	ADL	50	ADL	ADL	NA	NA	NA
		15.048	15.321	5000	75240	76605	75923	965	1.3%
		0.134	0.186	500000	67000	93000	80000	18385	23.0%
04-010517	Unfortified	7.812	8.005	50	391	400	395	7	1.7%
		BDL	BDL	5000	BDL	BDL	NA	NA	NA
		BDL	BDL	5000	BDL	BDL	NA	NA	NA
	Fortified	ADL	ADL	50	ADL	ADL	NA	NA	NA
		12.580	12.234	5000	62900	61170	62035	1223	2.0%
		BDL	0.12	500000	NA	60000	60000	NA	NA
11-011017	Unfortified	4.915	5.471	50	246	274	260	20	7.6%
		BDL	0.018	5000	BDL	90	90	NA	NA
		BDL	BDL	500000	BDL	NA	NA	NA	NA
	Fortified	ADL	ADL	50	BDL	BDL	NA	NA	NA
		9.762	9.856	5000	48810	49280	49045	332	0.7%
		BDL	0.018	500000	BDL	9000	9000	NA	NA

For all analysis:	**Mean (ng/mL)**	**SD**	**CV**
Unfortified	316	8	3%
Fortified	49667	840	2%
Fortification spike	50000		
Expected fortified concentration	50316		
Percent recovery of fortification standard	99%		

Determination of ½ the LOQ during VTG analysis for the 21-day exposure of fathead minnow (*Pimephales promelas*) to 2-ethylhexyl paraben

Plate #	Lowest Usable Concentration result from Standard Curve (ng/mL)	Mean multiplied by Lowest Dilution Factor (1:50)	1/2 of the LOQ
01-010417	0.063		
	0.078		
Mean:	0.071	3.53	1.763E+00
02-010417	0.346		
	0.309		
Mean:	0.328	16.38	8.188E+00
03-010417	0.186		
	0.180		
Mean:	0.183	9.15	4.575E+00
04-010517	0.419		
	0.374		
Mean:	0.397	19.83	9.913E+00
05-010517	0.061		
	0.071		
Mean:	0.066	3.30	1.650E+00
06-010517	0.976		
	0.909		
Mean:	0.943	47.13	2.356E+01
07-010517	0.082		
	0.100		
Mean:	0.091	4.55	2.275E+00
08-011017	0.099		
	0.061		
Mean:	0.080	4.00	2.000E+00
09-011017	0.058		
	0.058		
Mean:	0.058	2.90	1.450E+00
10-011017	0.429		
	0.404		
Mean:	0.417	20.83	1.041E+01
11-011017	0.444		
	0.364		
Mean:	0.404	20.20	1.010E+01

APPENDIX 9 – EPA-PRESCRIBED DATA ENTRY SPREADSHEET TEMPLATES (DESTS)

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Pre-test Body Weight

	Sex (M or F)	Pre-Test Animal ID	Initial Body Weight (g)
	M	1	5.19
	M	2	5.51
	M	3	4.47
	M	4	4.78
	M	5	4.60
	M	6	3.03
	M	7	3.86
	M	8	3.01
	M	9	4.95
	M	10	4.63
	M	11	4.23
	M	12	4.77
	M	13	4.22
	M	14	4.35
	M	15	3.65
	M	16	3.88
	M	17	4.16
	M	18	5.23
	M	19	4.40
	M	20	2.83
	M	21	3.20
	M	22	3.97
	M	23	3.59
	M	24	4.96
	M	25	4.69
	M	26	5.13
	M	27	4.41
	M	28	4.00
	M	29	4.56
	M	30	4.17
	M	31	4.79
	M	32	4.38
	M	33	5.12
	M	34	3.59
	M	35	3.93
	M	36	5.13
	M	37	4.69
	M	38	4.45
	M	39	4.38
	M	40	4.97
	M	41	5.13
	M	42	3.67
	M	43	4.73
	M	44	4.84
	M	45	4.78
	M	46	4.20
	M	47	4.12
	M	48	4.53
	M	49	5.10
	M	50	4.19
	M	51	4.16
	M	52	5.13

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Pre-test Body Weight

	Sex (M or F)	Pre-Test Animal ID	Initial Body Weight (g)
54	M	53	4.60
55	M	54	5.04
56	M	55	4.83
57	M	56	4.50
58	M	57	4.72
59	M	58	5.05
60	M	59	3.89
61	M	60	4.70
62	M	61	3.94
63	M	62	5.07
64	M	63	4.31
65	M	64	4.49
66	M	65	3.99
67	M	66	4.30
68	M	67	4.12
69	M	68	4.80
70	M	69	4.84
71	M	70	3.85
72	M	71	5.10
73	M	72	4.89
74	F	1	4.00
75	F	2	2.57
76	F	3	2.65
77	F	4	2.48
78	F	5	2.94
79	F	6	2.68
80	F	7	3.11
81	F	8	2.31
82	F	9	1.63
83	F	10	2.27
84	F	11	3.22
85	F	12	3.23
86	F	13	2.36
87	F	14	2.99
88	F	15	2.53
89	F	16	3.54
90	F	17	2.05
91	F	18	2.87
92	F	19	2.85
93	F	20	3.74
94	F	21	3.15
95	F	22	2.40
96	F	23	2.16
97	F	24	2.62
98	F	25	2.44
99	F	26	2.21
100	F	27	2.75
101	F	28	3.48
102	F	29	2.52
103	F	30	3.30
104	F	31	2.33
105	F	32	2.39

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Pre-test Body Weight

	Sex (M or F)	Pre-Test Animal ID	Initial Body Weight (g)
106	F	33	2.52
107	F	34	3.15
108	F	35	3.20
109	F	36	2.38
110	F	37	2.22
111	F	38	2.81
112	F	39	3.30
113	F	40	2.56
114	F	41	2.92
115	F	42	2.72
116	F	43	2.46
117	F	44	3.31
118	F	45	2.22
119	F	46	3.18
120	F	47	2.89
121	F	48	3.30
122	F	49	2.70
123	F	50	3.27
124	F	51	3.21
125	F	52	2.90
126	F	53	2.94
127	F	54	2.91
128	F	55	3.30
129	F	56	2.77
130	F	57	2.62
131	F	58	2.38
132	F	59	2.36
133	F	60	2.39
134	F	61	2.25
135	F	62	3.02
136	F	63	3.29
137	F	64	3.31
138	F	65	2.57
139	F	66	2.23
140	F	67	2.49
141	F	68	3.31
142	F	69	2.48
143	F	70	2.66
144	F	71	2.61
145	F	72	3.29
146	F	73	3.17
147	F	74	2.76
148	F	75	2.75
149	F	76	3.16
150	F	77	3.31
151	F	78	3.28
152	F	79	3.21
153	F	80	2.53
154	F	81	2.30
155	F	82	2.57
156	F	83	2.24
157	F	84	2.65

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Pre-test Body Weight

	Sex (M or F)	Pre-Test Animal ID	Initial Body Weight (g)
158	F	85	3.10
159	F	86	2.48
160	F	87	2.69
161	F	88	2.85
162	F	89	3.14
163	F	90	3.20
164	F	91	3.30
165	F	92	3.01
166	F	93	2.25
167	F	94	2.26
168	F	95	3.27
169	F	96	2.52
170	F	97	2.87
171	F	98	2.28
172	F	99	2.96
173	F	100	2.25
174	F	101	3.30
175	F	102	2.47
176	F	103	3.23
177	F	104	2.83
178	F	105	2.77
179	F	106	2.44
180	F	107	2.51
181	F	108	2.95
182	F	109	2.22
183	F	110	2.82
184	F	111	2.25
185	F	112	2.81
186	F	113	2.65
187	F	114	3.29
188	F	115	3.21
189	F	116	2.25
190	F	117	2.66
191	F	118	3.08
192	F	119	3.05
193	F	120	2.23
194	F	121	2.29
195	F	122	2.52
196	F	123	2.74
197	F	124	3.30
198	F	125	2.38
199	F	126	2.28
200	F	127	2.52
201	F	128	3.27
202	F	129	2.22
203	F	130	2.54
204	F	131	3.13
205	F	132	3.30
206	F	133	2.27
207	F	134	3.06
208	F	135	2.71
209	F	136	2.34

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Pre-test Body Weight

	Sex (M or F)	Pre-Test Animal ID	Initial Body Weight (g)
210	F	137	2.26
211	F	138	3.21
212	F	139	2.45
213	F	140	2.25
214	F	141	2.23
215	F	142	2.30
216	F	143	2.22
217	F	144	2.22

13784.6120 Biologica Data DESTS.xlsx

Pre-exposure Observations

	Tank ID	Pre-test Day (-24 to -1)	Number of Surviving Males	Number of Surviving Females	Number of Spawns	Number of Eggs	Number of Fertilized Eggs	Comments
	1	-16	2	4	1	160	NA	NA
	2	-16	2	4	2	41	NA	NA
	3	-16	2	4	1	103	NA	NA
	4	-16	2	4	0	0	NA	NA
	5	-16	2	4	1	209	NA	NA
	6	-16	2	4	2	287	NA	NA
	7	-16	2	4	0	0	NA	NA
	8	-16	2	4	0	0	NA	NA
	9	-16	2	4	0	0	NA	NA
	10	-16	2	4	0	0	NA	NA
	11	-16	2	4	0	0	NA	NA
	12	-16	2	4	2	225	NA	NA
	13	-16	2	4	1	225	NA	NA
	14	-16	2	4	0	0	NA	NA
	15	-16	2	4	0	0	NA	NA
	16	-16	2	4	0	0	NA	NA
	17	-16	2	4	1	96	NA	NA
	18	-16	2	4	1	42	NA	NA
	19	-16	2	4	0	0	NA	NA
	20	-16	2	4	0	0	NA	NA
	21	-16	2	4	0	0	NA	NA
	22	-16	2	4	1	212	NA	NA
	23	-16	2	4	2	136	NA	NA
	24	-16	2	4	0	0	NA	NA
	25	-16	2	4	0	0	NA	NA
	26	-16	2	4	0	0	NA	NA
	27	-16	2	4	0	0	NA	NA
	28	-16	2	4	2	157	NA	NA
	29	-16	2	4	1	298	NA	NA
	30	-16	2	4	0	0	NA	NA
	31	-16	2	4	1	312	NA	NA
	32	-16	2	4	0	0	NA	NA
	33	-16	2	4	0	0	NA	NA
	34	-16	2	4	1	78	NA	NA
	35	-16	2	4	2	199	NA	NA
	36	-16	2	4	0	0	NA	NA
	1	-15	2	4	1	270	NA	NA
	2	-15	2	4	2	541	NA	NA
	3	-15	2	4	2	231	NA	NA
	4	-15	2	4	1	89	NA	NA
	5	-15	2	4	1	92	NA	NA
	6	-15	2	4	2	140	NA	NA
	7	-15	2	4	2	250	NA	NA
	8	-15	2	4	2	39	NA	NA
	9	-15	2	4	2	366	NA	NA
	10	-15	2	4	1	408	NA	NA
	11	-15	2	4	1	46	NA	NA
	12	-15	2	4	1	235	NA	NA
	13	-15	2	4	1	246	NA	NA
	14	-15	2	4	1	21	NA	NA
	15	-15	2	4	1	16	NA	NA
	16	-15	2	4	1	178	NA	NA
	17	-15	2	4	2	227	NA	NA
	18	-15	2	4	2	262	NA	NA

13784.6120 Biologica Data DESTS.xlsx

Pre-exposure Observations

	Tank ID	Pre-test Day (-24 to -1)	Number of Surviving Males	Number of Surviving Females	Number of Spawns	Number of Eggs	Number of Fertilized Eggs	Comments
56	19	-15	2	4	0	0	NA	NA
57	20	-15	2	4	2	103	NA	NA
58	21	-15	2	4	1	323	NA	NA
59	22	-15	2	4	2	340	NA	NA
60	23	-15	2	4	2	344	NA	NA
61	24	-15	2	4	3	396	NA	NA
62	25	-15	2	4	2	408	NA	NA
63	26	-15	2	4	2	336	NA	NA
64	27	-15	2	4	2	260	NA	NA
65	28	-15	2	4	0	0	NA	NA
66	29	-15	2	4	2	362	NA	NA
67	30	-15	2	4	1	305	NA	NA
68	31	-15	2	4	2	444	NA	NA
69	32	-15	2	4	1	36	NA	NA
70	33	-15	2	4	1	72	NA	NA
71	34	-15	2	4	2	104	NA	NA
72	35	-15	2	4	1	40	NA	NA
73	36	-15	2	4	3	643	NA	NA
74	1	-14	2	4	1	145	NA	NA
75	2	-14	2	4	0	0	NA	NA
76	3	-14	2	4	2	206	NA	NA
77	4	-14	2	4	0	0	NA	NA
78	5	-14	2	4	0	0	NA	NA
79	6	-14	2	4	1	194	NA	NA
80	7	-14	2	4	0	0	NA	NA
81	8	-14	2	4	0	0	NA	NA
82	9	-14	2	4	1	159	NA	NA
83	10	-14	2	4	0	0	NA	NA
84	11	-14	2	4	1	198	NA	NA
85	12	-14	2	4	1	174	NA	NA
86	13	-14	2	4	0	0	NA	NA
87	14	-14	2	4	2	462	NA	NA
88	15	-14	2	4	3	59	NA	NA
89	16	-14	2	4	1	181	NA	NA
90	17	-14	2	4	0	0	NA	NA
91	18	-14	2	4	2	278	NA	NA
92	19	-14	2	4	1	54	NA	NA
93	20	-14	2	4	2	331	NA	NA
94	21	-14	2	4	2	160	NA	NA
95	22	-14	2	4	2	225	NA	NA
96	23	-14	2	4	1	208	NA	NA
97	24	-14	2	4	2	42	NA	NA
98	25	-14	2	4	0	0	NA	NA
99	26	-14	2	4	0	0	NA	NA
100	27	-14	2	4	2	262	NA	NA
101	28	-14	2	4	2	319	NA	NA
102	29	-14	2	4	2	167	NA	NA
103	30	-14	2	4	0	0	NA	NA
104	31	-14	2	4	0	0	NA	NA
105	32	-14	2	4	2	395	NA	NA
106	33	-14	2	4	2	169	NA	NA
107	34	-14	2	4	2	222	NA	NA
108	35	-14	2	4	1	38	NA	NA
109	36	-14	2	4	2	105	NA	NA

13784.6120 Biologica Data DESTS.xlsx

Pre-exposure Observations

	Tank ID	Pre-test Day (-24 to -1)	Number of Surviving Males	Number of Surviving Females	Number of Spawns	Number of Eggs	Number of Fertilized Eggs	Comments
110	1	-13	2	4	1	156	NA	NA
111	2	-13	2	4	2	404	NA	NA
112	3	-13	2	4	1	86	NA	NA
113	4	-13	2	4	2	127	NA	NA
114	5	-13	2	4	2	585	NA	NA
115	6	-13	2	4	2	436	NA	NA
116	7	-13	2	4	1	111	NA	NA
117	8	-13	2	4	1	98	NA	NA
118	9	-13	2	4	1	76	NA	NA
119	10	-13	2	4	1	213	NA	NA
120	11	-13	2	4	1	220	NA	NA
121	12	-13	2	4	0	0	NA	NA
122	13	-13	2	4	2	622	NA	NA
123	14	-13	2	4	2	149	NA	NA
124	15	-13	2	4	0	0	NA	NA
125	16	-13	2	4	1	162	NA	NA
126	17	-13	2	4	1	90	NA	NA
127	18	-13	2	4	1	46	NA	NA
128	19	-13	2	4	0	0	NA	NA
129	20	-13	2	4	0	0	NA	NA
130	21	-13	2	4	1	88	NA	NA
131	22	-13	2	4	2	232	NA	NA
132	23	-13	2	4	1	75	NA	NA
133	24	-13	2	4	1	336	NA	NA
134	25	-13	2	4	0	0	NA	NA
135	26	-13	2	4	1	22	NA	NA
136	27	-13	2	4	1	132	NA	NA
137	28	-13	2	4	1	61	NA	NA
138	29	-13	2	4	2	161	NA	NA
139	30	-13	2	4	1	103	NA	NA
140	31	-13	2	4	1	289	NA	NA
141	32	-13	2	4	2	64	NA	NA
142	33	-13	2	4	2	303	NA	NA
143	34	-13	2	4	1	35	NA	NA
144	35	-13	2	4	1	207	NA	NA
145	36	-13	2	4	1	74	NA	NA
146	1	-12	2	4	3	66	NA	NA
147	2	-12	2	4	2	92	NA	NA
148	3	-12	2	4	1	116	NA	NA
149	4	-12	2	4	2	86	NA	NA
150	5	-12	2	4	1	32	NA	NA
151	6	-12	2	4	2	111	NA	NA
152	7	-12	2	4	1	161	NA	NA
153	8	-12	2	4	2	97	NA	NA
154	9	-12	2	4	1	230	NA	NA
155	10	-12	2	4	1	301	NA	NA
156	11	-12	2	4	1	73	NA	NA
157	12	-12	2	4	2	365	NA	NA
158	13	-12	2	4	0	0	NA	NA
159	14	-12	2	4	2	84	NA	NA
160	15	-12	2	4	1	127	NA	NA
161	16	-12	2	4	1	97	NA	NA
162	17	-12	2	4	2	113	NA	NA
163	18	-12	2	4	2	201	NA	NA

13784.6120 Biologica Data DESTS.xlsx

Pre-exposure Observations

	Tank ID	Pre-test Day (-24 to -1)	Number of Surviving Males	Number of Surviving Females	Number of Spawns	Number of Eggs	Number of Fertilized Eggs	Comments
164	19	-12	2	4	1	30	NA	NA
165	20	-12	2	4	2	167	NA	NA
166	21	-12	2	4	2	382	NA	NA
167	22	-12	2	4	1	57	NA	NA
168	23	-12	2	4	2	198	NA	NA
169	24	-12	2	4	2	269	NA	NA
170	25	-12	2	4	2	202	NA	NA
171	26	-12	2	4	2	130	NA	NA
172	27	-12	2	4	1	244	NA	NA
173	28	-12	2	4	1	50	NA	NA
174	29	-12	2	4	1	106	NA	NA
175	30	-12	2	4	1	71	NA	NA
176	31	-12	2	4	2	392	NA	NA
177	32	-12	2	4	2	201	NA	NA
178	33	-12	2	4	1	17	NA	NA
179	34	-12	2	4	2	243	NA	NA
180	35	-12	2	4	1	83	NA	NA
181	36	-12	2	4	2	115	NA	NA
182	1	-11	2	4	1	52	NA	NA
183	2	-11	2	4	0	0	NA	NA
184	3	-11	2	4	1	99	NA	NA
185	4	-11	2	4	0	0	NA	NA
186	5	-11	2	4	0	0	NA	NA
187	6	-11	2	4	1	128	NA	NA
188	7	-11	2	4	1	67	NA	NA
189	8	-11	2	4	1	23	NA	NA
190	9	-11	2	4	1	38	NA	NA
191	10	-11	2	4	0	0	NA	NA
192	11	-11	2	4	1	130	NA	NA
193	12	-11	2	4	0	0	NA	NA
194	13	-11	2	4	2	451	NA	NA
195	14	-11	2	4	0	0	NA	NA
196	15	-11	2	4	0	0	NA	NA
197	16	-11	2	4	1	141	NA	NA
198	17	-11	2	4	1	105	NA	NA
199	18	-11	2	4	1	36	NA	NA
200	19	-11	2	4	2	44	NA	NA
201	20	-11	2	4	1	49	NA	NA
202	21	-11	2	4	0	0	NA	NA
203	22	-11	2	4	1	77	NA	NA
204	23	-11	2	4	1	31	NA	NA
205	24	-11	2	4	0	0	NA	NA
206	25	-11	2	4	1	118	NA	NA
207	26	-11	2	4	1	156	NA	NA
208	27	-11	2	4	0	0	NA	NA
209	28	-11	2	4	2	202	NA	NA
210	29	-11	2	4	0	0	NA	NA
211	30	-11	2	4	1	226	NA	NA
212	31	-11	2	4	2	59	NA	NA
213	32	-11	2	4	0	0	NA	NA
214	33	-11	2	4	1	92	NA	NA
215	34	-11	2	4	1	17	NA	NA
216	35	-11	2	4	0	0	NA	NA
217	36	-11	2	4	1	20	NA	NA

13784.6120 Biologica Data DESTS.xlsx

Pre-exposure Observations

	Tank ID	Pre-test Day (-24 to -1)	Number of Surviving Males	Number of Surviving Females	Number of Spawns	Number of Eggs	Number of Fertilized Eggs	Comments
218	1	-10	2	4	1	136	NA	NA
219	2	-10	2	4	2	388	NA	NA
220	3	-10	2	4	0	0	NA	NA
221	4	-10	2	4	1	271	NA	NA
222	5	-10	2	4	1	308	NA	NA
223	6	-10	2	4	1	189	NA	NA
224	7	-10	2	4	1	72	NA	NA
225	8	-10	2	4	1	121	NA	NA
226	9	-10	2	4	1	92	NA	NA
227	10	-10	2	4	2	197	NA	NA
228	11	-10	2	4	2	159	NA	NA
229	12	-10	2	4	0	0	NA	NA
230	13	-10	2	4	1	239	NA	NA
231	14	-10	2	4	1	115	NA	NA
232	15	-10	2	4	1	22	NA	NA
233	16	-10	2	4	1	59	NA	NA
234	17	-10	2	4	1	107	NA	NA
235	18	-10	2	4	1	112	NA	NA
236	19	-10	2	4	1	28	NA	NA
237	20	-10	2	4	1	211	NA	NA
238	21	-10	2	4	1	49	NA	NA
239	22	-10	2	4	1	160	NA	NA
240	23	-10	2	4	1	76	NA	NA
241	24	-10	2	4	0	0	NA	NA
242	25	-10	2	4	0	0	NA	NA
243	26	-10	2	4	1	131	NA	NA
244	27	-10	2	4	2	150	NA	NA
245	28	-10	2	4	0	0	NA	NA
246	29	-10	2	4	1	73	NA	NA
247	30	-10	2	4	1	118	NA	NA
248	31	-10	2	4	1	149	NA	NA
249	32	-10	2	4	1	107	NA	NA
250	33	-10	2	4	0	0	NA	NA
251	34	-10	2	4	1	38	NA	NA
252	35	-10	2	4	0	0	NA	NA
253	36	-10	2	4	2	88	NA	NA
254	1	-9	2	4	1	74	NA	NA
255	2	-9	2	4	0	0	NA	NA
256	3	-9	2	4	0	0	NA	NA
257	4	-9	2	4	1	260	NA	NA
258	5	-9	2	4	1	213	NA	NA
259	6	-9	2	4	1	197	NA	NA
260	7	-9	2	4	1	39	NA	NA
261	8	-9	2	4	1	56	NA	NA
262	9	-9	2	4	2	91	NA	NA
263	10	-9	2	4	1	319	NA	NA
264	11	-9	2	4	1	107	NA	NA
265	12	-9	2	4	1	48	NA	NA
266	13	-9	2	4	1	103	NA	NA
267	14	-9	2	4	1	68	NA	NA
268	15	-9	2	4	1	135	NA	NA
269	16	-9	2	4	0	0	NA	NA
270	17	-9	2	4	1	85	NA	NA
271	18	-9	2	4	0	0	NA	NA

13784.6120 Biologica Data DESTS.xlsx

Pre-exposure Observations

	Tank ID	Pre-test Day (-24 to -1)	Number of Surviving Males	Number of Surviving Females	Number of Spawns	Number of Eggs	Number of Fertilized Eggs	Comments
272	19	-9	2	4	0	0	NA	NA
273	20	-9	2	4	1	44	NA	NA
274	21	-9	2	4	1	213	NA	NA
275	22	-9	2	4	0	0	NA	NA
276	23	-9	2	4	1	56	NA	NA
277	24	-9	2	4	1	149	NA	NA
278	25	-9	2	4	1	102	NA	NA
279	26	-9	2	4	0	0	NA	NA
280	27	-9	2	4	1	188	NA	NA
281	28	-9	2	4	1	65	NA	NA
282	29	-9	2	4	2	204	NA	NA
283	30	-9	2	4	1	114	NA	NA
284	31	-9	2	4	1	302	NA	NA
285	32	-9	2	4	1	112	NA	NA
286	33	-9	2	4	1	19	NA	NA
287	34	-9	2	4	1	234	NA	NA
288	35	-9	2	4	1	96	NA	NA
289	36	-9	2	4	2	105	NA	NA
290	1	-8	2	4	1	73	NA	NA
291	2	-8	2	4	1	98	NA	NA
292	3	-8	2	4	1	121	NA	NA
293	4	-8	2	4	0	0	NA	NA
294	5	-8	2	4	0	0	NA	NA
295	6	-8	2	4	0	0	NA	NA
296	7	-8	2	4	1	155	NA	NA
297	8	-8	2	4	1	67	NA	NA
298	9	-8	2	4	1	138	NA	NA
299	10	-8	2	4	0	0	NA	NA
300	11	-8	2	4	2	243	NA	NA
301	12	-8	2	4	2	357	NA	NA
302	13	-8	2	4	0	0	NA	NA
303	14	-8	2	4	2	158	NA	NA
304	15	-8	2	4	3	218	NA	NA
305	16	-8	2	4	1	191	NA	NA
306	17	-8	2	4	0	0	NA	NA
307	18	-8	2	4	1	323	NA	NA
308	19	-8	2	4	1	142	NA	NA
309	20	-8	2	4	1	161	NA	NA
310	21	-8	2	4	3	237	NA	NA
311	22	-8	2	4	2	286	NA	NA
312	23	-8	2	4	1	175	NA	NA
313	24	-8	2	4	1	93	NA	NA
314	25	-8	2	4	1	108	NA	NA
315	26	-8	2	4	1	249	NA	NA
316	27	-8	2	4	2	167	NA	NA
317	28	-8	2	4	1	76	NA	NA
318	29	-8	2	4	1	35	NA	NA
319	30	-8	2	4	0	0	NA	NA
320	31	-8	2	4	1	211	NA	NA
321	32	-8	2	4	1	63	NA	NA
322	33	-8	2	4	1	154	NA	NA
323	34	-8	2	4	2	58	NA	NA
324	35	-8	2	4	2	59	NA	NA
325	36	-8	2	4	1	290	NA	NA

13784.6120 Biologica Data DESTS.xlsx

Pre-exposure Observations

	Tank ID	Pre-test Day (-24 to -1)	Number of Surviving Males	Number of Surviving Females	Number of Spawns	Number of Eggs	Number of Fertilized Eggs	Comments
326	1	-7	2	4	2	268	NA	NA
327	2	-7	2	4	2	149	NA	NA
328	3	-7	2	4	1	158	NA	NA
329	4	-7	2	4	0	0	NA	NA
330	5	-7	2	4	1	225	NA	NA
331	6	-7	2	4	1	504	NA	NA
332	7	-7	2	4	1	108	NA	NA
333	8	-7	2	4	1	162	NA	NA
334	9	-7	2	4	0	0	NA	NA
335	10	-7	2	4	2	341	NA	NA
336	11	-7	2	4	1	172	NA	NA
337	12	-7	2	4	0	0	NA	NA
338	13	-7	2	4	2	336	NA	NA
339	14	-7	2	4	1	144	NA	NA
340	15	-7	2	4	0	0	NA	NA
341	16	-7	2	4	1	92	NA	NA
342	17	-7	2	4	2	225	NA	NA
343	18	-7	2	4	0	0	NA	NA
344	19	-7	2	4	1	63	NA	NA
345	20	-7	2	4	1	117	NA	NA
346	21	-7	2	4	2	274	NA	NA
347	22	-7	2	4	1	298	NA	NA
348	23	-7	2	4	2	209	NA	NA
349	24	-7	2	4	1	166	NA	NA
350	25	-7	2	4	0	0	NA	NA
351	26	-7	2	4	1	126	NA	NA
352	27	-7	2	4	2	66	NA	NA
353	28	-7	2	4	1	319	NA	NA
354	29	-7	2	4	1	107	NA	NA
355	30	-7	2	4	1	68	NA	NA
356	31	-7	2	4	1	93	NA	NA
357	32	-7	2	4	1	162	NA	NA
358	33	-7	2	4	1	124	NA	NA
359	34	-7	2	4	2	180	NA	NA
360	35	-7	2	4	0	0	NA	NA
361	36	-7	2	4	2	261	NA	NA
362	1	-6	2	4	1	247	NA	NA
363	2	-6	2	4	2	198	NA	NA
364	3	-6	2	4	2	360	NA	NA
365	4	-6	2	4	0	0	NA	NA
366	5	-6	2	4	0	0	NA	NA
367	6	-6	2	4	1	260	NA	NA
368	7	-6	2	4	0	0	NA	NA
369	8	-6	2	4	0	0	NA	NA
370	9	-6	2	4	2	150	NA	NA
371	10	-6	2	4	2	211	NA	NA
372	11	-6	2	4	0	0	NA	NA
373	12	-6	2	4	2	244	NA	NA
374	13	-6	2	4	2	62	NA	NA
375	14	-6	2	4	2	128	NA	NA
376	15	-6	2	4	0	0	NA	NA
377	16	-6	2	4	1	78	NA	NA
378	17	-6	2	4	0	0	NA	NA
379	18	-6	2	4	1	84	NA	NA

13784.6120 Biologica Data DESTS.xlsx

Pre-exposure Observations

	Tank ID	Pre-test Day (-24 to -1)	Number of Surviving Males	Number of Surviving Females	Number of Spawns	Number of Eggs	Number of Fertilized Eggs	Comments
380	19	-6	2	4	2	32	NA	NA
381	20	-6	2	4	1	19	NA	NA
382	21	-6	2	4	0	0	NA	NA
383	22	-6	2	4	0	0	NA	NA
384	23	-6	2	4	0	0	NA	NA
385	24	-6	2	4	0	0	NA	NA
386	25	-6	2	4	1	94	NA	NA
387	26	-6	2	4	0	0	NA	NA
388	27	-6	2	4	1	218	NA	NA
389	28	-6	2	4	0	0	NA	NA
390	29	-6	2	4	0	0	NA	NA
391	30	-6	2	4	1	38	NA	NA
392	31	-6	2	4	1	115	NA	NA
393	32	-6	2	4	2	310	NA	NA
394	33	-6	2	4	1	145	NA	NA
395	34	-6	2	4	1	206	NA	NA
396	35	-6	2	4	0	0	NA	NA
397	36	-6	2	4	0	0	NA	NA
398	1	-5	2	4	1	578	NA	NA
399	2	-5	2	4	2	614	NA	NA
400	3	-5	2	4	2	444	NA	NA
401	4	-5	2	4	1	192	NA	NA
402	5	-5	2	4	3	296	NA	NA
403	6	-5	2	4	2	664	NA	NA
404	7	-5	2	4	1	118	NA	NA
405	8	-5	2	4	2	317	NA	NA
406	9	-5	2	4	2	289	NA	NA
407	10	-5	2	4	1	15	NA	NA
408	11	-5	2	4	1	555	NA	NA
409	12	-5	2	4	1	352	NA	NA
410	13	-5	2	4	1	160	NA	NA
411	14	-5	2	4	1	309	NA	NA
412	15	-5	2	4	2	571	NA	NA
413	16	-5	2	4	1	244	NA	NA
414	17	-5	2	4	0	0	NA	NA
415	18	-5	2	4	1	193	NA	NA
416	19	-5	2	4	2	311	NA	NA
417	20	-5	2	4	2	607	NA	NA
418	21	-5	2	4	1	307	NA	NA
419	22	-5	2	4	2	638	NA	NA
420	23	-5	2	4	2	585	NA	NA
421	24	-5	2	4	2	521	NA	NA
422	25	-5	2	4	2	347	NA	NA
423	26	-5	2	4	2	713	NA	NA
424	27	-5	2	4	1	313	NA	NA
425	28	-5	2	4	1	357	NA	NA
426	29	-5	2	4	1	70	NA	NA
427	30	-5	2	4	2	429	NA	NA
428	31	-5	2	4	1	696	NA	NA
429	32	-5	2	4	1	4	NA	NA
430	33	-5	2	4	0	0	NA	NA
431	34	-5	2	4	1	335	NA	NA
432	35	-5	2	4	2	828	NA	NA
433	36	-5	2	4	1	217	NA	NA

13784.6120 Biologica Data DESTS.xlsx

Pre-exposure Observations

	Tank ID	Pre-test Day (-24 to -1)	Number of Surviving Males	Number of Surviving Females	Number of Spawns	Number of Eggs	Number of Fertilized Eggs	Comments
434	1	-4	2	4	1	389	NA	NA
435	2	-4	2	4	1	275	NA	NA
436	3	-4	2	4	0	0	NA	NA
437	4	-4	2	4	0	0	NA	NA
438	5	-4	2	4	2	697	NA	NA
439	6	-4	2	4	1	215	NA	NA
440	7	-4	2	4	0	0	NA	NA
441	8	-4	2	4	1	178	NA	NA
442	9	-4	2	4	0	0	NA	NA
443	10	-4	2	4	1	305	NA	NA
444	11	-4	2	4	2	378	NA	NA
445	12	-4	2	4	1	402	NA	NA
446	13	-4	2	4	1	511	NA	NA
447	14	-4	2	4	1	107	NA	NA
448	15	-4	2	4	1	152	NA	NA
449	16	-4	2	4	2	219	NA	NA
450	17	-4	2	4	1	115	NA	NA
451	18	-4	2	4	0	0	NA	NA
452	19	-4	2	4	2	192	NA	NA
453	20	-4	2	4	1	271	NA	NA
454	21	-4	2	4	2	482	NA	NA
455	22	-4	2	4	2	351	NA	NA
456	23	-4	2	4	1	109	NA	NA
457	24	-4	2	4	1	78	NA	NA
458	25	-4	2	4	1	92	NA	NA
459	26	-4	2	4	1	197	NA	NA
460	27	-4	2	4	1	230	NA	NA
461	28	-4	2	4	1	381	NA	NA
462	29	-4	2	4	1	297	NA	NA
463	30	-4	2	4	1	214	NA	NA
464	31	-4	2	4	1	319	NA	NA
465	32	-4	2	4	1	478	NA	NA
466	33	-4	2	4	0	0	NA	NA
467	34	-4	2	4	1	501	NA	NA
468	35	-4	2	4	0	0	NA	NA
469	36	-4	2	4	1	108	NA	NA
470	1	-3	2	4	1	41	NA	NA
471	2	-3	2	4	2	256	NA	NA
472	3	-3	2	4	1	132	NA	NA
473	4	-3	2	4	1	107	NA	NA
474	5	-3	2	4	0	0	NA	NA
475	6	-3	2	4	1	119	NA	NA
476	7	-3	2	4	0	0	NA	NA
477	8	-3	2	4	2	148	NA	NA
478	9	-3	2	4	1	92	NA	NA
479	10	-3	2	4	1	181	NA	NA
480	11	-3	2	4	2	151	NA	NA
481	12	-3	2	4	1	278	NA	NA
482	13	-3	2	4	0	0	NA	NA
483	14	-3	2	4	2	306	NA	NA
484	15	-3	2	4	0	0	NA	NA
485	16	-3	2	4	0	0	NA	NA
486	17	-3	2	4	1	134	NA	NA
487	18	-3	2	4	0	0	NA	NA

13784.6120 Biologica Data DESTS.xlsx

Pre-exposure Observations

	Tank ID	Pre-test Day (-24 to -1)	Number of Surviving Males	Number of Surviving Females	Number of Spawns	Number of Eggs	Number of Fertilized Eggs	Comments
488	19	-3	2	4	0	0	NA	NA
489	20	-3	2	4	0	0	NA	NA
490	21	-3	2	4	0	0	NA	NA
491	22	-3	2	4	0	0	NA	NA
492	23	-3	2	4	1	246	NA	NA
493	24	-3	2	4	0	0	NA	NA
494	25	-3	2	4	1	171	NA	NA
495	26	-3	2	4	0	0	NA	NA
496	27	-3	2	4	2	266	NA	NA
497	28	-3	2	4	1	143	NA	NA
498	29	-3	2	4	0	0	NA	NA
499	30	-3	2	4	2	177	NA	NA
500	31	-3	2	4	1	190	NA	NA
501	32	-3	2	4	1	217	NA	NA
502	33	-3	2	4	1	143	NA	NA
503	34	-3	2	4	2	176	NA	NA
504	35	-3	2	4	1	134	NA	NA
505	36	-3	2	4	2	251	NA	NA
506	1	-2	2	4	1	197	NA	NA
507	2	-2	2	4	1	158	NA	NA
508	3	-2	2	4	1	365	NA	NA
509	4	-2	2	4	1	142	NA	NA
510	5	-2	2	4	2	174	NA	NA
511	6	-2	2	4	1	271	NA	NA
512	7	-2	2	4	2	320	NA	NA
513	8	-2	2	4	1	198	NA	NA
514	9	-2	2	4	2	235	NA	NA
515	10	-2	2	4	0	0	NA	NA
516	11	-2	2	4	2	130	NA	NA
517	12	-2	2	4	1	179	NA	NA
518	13	-2	2	4	2	234	NA	NA
519	14	-2	2	4	1	185	NA	NA
520	15	-2	2	4	3	333	NA	NA
521	16	-2	2	4	2	270	NA	NA
522	17	-2	2	4	0	0	NA	NA
523	18	-2	2	4	0	0	NA	NA
524	19	-2	2	4	1	215	NA	NA
525	20	-2	2	4	2	410	NA	NA
526	21	-2	2	4	1	138	NA	NA
527	22	-2	2	4	1	363	NA	NA
528	23	-2	2	4	2	452	NA	NA
529	24	-2	2	4	2	297	NA	NA
530	25	-2	2	4	1	63	NA	NA
531	26	-2	2	4	0	0	NA	NA
532	27	-2	2	4	1	251	NA	NA
533	28	-2	2	4	1	173	NA	NA
534	29	-2	2	4	2	161	NA	NA
535	30	-2	2	4	1	246	NA	NA
536	31	-2	2	4	1	184	NA	NA
537	32	-2	2	4	0	0	NA	NA
538	33	-2	2	4	0	0	NA	NA
539	34	-2	2	4	2	228	NA	NA
540	35	-2	2	4	2	126	NA	NA
541	36	-2	2	4	2	349	NA	NA

13784.6120 Biologica Data DESTS.xlsx

Pre-exposure Observations

	Tank ID	Pre-test Day (-24 to -1)	Number of Surviving Males	Number of Surviving Females	Number of Spawns	Number of Eggs	Number of Fertilized Eggs	Comments
542	1	-1	2	4	1	278	NA	NA
543	2	-1	2	4	1	215	NA	NA
544	3	-1	2	4	0	0	NA	NA
545	4	-1	2	4	1	166	NA	NA
546	5	-1	2	4	2	477	NA	NA
547	6	-1	2	4	1	109	NA	NA
548	7	-1	2	4	1	182	NA	NA
549	8	-1	2	4	1	72	NA	NA
550	9	-1	2	4	0	0	NA	NA
551	10	-1	2	4	1	361	NA	NA
552	11	-1	2	4	1	325	NA	NA
553	12	-1	2	4	1	271	NA	NA
554	13	-1	2	4	1	32	NA	NA
555	14	-1	2	4	2	228	NA	NA
556	15	-1	2	4	0	0	NA	NA
557	16	-1	2	4	1	55	NA	NA
558	17	-1	2	4	2	347	NA	NA
559	18	-1	2	4	1	40	NA	NA
560	19	-1	2	4	1	78	NA	NA
561	20	-1	2	4	0	0	NA	NA
562	21	-1	2	4	2	437	NA	NA
563	22	-1	2	4	2	197	NA	NA
564	23	-1	2	4	1	28	NA	NA
565	24	-1	2	4	0	0	NA	NA
566	25	-1	2	4	0	0	NA	NA
567	26	-1	2	4	2	340	NA	NA
568	27	-1	2	4	2	276	NA	NA
569	28	-1	2	4	1	23	NA	NA
570	29	-1	2	4	1	218	NA	NA
571	30	-1	2	4	2	203	NA	NA
572	31	-1	2	4	1	16	NA	NA
573	32	-1	2	4	0	0	NA	NA
574	33	-1	2	4	0	0	NA	NA
575	34	-1	2	4	1	142	NA	NA
576	35	-1	2	4	0	0	NA	NA
577	36	-1	2	4	1	197	NA	NA

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Survival & Reproduction (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Number of Surviving Males	Number of Surviving Females	Number of Eggs	Number of Infertile Eggs
	Control	A	5A	0	2	4	NA	NA
	Control	A	5A	1	2	4	239	0
	Control	A	5A	2	2	4	216	0
	Control	A	5A	3	2	4	93	0
	Control	A	5A	4	2	4	0	0
	Control	A	5A	5	2	4	123	2
	Control	A	5A	6	2	4	62	0
	Control	A	5A	7	2	4	26	2
	Control	A	5A	8	2	4	113	0
	Control	A	5A	9	2	4	527	0
	Control	A	5A	10	2	4	37	0
	Control	A	5A	11	2	4	331	0
	Control	A	5A	12	2	4	209	2
	Control	A	5A	13	2	4	0	0
	Control	A	5A	14	2	4	110	0
	Control	A	5A	15	2	4	360	0
	Control	A	5A	16	2	4	0	0
	Control	A	5A	17	2	4	115	0
	Control	A	5A	18	2	4	286	5
	Control	A	5A	19	2	4	223	1
	Control	A	5A	20	2	4	463	3
	Control	A	5A	21	2	4	290	0
	Control	B	5B	0	2	4	NA	NA
	Control	B	5B	1	2	4	137	0
	Control	B	5B	2	2	4	258	0
	Control	B	5B	3	2	4	179	0
	Control	B	5B	4	2	4	221	0
	Control	B	5B	5	2	4	161	0
	Control	B	5B	6	2	4	88	0
	Control	B	5B	7	2	4	234	0
	Control	B	5B	8	2	4	167	2
	Control	B	5B	9	2	4	626	0
	Control	B	5B	10	2	4	590	0
	Control	B	5B	11	2	4	0	0
	Control	B	5B	12	2	4	308	0
	Control	B	5B	13	2	4	394	2
	Control	B	5B	14	2	4	0	0
	Control	B	5B	15	2	4	322	0
	Control	B	5B	16	2	4	602	3
	Control	B	5B	17	2	4	0	0
	Control	B	5B	18	2	4	109	0
	Control	B	5B	19	2	4	404	6
	Control	B	5B	20	2	4	0	0
	Control	B	5B	21	2	4	197	2
	Control	C	5C	0	2	4	NA	NA
	Control	C	5C	1	2	4	115	0
	Control	C	5C	2	2	4	303	0
	Control	C	5C	3	2	4	206	0
	Control	C	5C	4	2	4	447	3
	Control	C	5C	5	2	4	191	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Survival & Reproduction (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Number of Surviving Males	Number of Surviving Females	Number of Eggs	Number of Infertile Eggs
52	Control	C	5C	6	2	4	0	0
53	Control	C	5C	7	2	4	360	0
54	Control	C	5C	8	2	4	165	0
55	Control	C	5C	9	2	4	0	0
56	Control	C	5C	10	2	4	75	0
57	Control	C	5C	11	2	4	376	8
58	Control	C	5C	12	2	4	0	0
59	Control	C	5C	13	2	4	173	2
60	Control	C	5C	14	2	4	196	0
61	Control	C	5C	15	2	4	202	0
62	Control	C	5C	16	2	4	31	0
63	Control	C	5C	17	2	4	210	1
64	Control	C	5C	18	2	4	98	0
65	Control	C	5C	19	2	4	250	0
66	Control	C	5C	20	2	4	376	1
67	Control	C	5C	21	2	4	218	0
68	Control	D	5D	0	2	4	NA	NA
69	Control	D	5D	1	2	4	158	0
70	Control	D	5D	2	2	4	66	0
71	Control	D	5D	3	2	4	217	0
72	Control	D	5D	4	2	4	84	0
73	Control	D	5D	5	2	4	180	0
74	Control	D	5D	6	2	4	201	0
75	Control	D	5D	7	2	4	0	0
76	Control	D	5D	8	2	4	16	0
77	Control	D	5D	9	2	4	476	37
78	Control	D	5D	10	2	4	0	0
79	Control	D	5D	11	2	4	608	3
80	Control	D	5D	12	2	4	0	0
81	Control	D	5D	13	2	4	0	0
82	Control	D	5D	14	2	4	291	0
83	Control	D	5D	15	2	4	150	0
84	Control	D	5D	16	2	4	87	0
85	Control	D	5D	17	2	4	133	0
86	Control	D	5D	18	2	4	53	0
87	Control	D	5D	19	2	4	248	0
88	Control	D	5D	20	2	4	0	0
89	Control	D	5D	21	2	4	306	3
90	14 µg/L	A	4A	0	2	4	NA	NA
91	14 µg/L	A	4A	1	2	4	343	7
92	14 µg/L	A	4A	2	2	4	133	1
93	14 µg/L	A	4A	3	2	4	176	0
94	14 µg/L	A	4A	4	2	4	0	0
95	14 µg/L	A	4A	5	2	4	0	0
96	14 µg/L	A	4A	6	2	4	0	0
97	14 µg/L	A	4A	7	2	4	97	0
98	14 µg/L	A	4A	8	2	4	270	0
99	14 µg/L	A	4A	9	2	4	151	15
100	14 µg/L	A	4A	10	2	4	0	0
101	14 µg/L	A	4A	11	2	4	116	3
102	14 µg/L	A	4A	12	2	4	0	0
103	14 µg/L	A	4A	13	2	4	27	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Survival & Reproduction (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Number of Surviving Males	Number of Surviving Females	Number of Eggs	Number of Infertile Eggs
104	14 µg/L	A	4A	14	2	4	46	0
105	14 µg/L	A	4A	15	2	4	0	0
106	14 µg/L	A	4A	16	2	4	112	0
107	14 µg/L	A	4A	17	2	4	25	0
108	14 µg/L	A	4A	18	2	4	207	0
109	14 µg/L	A	4A	19	2	4	336	0
110	14 µg/L	A	4A	20	2	4	4	0
111	14 µg/L	A	4A	21	2	3	272	0
112	14 µg/L	B	4B	0	2	4	NA	NA
113	14 µg/L	B	4B	1	2	4	71	5
114	14 µg/L	B	4B	2	2	4	190	1
115	14 µg/L	B	4B	3	2	4	182	1
116	14 µg/L	B	4B	4	2	4	105	1
117	14 µg/L	B	4B	5	2	4	0	0
118	14 µg/L	B	4B	6	2	4	0	0
119	14 µg/L	B	4B	7	2	4	142	0
120	14 µg/L	B	4B	8	2	4	0	0
121	14 µg/L	B	4B	9	2	4	71	0
122	14 µg/L	B	4B	10	2	4	128	0
123	14 µg/L	B	4B	11	2	3	0	0
124	14 µg/L	B	4B	12	2	3	327	0
125	14 µg/L	B	4B	13	2	3	0	0
126	14 µg/L	B	4B	14	2	3	0	0
127	14 µg/L	B	4B	15	2	3	242	0
128	14 µg/L	B	4B	16	2	3	79	0
129	14 µg/L	B	4B	17	2	3	0	0
130	14 µg/L	B	4B	18	2	3	413	0
131	14 µg/L	B	4B	19	2	3	145	0
132	14 µg/L	B	4B	20	2	3	0	0
133	14 µg/L	B	4B	21	2	3	107	1
134	14 µg/L	C	4C	0	2	4	NA	NA
135	14 µg/L	C	4C	1	2	4	159	0
136	14 µg/L	C	4C	2	2	4	348	5
137	14 µg/L	C	4C	3	2	4	0	0
138	14 µg/L	C	4C	4	2	4	278	1
139	14 µg/L	C	4C	5	2	4	357	0
140	14 µg/L	C	4C	6	2	4	0	0
141	14 µg/L	C	4C	7	2	4	0	0
142	14 µg/L	C	4C	8	2	4	88	0
143	14 µg/L	C	4C	9	2	4	26	0
144	14 µg/L	C	4C	10	2	4	402	1
145	14 µg/L	C	4C	11	2	4	179	3
146	14 µg/L	C	4C	12	2	4	215	0
147	14 µg/L	C	4C	13	2	4	300	0
148	14 µg/L	C	4C	14	2	4	82	0
149	14 µg/L	C	4C	15	2	4	98	0
150	14 µg/L	C	4C	16	2	4	12	0
151	14 µg/L	C	4C	17	2	4	442	1
152	14 µg/L	C	4C	18	2	4	0	0
153	14 µg/L	C	4C	19	2	4	179	0
154	14 µg/L	C	4C	20	2	4	221	2
155	14 µg/L	C	4C	21	2	4	263	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Survival & Reproduction (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Number of Surviving Males	Number of Surviving Females	Number of Eggs	Number of Infertile Eggs
156	14 µg/L	D	4D	0	2	4	NA	NA
157	14 µg/L	D	4D	1	2	4	0	0
158	14 µg/L	D	4D	2	2	4	311	0
159	14 µg/L	D	4D	3	2	4	0	0
160	14 µg/L	D	4D	4	2	4	53	0
161	14 µg/L	D	4D	5	2	4	215	0
162	14 µg/L	D	4D	6	2	4	135	0
163	14 µg/L	D	4D	7	2	4	197	2
164	14 µg/L	D	4D	8	2	4	153	0
165	14 µg/L	D	4D	9	2	4	332	0
166	14 µg/L	D	4D	10	2	4	0	0
167	14 µg/L	D	4D	11	2	4	400	0
168	14 µg/L	D	4D	12	2	4	0	0
169	14 µg/L	D	4D	13	2	4	284	0
170	14 µg/L	D	4D	14	2	4	74	0
171	14 µg/L	D	4D	15	2	4	0	0
172	14 µg/L	D	4D	16	2	4	0	0
173	14 µg/L	D	4D	17	2	4	351	0
174	14 µg/L	D	4D	18	2	4	160	2
175	14 µg/L	D	4D	19	2	4	0	0
176	14 µg/L	D	4D	20	2	4	436	0
177	14 µg/L	D	4D	21	2	4	77	0
178	35 µg/L	A	3A	0	2	4	NA	NA
179	35 µg/L	A	3A	1	2	4	154	0
180	35 µg/L	A	3A	2	2	4	0	0
181	35 µg/L	A	3A	3	2	4	0	0
182	35 µg/L	A	3A	4	2	3	503	52
183	35 µg/L	A	3A	5	2	3	0	0
184	35 µg/L	A	3A	6	2	3	0	0
185	35 µg/L	A	3A	7	2	3	329	3
186	35 µg/L	A	3A	8	2	3	0	0
187	35 µg/L	A	3A	9	2	3	210	0
188	35 µg/L	A	3A	10	2	3	268	0
189	35 µg/L	A	3A	11	2	3	168	0
190	35 µg/L	A	3A	12	2	3	0	0
191	35 µg/L	A	3A	13	2	3	245	0
192	35 µg/L	A	3A	14	2	3	435	0
193	35 µg/L	A	3A	15	2	3	0	0
194	35 µg/L	A	3A	16	2	3	0	0
195	35 µg/L	A	3A	17	2	3	0	0
196	35 µg/L	A	3A	18	2	3	0	0
197	35 µg/L	A	3A	19	2	3	0	0
198	35 µg/L	A	3A	20	2	3	272	1
199	35 µg/L	A	3A	21	2	3	174	2
200	35 µg/L	B	3B	0	2	4	NA	NA
201	35 µg/L	B	3B	1	2	4	106	0
202	35 µg/L	B	3B	2	2	4	142	0
203	35 µg/L	B	3B	3	2	4	289	1
204	35 µg/L	B	3B	4	2	4	418	3
205	35 µg/L	B	3B	5	2	4	0	0
206	35 µg/L	B	3B	6	2	4	0	0
207	35 µg/L	B	3B	7	2	4	239	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Survival & Reproduction (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Number of Surviving Males	Number of Surviving Females	Number of Eggs	Number of Infertile Eggs
208	35 µg/L	B	3B	8	2	4	200	2
209	35 µg/L	B	3B	9	2	4	0	0
210	35 µg/L	B	3B	10	2	4	687	0
211	35 µg/L	B	3B	11	2	4	200	0
212	35 µg/L	B	3B	12	2	4	0	0
213	35 µg/L	B	3B	13	2	4	0	0
214	35 µg/L	B	3B	14	2	4	0	0
215	35 µg/L	B	3B	15	2	4	121	0
216	35 µg/L	B	3B	16	2	4	42	0
217	35 µg/L	B	3B	17	2	4	337	2
218	35 µg/L	B	3B	18	2	4	320	2
219	35 µg/L	B	3B	19	2	4	201	0
220	35 µg/L	B	3B	20	2	4	251	0
221	35 µg/L	B	3B	21	2	4	401	2
222	35 µg/L	C	3C	0	2	4	NA	NA
223	35 µg/L	C	3C	1	2	4	89	0
224	35 µg/L	C	3C	2	2	4	135	0
225	35 µg/L	C	3C	3	2	4	169	4
226	35 µg/L	C	3C	4	2	4	452	5
227	35 µg/L	C	3C	5	2	4	203	0
228	35 µg/L	C	3C	6	2	4	0	0
229	35 µg/L	C	3C	7	2	4	0	0
230	35 µg/L	C	3C	8	2	4	67	0
231	35 µg/L	C	3C	9	2	4	125	0
232	35 µg/L	C	3C	10	2	4	209	0
233	35 µg/L	C	3C	11	2	4	307	0
234	35 µg/L	C	3C	12	2	4	0	0
235	35 µg/L	C	3C	13	2	4	0	0
236	35 µg/L	C	3C	14	2	4	0	0
237	35 µg/L	C	3C	15	2	4	0	0
238	35 µg/L	C	3C	16	2	4	216	2
239	35 µg/L	C	3C	17	2	4	0	0
240	35 µg/L	C	3C	18	2	4	273	2
241	35 µg/L	C	3C	19	2	4	343	0
242	35 µg/L	C	3C	20	2	4	0	0
243	35 µg/L	C	3C	21	2	4	79	0
244	35 µg/L	D	3D	0	2	4	NA	NA
245	35 µg/L	D	3D	1	2	4	0	0
246	35 µg/L	D	3D	2	2	4	232	4
247	35 µg/L	D	3D	3	2	4	220	42
248	35 µg/L	D	3D	4	2	4	147	14
249	35 µg/L	D	3D	5	2	4	213	0
250	35 µg/L	D	3D	6	2	4	278	12
251	35 µg/L	D	3D	7	2	4	167	6
252	35 µg/L	D	3D	8	2	4	29	0
253	35 µg/L	D	3D	9	2	4	343	0
254	35 µg/L	D	3D	10	2	4	0	0
255	35 µg/L	D	3D	11	2	4	147	0
256	35 µg/L	D	3D	12	2	4	497	10
257	35 µg/L	D	3D	13	2	4	127	0
258	35 µg/L	D	3D	14	2	4	37	0
259	35 µg/L	D	3D	15	2	4	305	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Survival & Reproduction (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Number of Surviving Males	Number of Surviving Females	Number of Eggs	Number of Infertile Eggs
260	35 µg/L	D	3D	16	2	4	59	0
261	35 µg/L	D	3D	17	2	4	283	0
262	35 µg/L	D	3D	18	2	4	140	0
263	35 µg/L	D	3D	19	2	4	0	0
264	35 µg/L	D	3D	20	2	4	67	0
265	35 µg/L	D	3D	21	2	4	371	5
266	88 µg/L	A	2A	0	2	4	NA	NA
267	88 µg/L	A	2A	1	2	4	0	0
268	88 µg/L	A	2A	2	2	4	0	0
269	88 µg/L	A	2A	3	2	4	126	0
270	88 µg/L	A	2A	4	2	3	157	0
271	88 µg/L	A	2A	5	2	3	0	0
272	88 µg/L	A	2A	6	2	3	97	0
273	88 µg/L	A	2A	7	2	3	291	0
274	88 µg/L	A	2A	8	2	3	25	0
275	88 µg/L	A	2A	9	2	3	345	0
276	88 µg/L	A	2A	10	2	3	338	8
277	88 µg/L	A	2A	11	2	3	151	0
278	88 µg/L	A	2A	12	2	3	292	0
279	88 µg/L	A	2A	13	2	3	0	0
280	88 µg/L	A	2A	14	2	3	0	0
281	88 µg/L	A	2A	15	2	3	108	0
282	88 µg/L	A	2A	16	2	3	81	1
283	88 µg/L	A	2A	17	2	3	225	2
284	88 µg/L	A	2A	18	2	3	203	3
285	88 µg/L	A	2A	19	2	3	179	0
286	88 µg/L	A	2A	20	2	3	635	1
287	88 µg/L	A	2A	21	2	3	401	6
288	88 µg/L	B	2B	0	2	4	NA	NA
289	88 µg/L	B	2B	1	2	4	0	0
290	88 µg/L	B	2B	2	2	4	208	0
291	88 µg/L	B	2B	3	2	4	0	0
292	88 µg/L	B	2B	4	2	4	350	0
293	88 µg/L	B	2B	5	2	4	0	0
294	88 µg/L	B	2B	6	2	4	178	0
295	88 µg/L	B	2B	7	2	4	288	0
296	88 µg/L	B	2B	8	2	4	28	0
297	88 µg/L	B	2B	9	2	4	434	5
298	88 µg/L	B	2B	10	2	4	256	0
299	88 µg/L	B	2B	11	2	4	210	0
300	88 µg/L	B	2B	12	2	4	256	12
301	88 µg/L	B	2B	13	2	4	109	0
302	88 µg/L	B	2B	14	2	4	0	0
303	88 µg/L	B	2B	15	2	4	245	19
304	88 µg/L	B	2B	16	2	4	0	0
305	88 µg/L	B	2B	17	2	4	48	0
306	88 µg/L	B	2B	18	2	4	246	0
307	88 µg/L	B	2B	19	2	4	629	7
308	88 µg/L	B	2B	20	2	4	164	0
309	88 µg/L	B	2B	21	2	4	71	4
310	88 µg/L	C	2C	0	2	4	NA	NA
311	88 µg/L	C	2C	1	2	4	224	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Survival & Reproduction (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Number of Surviving Males	Number of Surviving Females	Number of Eggs	Number of Infertile Eggs
312	88 µg/L	C	2C	2	2	4	359	0
313	88 µg/L	C	2C	3	2	4	0	0
314	88 µg/L	C	2C	4	2	4	0	0
315	88 µg/L	C	2C	5	2	4	0	0
316	88 µg/L	C	2C	6	2	4	66	0
317	88 µg/L	C	2C	7	2	4	23	0
318	88 µg/L	C	2C	8	2	4	0	0
319	88 µg/L	C	2C	9	2	4	457	0
320	88 µg/L	C	2C	10	2	4	0	0
321	88 µg/L	C	2C	11	2	4	0	0
322	88 µg/L	C	2C	12	2	4	509	0
323	88 µg/L	C	2C	13	2	4	0	0
324	88 µg/L	C	2C	14	2	4	0	0
325	88 µg/L	C	2C	15	2	4	299	0
326	88 µg/L	C	2C	16	2	4	0	0
327	88 µg/L	C	2C	17	2	4	27	0
328	88 µg/L	C	2C	18	2	4	186	0
329	88 µg/L	C	2C	19	2	4	0	0
330	88 µg/L	C	2C	20	2	4	0	0
331	88 µg/L	C	2C	21	2	4	58	0
332	88 µg/L	D	2D	0	2	4	NA	NA
333	88 µg/L	D	2D	1	2	4	0	0
334	88 µg/L	D	2D	2	2	4	306	0
335	88 µg/L	D	2D	3	2	4	169	1
336	88 µg/L	D	2D	4	2	4	170	0
337	88 µg/L	D	2D	5	2	4	226	0
338	88 µg/L	D	2D	6	2	4	191	0
339	88 µg/L	D	2D	7	2	4	69	0
340	88 µg/L	D	2D	8	2	4	178	2
341	88 µg/L	D	2D	9	2	4	276	0
342	88 µg/L	D	2D	10	2	4	307	0
343	88 µg/L	D	2D	11	2	4	0	0
344	88 µg/L	D	2D	12	2	4	411	0
345	88 µg/L	D	2D	13	2	4	407	0
346	88 µg/L	D	2D	14	2	4	97	0
347	88 µg/L	D	2D	15	2	4	225	0
348	88 µg/L	D	2D	16	2	4	197	1
349	88 µg/L	D	2D	17	2	4	0	0
350	88 µg/L	D	2D	18	2	4	259	0
351	88 µg/L	D	2D	19	2	4	197	0
352	88 µg/L	D	2D	20	2	4	0	0
353	88 µg/L	D	2D	21	2	4	0	0
354	220 µg/L	A	1A	0	2	4	NA	NA
355	220 µg/L	A	1A	1	2	4	50	0
356	220 µg/L	A	1A	2	2	4	62	2
357	220 µg/L	A	1A	3	2	4	271	0
358	220 µg/L	A	1A	4	2	4	0	0
359	220 µg/L	A	1A	5	2	4	77	0
360	220 µg/L	A	1A	6	2	4	270	0
361	220 µg/L	A	1A	7	2	4	0	0
362	220 µg/L	A	1A	8	2	4	129	0
363	220 µg/L	A	1A	9	2	4	227	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Survival & Reproduction (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Number of Surviving Males	Number of Surviving Females	Number of Eggs	Number of Infertile Eggs
364	220 µg/L	A	1A	10	2	4	0	0
365	220 µg/L	A	1A	11	2	4	396	1
366	220 µg/L	A	1A	12	2	4	357	0
367	220 µg/L	A	1A	13	2	4	0	0
368	220 µg/L	A	1A	14	2	4	191	5
369	220 µg/L	A	1A	15	2	4	156	0
370	220 µg/L	A	1A	16	2	4	169	3
371	220 µg/L	A	1A	17	2	4	317	2
372	220 µg/L	A	1A	18	2	4	114	0
373	220 µg/L	A	1A	19	2	4	42	0
374	220 µg/L	A	1A	20	2	4	213	0
375	220 µg/L	A	1A	21	2	4	192	0
376	220 µg/L	B	1B	0	2	4	NA	NA
377	220 µg/L	B	1B	1	2	4	0	0
378	220 µg/L	B	1B	2	2	4	0	0
379	220 µg/L	B	1B	3	2	4	0	0
380	220 µg/L	B	1B	4	2	4	337	0
381	220 µg/L	B	1B	5	2	4	143	0
382	220 µg/L	B	1B	6	2	4	150	0
383	220 µg/L	B	1B	7	2	4	208	2
384	220 µg/L	B	1B	8	2	4	256	5
385	220 µg/L	B	1B	9	2	4	207	0
386	220 µg/L	B	1B	10	2	4	452	0
387	220 µg/L	B	1B	11	2	4	318	0
388	220 µg/L	B	1B	12	2	4	359	0
389	220 µg/L	B	1B	13	2	4	121	0
390	220 µg/L	B	1B	14	2	4	392	0
391	220 µg/L	B	1B	15	2	4	0	0
392	220 µg/L	B	1B	16	2	4	71	0
393	220 µg/L	B	1B	17	2	4	115	0
394	220 µg/L	B	1B	18	2	4	0	0
395	220 µg/L	B	1B	19	2	4	107	2
396	220 µg/L	B	1B	20	2	4	301	6
397	220 µg/L	B	1B	21	2	4	0	0
398	220 µg/L	C	1C	0	2	4	NA	NA
399	220 µg/L	C	1C	1	2	4	0	0
400	220 µg/L	C	1C	2	2	4	196	2
401	220 µg/L	C	1C	3	2	4	188	0
402	220 µg/L	C	1C	4	2	4	405	5
403	220 µg/L	C	1C	5	2	4	309	12
404	220 µg/L	C	1C	6	2	4	0	0
405	220 µg/L	C	1C	7	2	4	71	0
406	220 µg/L	C	1C	8	2	4	218	0
407	220 µg/L	C	1C	9	2	4	0	0
408	220 µg/L	C	1C	10	2	4	0	0
409	220 µg/L	C	1C	11	2	4	321	0
410	220 µg/L	C	1C	12	2	4	0	0
411	220 µg/L	C	1C	13	2	4	0	0
412	220 µg/L	C	1C	14	2	4	151	0
413	220 µg/L	C	1C	15	2	4	207	0
414	220 µg/L	C	1C	16	2	4	42	0
415	220 µg/L	C	1C	17	2	4	196	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Survival & Reproduction (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Number of Surviving Males	Number of Surviving Females	Number of Eggs	Number of Infertile Eggs
416	220 µg/L	C	1C	18	2	4	0	0
417	220 µg/L	C	1C	19	2	4	0	0
418	220 µg/L	C	1C	20	2	4	138	0
419	220 µg/L	C	1C	21	2	4	0	0
420	220 µg/L	D	1D	0	2	4	NA	NA
421	220 µg/L	D	1D	1	2	4	0	0
422	220 µg/L	D	1D	2	2	4	251	0
423	220 µg/L	D	1D	3	2	4	324	9
424	220 µg/L	D	1D	4	2	4	10	3
425	220 µg/L	D	1D	5	2	4	0	0
426	220 µg/L	D	1D	6	2	4	178	0
427	220 µg/L	D	1D	7	2	4	210	0
428	220 µg/L	D	1D	8	2	4	311	4
429	220 µg/L	D	1D	9	2	4	481	0
430	220 µg/L	D	1D	10	2	4	0	0
431	220 µg/L	D	1D	11	2	4	702	0
432	220 µg/L	D	1D	12	2	4	0	0
433	220 µg/L	D	1D	13	2	4	341	0
434	220 µg/L	D	1D	14	2	4	0	0
435	220 µg/L	D	1D	15	2	4	0	0
436	220 µg/L	D	1D	16	2	4	0	0
437	220 µg/L	D	1D	17	2	4	102	3
438	220 µg/L	D	1D	18	2	4	122	0
439	220 µg/L	D	1D	19	2	4	0	0
440	220 µg/L	D	1D	20	2	4	107	2
441	220 µg/L	D	1D	21	2	4	88	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	G	H	I
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Measured Treatment Concentration (µg a.i./L)	Temperature (°C)	Dissolved Oxygen (mg/L)
2	Control	0	4	A	5A	0	NA	25.5	8.05
3	Control	0	1	B	5B	0	NA	26.1	7.90
4	Control	0	3	C	5C	0	<1.1	25.5	8.09
5	Control	0	2	D	5D	0	<1.1	26.0	8.13
6	14	14	4	A	4A	0	NA	24.4	8.20
7	14	14	3	B	4B	0	NA	25.8	7.97
8	14	14	2	C	4C	0	16	25.5	8.01
9	14	14	1	D	4D	0	12	25.7	7.98
10	35	35	4	A	3A	0	NA	25.5	8.09
11	35	35	1	B	3B	0	NA	24.9	8.09
12	35	35	3	C	3C	0	26	26.0	7.96
13	35	35	2	D	3D	0	27	25.9	7.94
14	88	88	2	A	2A	0	NA	25.5	8.09
15	88	88	4	B	2B	0	NA	26.0	7.94
16	88	88	3	C	2C	0	60	25.3	7.82
17	88	88	1	D	2D	0	51	25.3	8.14
18	220	220	2	A	1A	0	NA	25.5	8.11
19	220	220	4	B	1B	0	NA	26.1	7.93
20	220	220	1	C	1C	0	114	25.4	8.05
21	220	220	3	D	1D	0	156	26.7	7.92
22	Control	0	4	A	5A	0	NA	25.5	7.61
23	14	14	4	A	4A	0	NA	25.5	7.75
24	35	35	4	A	3A	0	NA	25.5	7.60
25	88	88	2	A	2A	0	NA	25.5	7.65
26	220	220	2	A	1A	0	NA	25.5	7.64
27	Control	0	1	B	5B	0	NA	25.5	7.42
28	14	14	3	B	4B	0	NA	25.6	7.55
29	35	35	1	B	3B	0	NA	25.5	7.52
30	88	88	4	B	2B	0	NA	25.5	7.56
31	220	220	4	B	1B	0	NA	25.6	7.69
32	Control	0	3	C	5C	0	NA	26.0	7.55
33	14	14	2	C	4C	0	NA	26.0	7.30
34	35	35	3	C	3C	0	NA	26.1	7.42
35	88	88	3	C	2C	0	NA	26.1	7.30
36	220	220	1	C	1C	0	NA	26.1	7.50
37	Control	0	2	D	5D	0	NA	26.2	7.29
38	14	14	1	D	4D	0	NA	26.2	7.52
39	35	35	2	D	3D	0	NA	26.3	7.34
40	88	88	1	D	2D	0	NA	26.3	7.40
41	220	220	3	D	1D	0	NA	26.3	7.49
42	Control	0	4	A	5A	1	NA	25.3	7.51
43	14	14	4	A	4A	1	NA	25.3	7.43
44	35	35	4	A	3A	1	NA	25.2	7.43
45	88	88	2	A	2A	1	NA	25.2	7.34
46	220	220	2	A	1A	1	NA	25.2	7.27
47	Control	0	1	B	5B	2	NA	24.8	7.22
48	14	14	3	B	4B	2	NA	24.7	7.59
49	35	35	1	B	3B	2	NA	24.8	7.23
50	88	88	4	B	2B	2	NA	24.8	7.55
51	220	220	4	B	1B	2	NA	24.7	7.69
52	Control	0	3	C	5C	3	NA	24.6	7.50
53	14	14	2	C	4C	3	NA	24.5	7.42
54	35	35	3	C	3C	3	NA	24.5	7.16
55	88	88	3	C	2C	3	NA	24.5	6.89
56	220	220	1	C	1C	3	NA	24.5	7.53
57	Control	0	2	D	5D	4	NA	25.0	5.89
58	14	14	1	D	4D	4	NA	25.1	7.41

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	G	H	I
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Measured Treatment Concentration (µg a.i./L)	Temperature (°C)	Dissolved Oxygen (mg/L)
59	35	35	2	D	3D	4	NA	25.0	7.41
60	88	88	1	D	2D	4	NA	25.1	7.76
61	220	220	3	D	1D	4	NA	24.9	7.85
62	Control	0	4	A	5A	5	NA	25.1	7.84
63	14	14	4	A	4A	5	NA	25.0	7.68
64	35	35	4	A	3A	5	NA	25.0	7.91
65	88	88	2	A	2A	5	NA	24.9	7.79
66	220	220	2	A	1A	5	NA	24.9	7.70
67	Control	0	1	B	5B	6	NA	24.9	6.45
68	14	14	3	B	4B	6	NA	24.8	6.73
69	35	35	1	B	3B	6	NA	24.9	7.35
70	88	88	4	B	2B	6	NA	24.8	7.31
71	220	220	4	B	1B	6	NA	24.8	7.30
72	Control	0	4	A	5A	7	<1.0	NA	NA
73	Control	0	1	B	5B	7	<1.0	NA	NA
74	Control	0	3	C	5C	7	NA	24.6	7.18
75	Control	0	2	D	5D	7	NA	NA	NA
76	14	14	4	A	4A	7	4.8	NA	NA
77	14	14	3	B	4B	7	5.6	NA	NA
78	14	14	2	C	4C	7	NA	24.5	7.50
79	14	14	1	D	4D	7	NA	NA	NA
80	35	35	4	A	3A	7	12	NA	NA
81	35	35	1	B	3B	7	8.5	NA	NA
82	35	35	3	C	3C	7	NA	24.5	7.02
83	35	35	2	D	3D	7	NA	NA	NA
84	88	88	2	A	2A	7	34	NA	NA
85	88	88	4	B	2B	7	34	NA	NA
86	88	88	3	C	2C	7	NA	24.5	7.06
87	88	88	1	D	2D	7	NA	NA	NA
88	220	220	2	A	1A	7	58	NA	NA
89	220	220	4	B	1B	7	49	NA	NA
90	220	220	1	C	1C	7	NA	24.5	7.73
91	220	220	3	D	1D	7	NA	NA	NA
92	Control	0	2	D	5D	8	NA	24.6	7.39
93	14	14	1	D	4D	8	NA	24.6	7.60
94	35	35	2	D	3D	8	NA	24.6	7.51
95	88	88	1	D	2D	8	NA	24.7	7.88
96	220	220	3	D	1D	8	NA	24.5	7.73
97	Control	0	4	A	5A	9	NA	24.9	7.35
98	14	14	4	A	4A	9	NA	24.9	6.75
99	35	35	4	A	3A	9	NA	24.9	7.52
100	88	88	2	A	2A	9	NA	24.9	7.11
101	220	220	2	A	1A	9	NA	24.9	6.80
102	Control	0	1	B	5B	10	NA	24.9	7.27
103	14	14	3	B	4B	10	NA	24.9	7.32
104	35	35	1	B	3B	10	NA	25.0	7.39
105	88	88	4	B	2B	10	NA	25.0	7.05
106	220	220	4	B	1B	10	NA	24.9	6.83
107	Control	0	3	C	5C	11	NA	24.6	7.26
108	14	14	2	C	4C	11	NA	24.5	7.21
109	35	35	3	C	3C	11	NA	24.6	6.99
110	88	88	3	C	2C	11	NA	24.6	6.64
111	220	220	1	C	1C	11	NA	24.5	7.01
112	Control	0	2	D	5D	12	NA	25.0	8.34
113	14	14	1	D	4D	12	NA	24.9	8.25
114	35	35	2	D	3D	12	NA	24.9	8.47

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	G	H	I
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Measured Treatment Concentration (µg a.i./L)	Temperature (°C)	Dissolved Oxygen (mg/L)
115	88	88	1	D	2D	12	NA	24.9	8.04
116	220	220	3	D	1D	12	NA	24.8	8.43
117	Control	0	4	A	5A	13	NA	25.2	7.62
118	14	14	4	A	4A	13	NA	25.2	6.67
119	35	35	4	A	3A	13	NA	25.1	7.36
120	88	88	2	A	2A	13	NA	25.2	7.01
121	220	220	2	A	1A	13	NA	25.2	6.82
122	Control	0	4	A	5A	14	NA	NA	NA
123	Control	0	1	B	5B	14	NA	25.0	7.08
124	Control	0	3	C	5C	14	<1.4	NA	NA
125	Control	0	2	D	5D	14	<1.4	NA	NA
126	14	14	4	A	4A	14	NA	NA	NA
127	14	14	3	B	4B	14	NA	25.0	5.56
128	14	14	2	C	4C	14	8.8	NA	NA
129	14	14	1	D	4D	14	6.8	NA	NA
130	35	35	4	A	3A	14	NA	NA	NA
131	35	35	1	B	3B	14	NA	25.0	4.63
132	35	35	3	C	3C	14	14	NA	NA
133	35	35	2	D	3D	14	12	NA	NA
134	88	88	2	A	2A	14	NA	NA	NA
135	88	88	4	B	2B	14	NA	25.0	6.88
136	88	88	3	C	2C	14	51	NA	NA
137	88	88	1	D	2D	14	32	NA	NA
138	220	220	2	A	1A	14	NA	NA	NA
139	220	220	4	B	1B	14	NA	25.0	6.76
140	220	220	1	C	1C	14	100	NA	NA
141	220	220	3	D	1D	14	60	NA	NA
142	14	14	3	B	4B	14	NA	24.9	6.93
143	35	35	1	B	3B	14	NA	25.0	7.47
144	Control	0	3	C	5C	15	NA	24.9	8.07
145	14	14	2	C	4C	15	NA	24.7	8.08
146	35	35	3	C	3C	15	NA	24.8	7.38
147	88	88	3	C	2C	15	NA	24.8	7.60
148	220	220	1	C	1C	15	NA	24.8	8.06
149	Control	0	2	D	5D	16	NA	24.7	7.78
150	14	14	1	D	4D	16	NA	24.7	7.81
151	35	35	2	D	3D	16	NA	24.6	7.89
152	88	88	1	D	2D	16	NA	24.7	7.88
153	220	220	3	D	1D	16	NA	24.5	7.78
154	Control	0	4	A	5A	17	NA	24.6	7.65
155	14	14	4	A	4A	17	NA	24.6	7.34
156	35	35	4	A	3A	17	NA	24.6	7.57
157	88	88	2	A	2A	17	NA	24.7	7.42
158	220	220	2	A	1A	17	NA	24.6	7.23
159	Control	0	1	B	5B	18	NA	25.1	6.82
160	14	14	3	B	4B	18	NA	25.0	6.87
161	35	35	1	B	3B	18	NA	25.2	6.90
162	88	88	4	B	2B	18	NA	25.1	6.84
163	220	220	4	B	1B	18	NA	25.0	6.81
164	Control	0	3	C	5C	19	NA	25.0	6.97
165	14	14	2	C	4C	19	NA	24.9	6.97
166	35	35	3	C	3C	19	NA	25.0	6.46
167	88	88	3	C	2C	19	NA	24.8	7.11
168	220	220	1	C	1C	19	NA	24.8	7.31
169	Control	0	2	D	5D	20	NA	24.8	8.23
170	14	14	1	D	4D	20	NA	24.7	7.73
171	35	35	2	D	3D	20	NA	24.6	8.09

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	G	H	I
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Measured Treatment Concentration (µg a.i./L)	Temperature (°C)	Dissolved Oxygen (mg/L)
172	88	88	1	D	2D	20	NA	24.7	8.12
173	220	220	3	D	1D	20	NA	24.5	8.04
174	Control	0	4	A	5A	21	<1.1	25.1	7.64
175	Control	0	1	B	5B	21	<1.1	NA	NA
176	Control	0	3	C	5C	21	NA	NA	NA
177	Control	0	2	D	5D	21	NA	NA	NA
178	14	14	4	A	4A	21	11	25.1	6.95
179	14	14	3	B	4B	21	12	NA	NA
180	14	14	2	C	4C	21	NA	NA	NA
181	14	14	1	D	4D	21	NA	NA	NA
182	35	35	4	A	3A	21	28	NA	NA
183	35	35	1	B	3B	21	30	25.1	7.67
184	35	35	3	C	3C	21	NA	NA	NA
185	35	35	2	D	3D	21	NA	NA	NA
186	88	88	2	A	2A	21	80	NA	NA
187	88	88	4	B	2B	21	83	25.1	7.41
188	88	88	3	C	2C	21	NA	NA	NA
189	88	88	1	D	2D	21	NA	NA	NA
190	220	220	2	A	1A	21	164	25.1	7.08
191	220	220	4	B	1B	21	170	NA	NA
192	220	220	1	C	1C	21	NA	NA	NA
193	220	220	3	D	1D	21	NA	NA	NA
194									
195	*pH was inaddvertently recorded as 24.60. This value was removed from the data set.							24.4	
196								26.7	

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	J	K	L
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Dissolved Oxygen (% saturation)	pH	Total Hardness (mg/L as $CaCO_3$)
2	Control	0	4	A	5A	0	98.5	7.44	88
3	Control	0	1	B	5B	0	97.8	7.46	NA
4	Control	0	3	C	5C	0	99.1	7.43	NA
5	Control	0	2	D	5D	0	100.4	7.40	NA
6	14	14	4	A	4A	0	98.4	7.47	NA
7	14	14	3	B	4B	0	98.1	7.47	NA
8	14	14	2	C	4C	0	98.0	7.24	NA
9	14	14	1	D	4D	0	98.1	NA	NA
10	35	35	4	A	3A	0	99.1	7.46	NA
11	35	35	1	B	3B	0	97.9	7.46	NA
12	35	35	3	C	3C	0	98.3	7.45	NA
13	35	35	2	D	3D	0	97.9	7.48	NA
14	88	88	2	A	2A	0	99.1	7.46	NA
15	88	88	4	B	2B	0	98.1	7.45	NA
16	88	88	3	C	2C	0	95.4	7.46	NA
17	88	88	1	D	2D	0	99.3	7.45	NA
18	220	220	2	A	1A	0	99.3	7.44	88
19	220	220	4	B	1B	0	98.1	7.47	NA
20	220	220	1	C	1C	0	98.4	7.48	NA
21	220	220	3	D	1D	0	99.1	7.47	NA
22	Control	0	4	A	5A	0	93.2	7.44	NA
23	14	14	4	A	4A	0	94.9	7.43	NA
24	35	35	4	A	3A	0	93.0	7.42	NA
25	88	88	2	A	2A	0	93.7	7.42	NA
26	220	220	2	A	1A	0	93.5	7.44	NA
27	Control	0	1	B	5B	0	90.8	7.44	NA
28	14	14	3	B	4B	0	92.6	7.42	NA
29	35	35	1	B	3B	0	92.0	7.41	NA
30	88	88	4	B	2B	0	92.6	7.41	NA
31	220	220	4	B	1B	0	94.3	7.44	NA
32	Control	0	3	C	5C	0	93.3	7.41	NA
33	14	14	2	C	4C	0	90.2	7.39	NA
34	35	35	3	C	3C	0	91.9	7.40	NA
35	88	88	3	C	2C	0	90.3	7.43	NA
36	220	220	1	C	1C	0	92.8	7.44	NA
37	Control	0	2	D	5D	0	90.4	7.40	NA
38	14	14	1	D	4D	0	93.2	7.45	NA
39	35	35	2	D	3D	0	91.2	7.41	NA
40	88	88	1	D	2D	0	91.9	7.42	NA
41	220	220	3	D	1D	0	93.1	7.44	NA
42	Control	0	4	A	5A	1	91.6	7.20	NA
43	14	14	4	A	4A	1	90.6	7.21	NA
44	35	35	4	A	3A	1	90.4	7.19	NA
45	88	88	2	A	2A	1	89.4	7.19	NA
46	220	220	2	A	1A	1	88.5	7.17	NA
47	Control	0	1	B	5B	2	87.2	7.36	NA
48	14	14	3	B	4B	2	91.5	7.37	NA
49	35	35	1	B	3B	2	87.4	7.33	NA
50	88	88	4	B	2B	2	91.2	7.35	NA
51	220	220	4	B	1B	2	92.7	7.38	NA
52	Control	0	3	C	5C	3	90.3	7.50	NA
53	14	14	2	C	4C	3	89.2	7.46	NA
54	35	35	3	C	3C	3	86.0	7.40	NA
55	88	88	3	C	2C	3	82.8	7.39	NA
56	220	220	1	C	1C	3	90.5	7.41	NA
57	Control	0	2	D	5D	4	71.4	7.17	NA
58	14	14	1	D	4D	4	90.1	7.19	NA

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	J	K	L
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Dissolved Oxygen (% saturation)	pH	Total Hardness (mg/L as $CaCO_3$)
59	35	35	2	D	3D	4	89.9	7.24	NA
60	88	88	1	D	2D	4	94.3	7.36	NA
61	220	220	3	D	1D	4	95.0	7.38	NA
62	Control	0	4	A	5A	5	95.3	7.34	NA
63	14	14	4	A	4A	5	93.1	7.42	NA
64	35	35	4	A	3A	5	96.0	7.41	NA
65	88	88	2	A	2A	5	94.3	7.42	NA
66	220	220	2	A	1A	5	93.2	7.40	NA
67	Control	0	1	B	5B	6	78.1	7.19	NA
68	14	14	3	B	4B	6	81.3	7.20	NA
69	35	35	1	B	3B	6	89.0	7.22	NA
70	88	88	4	B	2B	6	88.4	7.23	NA
71	220	220	4	B	1B	6	88.2	7.22	NA
72	Control	0	4	A	5A	7	NA	NA	NA
73	Control	0	1	B	5B	7	NA	NA	NA
74	Control	0	3	C	5C	7	86.5	7.32	NA
75	Control	0	2	D	5D	7	NA	NA	NA
76	14	14	4	A	4A	7	NA	NA	NA
77	14	14	3	B	4B	7	NA	NA	NA
78	14	14	2	C	4C	7	90.1	7.32	NA
79	14	14	1	D	4D	7	NA	NA	NA
80	35	35	4	A	3A	7	NA	NA	NA
81	35	35	1	B	3B	7	NA	NA	NA
82	35	35	3	C	3C	7	84.4	7.28	NA
83	35	35	2	D	3D	7	NA	NA	NA
84	88	88	2	A	2A	7	NA	NA	NA
85	88	88	4	B	2B	7	NA	NA	NA
86	88	88	3	C	2C	7	84.8	7.29	NA
87	88	88	1	D	2D	7	NA	NA	NA
88	220	220	2	A	1A	7	NA	NA	NA
89	220	220	4	B	1B	7	NA	NA	NA
90	220	220	1	C	1C	7	92.9	7.29	NA
91	220	220	3	D	1D	7	NA	NA	NA
92	Control	0	2	D	5D	8	89.0	7.47	68
93	14	14	1	D	4D	8	91.5	7.45	72
94	35	35	2	D	3D	8	90.4	7.50	NA
95	88	88	1	D	2D	8	95.1	7.50	NA
96	220	220	3	D	1D	8	92.9	7.48	NA
97	Control	0	4	A	5A	9	89.0	7.32	NA
98	14	14	4	A	4A	9	81.7	7.32	NA
99	35	35	4	A	3A	9	91.1	7.28	NA
100	88	88	2	A	2A	9	86.1	7.26	NA
101	220	220	2	A	1A	9	82.3	7.21	NA
102	Control	0	1	B	5B	10	88.0	7.26	NA
103	14	14	3	B	4B	10	88.6	7.39	NA
104	35	35	1	B	3B	10	89.6	7.34	NA
105	88	88	4	B	2B	10	85.5	7.32	NA
106	220	220	4	B	1B	10	82.7	7.25	NA
107	Control	0	3	C	5C	11	87.4	7.37	NA
108	14	14	2	C	4C	11	86.7	7.30	NA
109	35	35	3	C	3C	11	84.1	7.19	NA
110	88	88	3	C	2C	11	79.9	7.17	NA
111	220	220	1	C	1C	11	84.3	7.23	NA
112	Control	0	2	D	5D	12	101.2	7.15	NA
113	14	14	1	D	4D	12	99.9	7.32	NA
114	35	35	2	D	3D	12	102.5	7.41	NA

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	J	K	L
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Dissolved Oxygen (% saturation)	pH	Total Hardness (mg/L as $CaCO_3$)
115	88	88	1	D	2D	12	97.4	7.35	NA
116	220	220	3	D	1D	12	101.9	7.38	NA
117	Control	0	4	A	5A	13	92.8	7.00	NA
118	14	14	4	A	4A	13	81.2	6.98	NA
119	35	35	4	A	3A	13	89.5	7.14	NA
120	88	88	2	A	2A	13	85.4	7.13	NA
121	220	220	2	A	1A	13	83.0	7.10	NA
122	Control	0	4	A	5A	14	NA	NA	NA
123	Control	0	1	B	5B	14	85.9	7.29	NA
124	Control	0	3	C	5C	14	NA	NA	72
125	Control	0	2	D	5D	14	NA	NA	NA
126	14	14	4	A	4A	14	NA	NA	NA
127	14	14	3	B	4B	14	67.5	7.04	NA
128	14	14	2	C	4C	14	NA	NA	NA
129	14	14	1	D	4D	14	NA	NA	NA
130	35	35	4	A	3A	14	NA	NA	NA
131	35	35	1	B	3B	14	56.1	6.78	NA
132	35	35	3	C	3C	14	NA	NA	NA
133	35	35	2	D	3D	14	NA	NA	72
134	88	88	2	A	2A	14	NA	NA	NA
135	88	88	4	B	2B	14	83.5	7.02	NA
136	88	88	3	C	2C	14	NA	NA	NA
137	88	88	1	D	2D	14	NA	NA	NA
138	220	220	2	A	1A	14	NA	NA	NA
139	220	220	4	B	1B	14	82.0	7.06	NA
140	220	220	1	C	1C	14	NA	NA	NA
141	220	220	3	D	1D	14	NA	NA	NA
142	14	14	3	B	4B	14	83.9	7.09	NA
143	35	35	1	B	3B	14	90.6	7.10	NA
144	Control	0	3	C	5C	15	97.7	7.25	NA
145	14	14	2	C	4C	15	97.5	7.26	NA
146	35	35	3	C	3C	15	89.2	7.13	NA
147	88	88	3	C	2C	15	91.9	7.15	NA
148	220	220	1	C	1C	15	97.4	7.25	NA
149	Control	0	2	D	5D	16	93.9	7.35	NA
150	14	14	1	D	4D	16	94.2	7.39	NA
151	35	35	2	D	3D	16	95.0	7.47	NA
152	88	88	1	D	2D	16	95.1	7.43	NA
153	220	220	3	D	1D	16	93.5	7.41	NA
154	Control	0	4	A	5A	17	92.1	7.35	NA
155	14	14	4	A	4A	17	88.4	7.28	NA
156	35	35	4	A	3A	17	91.1	7.33	NA
157	88	88	2	A	2A	17	89.5	7.31	NA
158	220	220	2	A	1A	17	87.1	7.21	NA
159	Control	0	1	B	5B	18	82.9	7.31	NA
160	14	14	3	B	4B	18	83.3	7.29	NA
161	35	35	1	B	3B	18	84.0	7.35	NA
162	88	88	4	B	2B	18	83.1	7.28	NA
163	220	220	4	B	1B	18	82.6	7.25	NA
164	Control	0	3	C	5C	19	84.5	7.25	NA
165	14	14	2	C	4C	19	84.4	7.26	NA
166	35	35	3	C	3C	19	78.4	7.22	NA
167	88	88	3	C	2C	19	85.9	7.20	NA
168	220	220	1	C	1C	19	88.3	7.23	NA
169	Control	0	2	D	5D	20	99.4	7.39	NA
170	14	14	1	D	4D	20	93.3	7.35	NA
171	35	35	2	D	3D	20	97.4	7.42	NA

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	J	K	L
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Dissolved Oxygen (% saturation)	pH	Total Hardness (mg/L as $CaCO_3$)
172	88	88	1	D	2D	20	98.0	7.40	NA
173	220	220	3	D	1D	20	96.6	7.39	NA
174	Control	0	4	A	5A	21	92.8	7.36	NA
175	Control	0	1	B	5B	21	NA	NA	NA
176	Control	0	3	C	5C	21	NA	NA	NA
177	Control	0	2	D	5D	21	NA	NA	72
178	14	14	4	A	4A	21	84.4	7.27	NA
179	14	14	3	B	4B	21	NA	NA	NA
180	14	14	2	C	4C	21	NA	NA	NA
181	14	14	1	D	4D	21	NA	NA	NA
182	35	35	4	A	3A	21	93.2	NA	NA
183	35	35	1	B	3B	21	NA	7.25	NA
184	35	35	3	C	3C	21	NA	NA	NA
185	35	35	2	D	3D	21	NA	NA	NA
186	88	88	2	A	2A	21	90.0	NA	NA
187	88	88	4	B	2B	21	NA	7.31	NA
188	88	88	3	C	2C	21	NA	NA	NA
189	88	88	1	D	2D	21	NA	NA	68
190	220	220	2	A	1A	21	86.0	7.24	NA
191	220	220	4	B	1B	21	NA	NA	NA
192	220	220	1	C	1C	21	NA	NA	NA
193	220	220	3	D	1D	21	NA	NA	NA
194									
195	*pH was inaddvertently recorded as 24.60. This value was removed from the data set								
196									

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	M	N
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Alkalinity (mg/L as $CaCO_3$)	Conductivity (uS/cm)
2	Control	0	4	A	5A	0	20	602
3	Control	0	1	B	5B	0	NA	NA
4	Control	0	3	C	5C	0	NA	NA
5	Control	0	2	D	5D	0	NA	NA
6	14	14	4	A	4A	0	NA	NA
7	14	14	3	B	4B	0	NA	NA
8	14	14	2	C	4C	0	NA	NA
9	14	14	1	D	4D	0	NA	NA
10	35	35	4	A	3A	0	NA	NA
11	35	35	1	B	3B	0	NA	NA
12	35	35	3	C	3C	0	NA	NA
13	35	35	2	D	3D	0	NA	NA
14	88	88	2	A	2A	0	NA	NA
15	88	88	4	B	2B	0	NA	NA
16	88	88	3	C	2C	0	NA	NA
17	88	88	1	D	2D	0	NA	NA
18	220	220	2	A	1A	0	20	464
19	220	220	4	B	1B	0	NA	NA
20	220	220	1	C	1C	0	NA	NA
21	220	220	3	D	1D	0	NA	NA
22	Control	0	4	A	5A	0	NA	NA
23	14	14	4	A	4A	0	NA	NA
24	35	35	4	A	3A	0	NA	NA
25	88	88	2	A	2A	0	NA	NA
26	220	220	2	A	1A	0	NA	NA
27	Control	0	1	B	5B	0	NA	NA
28	14	14	3	B	4B	0	NA	NA
29	35	35	1	B	3B	0	NA	NA
30	88	88	4	B	2B	0	NA	NA
31	220	220	4	B	1B	0	NA	NA
32	Control	0	3	C	5C	0	NA	NA
33	14	14	2	C	4C	0	NA	NA
34	35	35	3	C	3C	0	NA	NA
35	88	88	3	C	2C	0	NA	NA
36	220	220	1	C	1C	0	NA	NA
37	Control	0	2	D	5D	0	NA	NA
38	14	14	1	D	4D	0	NA	NA
39	35	35	2	D	3D	0	NA	NA
40	88	88	1	D	2D	0	NA	NA
41	220	220	3	D	1D	0	NA	NA
42	Control	0	4	A	5A	1	NA	NA
43	14	14	4	A	4A	1	NA	NA
44	35	35	4	A	3A	1	NA	NA
45	88	88	2	A	2A	1	NA	NA
46	220	220	2	A	1A	1	NA	NA
47	Control	0	1	B	5B	2	NA	NA
48	14	14	3	B	4B	2	NA	NA
49	35	35	1	B	3B	2	NA	NA
50	88	88	4	B	2B	2	NA	NA
51	220	220	4	B	1B	2	NA	NA
52	Control	0	3	C	5C	3	NA	NA
53	14	14	2	C	4C	3	NA	NA
54	35	35	3	C	3C	3	NA	NA
55	88	88	3	C	2C	3	NA	NA
56	220	220	1	C	1C	3	NA	NA
57	Control	0	2	D	5D	4	NA	NA
58	14	14	1	D	4D	4	NA	NA

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	M	N
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Alkalinity (mg/L as $CaCO_3$)	Conductivity (uS/cm)
59	35	35	2	D	3D	4	NA	NA
60	88	88	1	D	2D	4	NA	NA
61	220	220	3	D	1D	4	NA	NA
62	Control	0	4	A	5A	5	NA	NA
63	14	14	4	A	4A	5	NA	NA
64	35	35	4	A	3A	5	NA	NA
65	88	88	2	A	2A	5	NA	NA
66	220	220	2	A	1A	5	NA	NA
67	Control	0	1	B	5B	6	NA	NA
68	14	14	3	B	4B	6	NA	NA
69	35	35	1	B	3B	6	NA	NA
70	88	88	4	B	2B	6	NA	NA
71	220	220	4	B	1B	6	NA	NA
72	Control	0	4	A	5A	7	NA	NA
73	Control	0	1	B	5B	7	NA	NA
74	Control	0	3	C	5C	7	NA	NA
75	Control	0	2	D	5D	7	NA	NA
76	14	14	4	A	4A	7	NA	NA
77	14	14	3	B	4B	7	NA	NA
78	14	14	2	C	4C	7	NA	NA
79	14	14	1	D	4D	7	NA	NA
80	35	35	4	A	3A	7	NA	NA
81	35	35	1	B	3B	7	NA	NA
82	35	35	3	C	3C	7	NA	NA
83	35	35	2	D	3D	7	NA	NA
84	88	88	2	A	2A	7	NA	NA
85	88	88	4	B	2B	7	NA	NA
86	88	88	3	C	2C	7	NA	NA
87	88	88	1	D	2D	7	NA	NA
88	220	220	2	A	1A	7	NA	NA
89	220	220	4	B	1B	7	NA	NA
90	220	220	1	C	1C	7	NA	NA
91	220	220	3	D	1D	7	NA	NA
92	Control	0	2	D	5D	8	26	711
93	14	14	1	D	4D	8	24	459
94	35	35	2	D	3D	8	NA	NA
95	88	88	1	D	2D	8	NA	NA
96	220	220	3	D	1D	8	NA	NA
97	Control	0	4	A	5A	9	NA	NA
98	14	14	4	A	4A	9	NA	NA
99	35	35	4	A	3A	9	NA	NA
100	88	88	2	A	2A	9	NA	NA
101	220	220	2	A	1A	9	NA	NA
102	Control	0	1	B	5B	10	NA	NA
103	14	14	3	B	4B	10	NA	NA
104	35	35	1	B	3B	10	NA	NA
105	88	88	4	B	2B	10	NA	NA
106	220	220	4	B	1B	10	NA	NA
107	Control	0	3	C	5C	11	NA	NA
108	14	14	2	C	4C	11	NA	NA
109	35	35	3	C	3C	11	NA	NA
110	88	88	3	C	2C	11	NA	NA
111	220	220	1	C	1C	11	NA	NA
112	Control	0	2	D	5D	12	NA	NA
113	14	14	1	D	4D	12	NA	NA
114	35	35	2	D	3D	12	NA	NA

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	M	N
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Alkalinity (mg/L as $CaCO_3$)	Conductivity (uS/cm)
115	88	88	1	D	2D	12	NA	NA
116	220	220	3	D	1D	12	NA	NA
117	Control	0	4	A	5A	13	NA	NA
118	14	14	4	A	4A	13	NA	NA
119	35	35	4	A	3A	13	NA	NA
120	88	88	2	A	2A	13	NA	NA
121	220	220	2	A	1A	13	NA	NA
122	Control	0	4	A	5A	14	NA	NA
123	Control	0	1	B	5B	14	NA	NA
124	Control	0	3	C	5C	14	24	541
125	Control	0	2	D	5D	14	NA	NA
126	14	14	4	A	4A	14	NA	NA
127	14	14	3	B	4B	14	NA	NA
128	14	14	2	C	4C	14	NA	NA
129	14	14	1	D	4D	14	NA	NA
130	35	35	4	A	3A	14	NA	NA
131	35	35	1	B	3B	14	NA	NA
132	35	35	3	C	3C	14	NA	NA
133	35	35	2	D	3D	14	24	525
134	88	88	2	A	2A	14	NA	NA
135	88	88	4	B	2B	14	NA	NA
136	88	88	3	C	2C	14	NA	NA
137	88	88	1	D	2D	14	NA	NA
138	220	220	2	A	1A	14	NA	NA
139	220	220	4	B	1B	14	NA	NA
140	220	220	1	C	1C	14	NA	NA
141	220	220	3	D	1D	14	NA	NA
142	14	14	3	B	4B	14	NA	NA
143	35	35	1	B	3B	14	NA	NA
144	Control	0	3	C	5C	15	NA	NA
145	14	14	2	C	4C	15	NA	NA
146	35	35	3	C	3C	15	NA	NA
147	88	88	3	C	2C	15	NA	NA
148	220	220	1	C	1C	15	NA	NA
149	Control	0	2	D	5D	16	NA	NA
150	14	14	1	D	4D	16	NA	NA
151	35	35	2	D	3D	16	NA	NA
152	88	88	1	D	2D	16	NA	NA
153	220	220	3	D	1D	16	NA	NA
154	Control	0	4	A	5A	17	NA	NA
155	14	14	4	A	4A	17	NA	NA
156	35	35	4	A	3A	17	NA	NA
157	88	88	2	A	2A	17	NA	NA
158	220	220	2	A	1A	17	NA	NA
159	Control	0	1	B	5B	18	NA	NA
160	14	14	3	B	4B	18	NA	NA
161	35	35	1	B	3B	18	NA	NA
162	88	88	4	B	2B	18	NA	NA
163	220	220	4	B	1B	18	NA	NA
164	Control	0	3	C	5C	19	NA	NA
165	14	14	2	C	4C	19	NA	NA
166	35	35	3	C	3C	19	NA	NA
167	88	88	3	C	2C	19	NA	NA
168	220	220	1	C	1C	19	NA	NA
169	Control	0	2	D	5D	20	NA	NA
170	14	14	1	D	4D	20	NA	NA
171	35	35	2	D	3D	20	NA	NA

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\9. DEST forms + Stats Report\13784.6120 Biologica Data DESTS.final

	A	B	C	D	E	F	M	N
1	Name of Treatment Group	Nominal Treatment Concentration (µg a.i./L)	Block ID	Replicate ID	Tank ID	Test Day (0 to 21)	Alkalinity (mg/L as $CaCO_3$)	Conductivity (uS/cm)
172	88	88	1	D	2D	20	NA	NA
173	220	220	3	D	1D	20	NA	NA
174	Control	0	4	A	5A	21	NA	NA
175	Control	0	1	B	5B	21	NA	NA
176	Control	0	3	C	5C	21	NA	NA
177	Control	0	2	D	5D	21	22	488
178	14	14	4	A	4A	21	NA	NA
179	14	14	3	B	4B	21	NA	NA
180	14	14	2	C	4C	21	NA	NA
181	14	14	1	D	4D	21	NA	NA
182	35	35	4	A	3A	21	NA	NA
183	35	35	1	B	3B	21	NA	NA
184	35	35	3	C	3C	21	NA	NA
185	35	35	2	D	3D	21	NA	NA
186	88	88	2	A	2A	21	NA	NA
187	88	88	4	B	2B	21	NA	NA
188	88	88	3	C	2C	21	NA	NA
189	88	88	1	D	2D	21	20	488
190	220	220	2	A	1A	21	NA	NA
191	220	220	4	B	1B	21	NA	NA
192	220	220	1	C	1C	21	NA	NA
193	220	220	3	D	1D	21	NA	NA
194								
195	*pH was inaddvertently recorded as 24.60. This value was removed from the data set							
196								

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
	Control	A	5A	0	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	1	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	2	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	3	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	4	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	0	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	5	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	0	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	6	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	0	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	7	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	1
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	8	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	9	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	10	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	11	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	12	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	13	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	0
					Territorial Agression	2	0
					Bloated	0	0
	Control	A	5A	14	Tubercles Present	2	0
					Fatpad Present	2	0
					Color Bars Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
75					Territorial Agression	2	0
76					Bloated	0	0
77	Control	A	5A	15	Tubercles Present	2	0
78					Fatpad Present	2	0
79					Color Bars Present	2	0
80					Territorial Agression	2	0
81					Bloated	0	0
82	Control	A	5A	16	Tubercles Present	2	0
83					Fatpad Present	2	0
84					Color Bars Present	2	0
85					Territorial Agression	2	0
86					Bloated	0	0
87	Control	A	5A	17	Tubercles Present	2	0
88					Fatpad Present	2	0
89					Color Bars Present	2	0
90					Territorial Agression	2	0
91					Bloated	0	0
92	Control	A	5A	18	Tubercles Present	2	0
93					Fatpad Present	2	0
94					Color Bars Present	2	0
95					Territorial Agression	2	0
96					Bloated	0	0
97	Control	A	5A	19	Tubercles Present	2	0
98					Fatpad Present	2	0
99					Color Bars Present	2	0
100					Territorial Agression	2	0
101					Bloated	0	0
102	Control	A	5A	20	Tubercles Present	2	0
103					Fatpad Present	2	0
104					Color Bars Present	2	0
105					Territorial Agression	2	0
106					Bloated	0	0
107	Control	A	5A	21	Tubercles Present	2	0
108					Fatpad Present	2	0
109					Color Bars Present	2	0
110					Territorial Agression	2	0
111					Bloated	0	0
112	Control	B	5B	0	Tubercles Present	2	0
113					Fatpad Present	2	0
114					Color Bars Present	2	0
115					Territorial Agression	2	0
116					Bloated	0	0
117	Control	B	5B	1	Tubercles Present	2	0
118					Fatpad Present	2	0
119					Color Bars Present	2	0
120					Territorial Agression	2	0
121					Bloated	0	0
122	Control	B	5B	2	Tubercles Present	2	0
123					Fatpad Present	2	0
124					Color Bars Present	2	0
125					Territorial Agression	2	0
126					Bloated	0	0
127	Control	B	5B	3	Tubercles Present	2	0
128					Fatpad Present	2	0
129					Color Bars Present	2	0
130					Territorial Agression	2	0
131					Bloated	0	0
132	Control	B	5B	4	Tubercles Present	2	0
133					Fatpad Present	2	0
134					Color Bars Present	2	0
135					Territorial Agression	2	0
136					Bloated	0	0
137	Control	B	5B	5	Tubercles Present	2	0
138					Fatpad Present	2	0
139					Color Bars Present	2	0
140					Territorial Agression	2	0
141					Bloated	0	0
142	Control	B	5B	6	Tubercles Present	2	0
143					Fatpad Present	2	0
144					Color Bars Present	2	0
145					Territorial Agression	2	0
146					Bloated	0	0
147	Control	B	5B	7	Tubercles Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
148					Fatpad Present	2	0
149					Color Bars Present	2	0
150					Territorial Agression	2	0
151					Bloated	0	0
152	Control	B	5B	8	Tubercles Present	2	0
153					Fatpad Present	2	0
154					Color Bars Present	2	0
155					Territorial Agression	2	0
156					Bloated	0	0
157	Control	B	5B	9	Tubercles Present	2	0
158					Fatpad Present	2	0
159					Color Bars Present	2	0
160					Territorial Agression	2	0
161					Bloated	0	0
162	Control	B	5B	10	Tubercles Present	2	0
163					Fatpad Present	2	0
164					Color Bars Present	2	0
165					Territorial Agression	2	0
166					Bloated	0	0
167	Control	B	5B	11	Tubercles Present	2	0
168					Fatpad Present	2	0
169					Color Bars Present	2	0
170					Territorial Agression	2	0
171					Bloated	0	0
172	Control	B	5B	12	Tubercles Present	2	0
173					Fatpad Present	2	0
174					Color Bars Present	2	0
175					Territorial Agression	2	0
176					Bloated	0	0
177	Control	B	5B	13	Tubercles Present	2	0
178					Fatpad Present	2	0
179					Color Bars Present	2	0
180					Territorial Agression	2	0
181					Bloated	0	0
182	Control	B	5B	14	Tubercles Present	2	0
183					Fatpad Present	2	0
184					Color Bars Present	2	0
185					Territorial Agression	2	0
186					Bloated	0	0
187	Control	B	5B	15	Tubercles Present	2	0
188					Fatpad Present	2	0
189					Color Bars Present	2	0
190					Territorial Agression	2	0
191					Bloated	0	0
192	Control	B	5B	16	Tubercles Present	2	0
193					Fatpad Present	2	0
194					Color Bars Present	2	0
195					Territorial Agression	2	0
196					Bloated	0	0
197	Control	B	5B	17	Tubercles Present	2	0
198					Fatpad Present	2	0
199					Color Bars Present	2	0
200					Territorial Agression	2	0
201					Bloated	0	0
202	Control	B	5B	18	Tubercles Present	2	0
203					Fatpad Present	2	0
204					Color Bars Present	2	0
205					Territorial Agression	2	0
206					Bloated	0	0
207	Control	B	5B	19	Tubercles Present	2	0
208					Fatpad Present	2	0
209					Color Bars Present	2	0
210					Territorial Agression	2	0
211					Bloated	0	0
212	Control	B	5B	20	Tubercles Present	2	0
213					Fatpad Present	2	0
214					Color Bars Present	2	0
215					Territorial Agression	2	0
216					Bloated	0	0
217	Control	B	5B	21	Tubercles Present	2	0
218					Fatpad Present	2	0
219					Color Bars Present	2	0
220					Territorial Agression	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
221					Bloated	0	0
222	Control	C	5C	0	Tubercles Present	2	0
223					Fatpad Present	2	0
224					Color Bars Present	2	0
225					Territorial Agression	2	0
226					Bloated	0	0
227	Control	C	5C	1	Tubercles Present	2	0
228					Fatpad Present	2	0
229					Color Bars Present	2	0
230					Territorial Agression	2	0
231					Bloated	0	0
232	Control	C	5C	2	Tubercles Present	2	0
233					Fatpad Present	2	0
234					Color Bars Present	2	0
235					Territorial Agression	2	0
236					Bloated	0	0
237	Control	C	5C	3	Tubercles Present	2	0
238					Fatpad Present	2	0
239					Color Bars Present	2	0
240					Territorial Agression	2	0
241					Bloated	0	0
242	Control	C	5C	4	Tubercles Present	0	0
243					Fatpad Present	2	0
244					Color Bars Present	1	0
245					Territorial Agression	2	0
246					Bloated	0	0
247	Control	C	5C	5	Tubercles Present	2	0
248					Fatpad Present	2	0
249					Color Bars Present	2	0
250					Territorial Agression	2	0
251					Bloated	0	0
252	Control	C	5C	6	Tubercles Present	2	0
253					Fatpad Present	2	0
254					Color Bars Present	2	0
255					Territorial Agression	2	0
256					Bloated	0	0
257	Control	C	5C	7	Tubercles Present	2	0
258					Fatpad Present	2	0
259					Color Bars Present	2	0
260					Territorial Agression	2	0
261					Bloated	0	0
262	Control	C	5C	8	Tubercles Present	2	0
263					Fatpad Present	2	0
264					Color Bars Present	2	0
265					Territorial Agression	2	0
266					Bloated	0	0
267	Control	C	5C	9	Tubercles Present	2	0
268					Fatpad Present	2	0
269					Color Bars Present	2	0
270					Territorial Agression	2	0
271					Bloated	0	0
272	Control	C	5C	10	Tubercles Present	2	0
273					Fatpad Present	2	0
274					Color Bars Present	2	0
275					Territorial Agression	2	0
276					Bloated	0	0
277	Control	C	5C	11	Tubercles Present	2	0
278					Fatpad Present	2	0
279					Color Bars Present	2	0
280					Territorial Agression	2	0
281					Bloated	0	0
282	Control	C	5C	12	Tubercles Present	2	0
283					Fatpad Present	2	0
284					Color Bars Present	2	0
285					Territorial Agression	2	0
286					Bloated	0	0
287	Control	C	5C	13	Tubercles Present	2	0
288					Fatpad Present	2	0
289					Color Bars Present	2	0
290					Territorial Agression	2	0
291					Bloated	0	0
292	Control	C	5C	14	Tubercles Present	2	0
293					Fatpad Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
294					Color Bars Present	2	0
295					Territorial Agression	2	0
296					Bloated	0	0
297	Control	C	5C	15	Tubercles Present	2	0
298					Fatpad Present	2	0
299					Color Bars Present	2	0
300					Territorial Agression	2	0
301					Bloated	0	0
302	Control	C	5C	16	Tubercles Present	2	0
303					Fatpad Present	2	0
304					Color Bars Present	2	0
305					Territorial Agression	2	0
306					Bloated	0	0
307	Control	C	5C	17	Tubercles Present	2	0
308					Fatpad Present	2	0
309					Color Bars Present	2	0
310					Territorial Agression	2	0
311					Bloated	0	0
312	Control	C	5C	18	Tubercles Present	2	0
313					Fatpad Present	2	0
314					Color Bars Present	2	0
315					Territorial Agression	2	0
316					Bloated	0	0
317	Control	C	5C	19	Tubercles Present	2	0
318					Fatpad Present	2	0
319					Color Bars Present	2	0
320					Territorial Agression	2	0
321					Bloated	0	0
322	Control	C	5C	20	Tubercles Present	2	0
323					Fatpad Present	2	0
324					Color Bars Present	2	0
325					Territorial Agression	2	0
326					Bloated	0	0
327	Control	C	5C	21	Tubercles Present	2	0
328					Fatpad Present	2	0
329					Color Bars Present	2	0
330					Territorial Agression	2	0
331					Bloated	0	0
332	Control	D	5D	0	Tubercles Present	2	0
333					Fatpad Present	2	0
334					Color Bars Present	2	0
335					Territorial Agression	2	0
336					Bloated	0	0
337	Control	D	5D	1	Tubercles Present	2	0
338					Fatpad Present	2	0
339					Color Bars Present	2	0
340					Territorial Agression	2	0
341					Bloated	0	0
342	Control	D	5D	2	Tubercles Present	2	0
343					Fatpad Present	2	0
344					Color Bars Present	2	0
345					Territorial Agression	2	0
346					Bloated	0	0
347	Control	D	5D	3	Tubercles Present	2	0
348					Fatpad Present	2	0
349					Color Bars Present	2	0
350					Territorial Agression	2	0
351					Bloated	0	0
352	Control	D	5D	4	Tubercles Present	2	0
353					Fatpad Present	2	0
354					Color Bars Present	2	0
355					Territorial Agression	2	0
356					Bloated	0	0
357	Control	D	5D	5	Tubercles Present	2	0
358					Fatpad Present	2	0
359					Color Bars Present	2	0
360					Territorial Agression	2	0
361					Bloated	0	0
362	Control	D	5D	6	Tubercles Present	2	0
363					Fatpad Present	2	0
364					Color Bars Present	2	0
365					Territorial Agression	2	0
366					Bloated	0	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
367	Control	D	5D	7	Tubercles Present	2	0
368					Fatpad Present	2	0
369					Color Bars Present	2	0
370					Territorial Agression	2	0
371					Bloated	0	0
372	Control	D	5D	8	Tubercles Present	2	0
373					Fatpad Present	2	0
374					Color Bars Present	2	0
375					Territorial Agression	2	0
376					Bloated	0	0
377	Control	D	5D	9	Tubercles Present	2	0
378					Fatpad Present	2	0
379					Color Bars Present	2	0
380					Territorial Agression	2	0
381					Bloated	0	0
382	Control	D	5D	10	Tubercles Present	2	0
383					Fatpad Present	2	0
384					Color Bars Present	2	0
385					Territorial Agression	2	0
386					Bloated	0	0
387	Control	D	5D	11	Tubercles Present	2	0
388					Fatpad Present	2	0
389					Color Bars Present	2	0
390					Territorial Agression	2	0
391					Bloated	0	0
392	Control	D	5D	12	Tubercles Present	2	0
393					Fatpad Present	2	0
394					Color Bars Present	2	0
395					Territorial Agression	2	0
396					Bloated	0	0
397	Control	D	5D	13	Tubercles Present	2	0
398					Fatpad Present	2	0
399					Color Bars Present	2	0
400					Territorial Agression	2	0
401					Bloated	0	0
402	Control	D	5D	14	Tubercles Present	2	0
403					Fatpad Present	2	0
404					Color Bars Present	2	0
405					Territorial Agression	2	0
406					Bloated	0	0
407	Control	D	5D	15	Tubercles Present	2	0
408					Fatpad Present	2	0
409					Color Bars Present	2	0
410					Territorial Agression	2	0
411					Bloated	0	0
412	Control	D	5D	16	Tubercles Present	2	0
413					Fatpad Present	2	0
414					Color Bars Present	2	0
415					Territorial Agression	2	0
416					Bloated	0	0
417	Control	D	5D	17	Tubercles Present	2	0
418					Fatpad Present	2	0
419					Color Bars Present	2	0
420					Territorial Agression	2	0
421					Bloated	0	0
422	Control	D	5D	18	Tubercles Present	2	0
423					Fatpad Present	2	0
424					Color Bars Present	2	0
425					Territorial Agression	2	0
426					Bloated	0	0
427	Control	D	5D	19	Tubercles Present	2	0
428					Fatpad Present	2	0
429					Color Bars Present	2	0
430					Territorial Agression	2	0
431					Bloated	0	0
432	Control	D	5D	20	Tubercles Present	2	0
433					Fatpad Present	2	0
434					Color Bars Present	2	0
435					Territorial Agression	2	0
436					Bloated	0	0
437	Control	D	5D	21	Tubercles Present	2	0
438					Fatpad Present	2	0
439					Color Bars Present	2	0
440					Territorial Agression	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
441					Bloated	0	0
442	14 µg/L	A	4A	0	Tubercles Present	2	0
443					Fatpad Present	2	0
444					Color Bars Present	2	0
445					Territorial Agression	2	0
446					Bloated	0	0
447	14 µg/L	A	4A	1	Tubercles Present	2	0
448					Fatpad Present	2	0
449					Color Bars Present	2	0
450					Territorial Agression	2	0
451					Bloated	0	0
452	14 µg/L	A	4A	2	Tubercles Present	2	0
453					Fatpad Present	2	0
454					Color Bars Present	2	0
455					Territorial Agression	2	0
456					Bloated	0	0
457	14 µg/L	A	4A	3	Tubercles Present	2	0
458					Fatpad Present	2	0
459					Color Bars Present	2	0
460					Territorial Agression	2	0
461					Bloated	0	0
462	14 µg/L	A	4A	4	Tubercles Present	2	0
463					Fatpad Present	2	0
464					Color Bars Present	2	0
465					Territorial Agression	2	0
466					Bloated	0	0
467	14 µg/L	A	4A	5	Tubercles Present	2	0
468					Fatpad Present	2	0
469					Color Bars Present	2	0
470					Territorial Agression	2	0
471					Bloated	0	0
472	14 µg/L	A	4A	6	Tubercles Present	2	0
473					Fatpad Present	2	0
474					Color Bars Present	2	0
475					Territorial Agression	2	0
476					Bloated	0	0
477	14 µg/L	A	4A	7	Tubercles Present	2	0
478					Fatpad Present	2	0
479					Color Bars Present	2	0
480					Territorial Agression	2	0
481					Bloated	0	0
482	14 µg/L	A	4A	8	Tubercles Present	2	0
483					Fatpad Present	2	0
484					Color Bars Present	2	0
485					Territorial Agression	2	0
486					Bloated	0	0
487	14 µg/L	A	4A	9	Tubercles Present	2	0
488					Fatpad Present	2	0
489					Color Bars Present	2	0
490					Territorial Agression	2	0
491					Bloated	0	0
492	14 µg/L	A	4A	10	Tubercles Present	2	0
493					Fatpad Present	2	0
494					Color Bars Present	2	0
495					Territorial Agression	2	0
496					Bloated	0	0
497	14 µg/L	A	4A	11	Tubercles Present	2	0
498					Fatpad Present	2	0
499					Color Bars Present	2	0
500					Territorial Agression	2	0
501					Bloated	0	0
502	14 µg/L	A	4A	12	Tubercles Present	2	0
503					Fatpad Present	2	0
504					Color Bars Present	2	0
505					Territorial Agression	2	0
506					Bloated	0	0
507	14 µg/L	A	4A	13	Tubercles Present	2	0
508					Fatpad Present	2	0
509					Color Bars Present	2	0
510					Territorial Agression	2	0
511					Bloated	0	0
512	14 µg/L	A	4A	14	Tubercles Present	2	0
513					Fatpad Present	2	0
514					Color Bars Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
515					Territorial Agression	2	0
516					Bloated	0	0
517	14 µg/L	A	4A	15	Tubercles Present	2	0
518					Fatpad Present	2	0
519					Color Bars Present	2	0
520					Territorial Agression	2	0
521					Bloated	0	0
522	14 µg/L	A	4A	16	Tubercles Present	2	0
523					Fatpad Present	2	0
524					Color Bars Present	2	0
525					Territorial Agression	2	0
526					Bloated	0	0
527	14 µg/L	A	4A	17	Tubercles Present	2	0
528					Fatpad Present	2	0
529					Color Bars Present	2	0
530					Territorial Agression	2	0
531					Bloated	0	0
532	14 µg/L	A	4A	18	Tubercles Present	2	0
533					Fatpad Present	2	0
534					Color Bars Present	2	0
535					Territorial Agression	2	0
536					Bloated	0	0
537	14 µg/L	A	4A	19	Tubercles Present	2	0
538					Fatpad Present	2	0
539					Color Bars Present	2	0
540					Territorial Agression	2	0
541					Bloated	0	0
542	14 µg/L	A	4A	20	Tubercles Present	2	0
543					Fatpad Present	2	0
544					Color Bars Present	2	0
545					Territorial Agression	2	0
546					Bloated	0	0
547	14 µg/L	A	4A	21	Tubercles Present	2	0
548					Fatpad Present	2	0
549					Color Bars Present	2	0
550					Territorial Agression	2	0
551					Bloated	0	0
552	14 µg/L	B	4B	0	Tubercles Present	2	0
553					Fatpad Present	2	0
554					Color Bars Present	2	0
555					Territorial Agression	2	0
556					Bloated	0	0
557	14 µg/L	B	4B	1	Tubercles Present	2	0
558					Fatpad Present	2	0
559					Color Bars Present	2	0
560					Territorial Agression	2	0
561					Bloated	0	0
562	14 µg/L	B	4B	2	Tubercles Present	2	0
563					Fatpad Present	2	0
564					Color Bars Present	2	0
565					Territorial Agression	2	0
566					Bloated	0	0
567	14 µg/L	B	4B	3	Tubercles Present	2	0
568					Fatpad Present	2	0
569					Color Bars Present	2	0
570					Territorial Agression	2	0
571					Bloated	0	0
572	14 µg/L	B	4B	4	Tubercles Present	2	0
573					Fatpad Present	2	0
574					Color Bars Present	2	0
575					Territorial Agression	2	0
576					Bloated	0	0
577	14 µg/L	B	4B	5	Tubercles Present	2	0
578					Fatpad Present	2	0
579					Color Bars Present	2	0
580					Territorial Agression	2	0
581					Bloated	0	0
582	14 µg/L	B	4B	6	Tubercles Present	2	0
583					Fatpad Present	2	0
584					Color Bars Present	2	0
585					Territorial Agression	2	0
586					Bloated	0	0
587	14 µg/L	B	4B	7	Tubercles Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
588					Fatpad Present	2	0
589					Color Bars Present	2	0
590					Territorial Agression	2	0
591					Bloated	0	0
592	14 µg/L	B	4B	8	Tubercles Present	2	0
593					Fatpad Present	2	0
594					Color Bars Present	2	0
595					Territorial Agression	2	0
596					Bloated	0	0
597	14 µg/L	B	4B	9	Tubercles Present	2	0
598					Fatpad Present	2	0
599					Color Bars Present	2	0
600					Territorial Agression	2	0
601					Bloated	0	0
602	14 µg/L	B	4B	10	Tubercles Present	2	0
603					Fatpad Present	2	0
604					Color Bars Present	2	0
605					Territorial Agression	2	0
606					Bloated	0	0
607	14 µg/L	B	4B	11	Tubercles Present	2	0
608					Fatpad Present	2	0
609					Color Bars Present	2	0
610					Territorial Agression	2	0
611					Bloated	0	0
612	14 µg/L	B	4B	12	Tubercles Present	2	0
613					Fatpad Present	2	0
614					Color Bars Present	2	0
615					Territorial Agression	2	0
616					Bloated	0	0
617	14 µg/L	B	4B	13	Tubercles Present	2	0
618					Fatpad Present	2	0
619					Color Bars Present	2	0
620					Territorial Agression	2	0
621					Bloated	0	0
622	14 µg/L	B	4B	14	Tubercles Present	2	0
623					Fatpad Present	2	0
624					Color Bars Present	2	0
625					Territorial Agression	2	0
626					Bloated	0	0
627	14 µg/L	B	4B	15	Tubercles Present	2	0
628					Fatpad Present	2	0
629					Color Bars Present	2	0
630					Territorial Agression	2	0
631					Bloated	0	0
632	14 µg/L	B	4B	16	Tubercles Present	2	0
633					Fatpad Present	2	0
634					Color Bars Present	2	0
635					Territorial Agression	2	0
636					Bloated	0	0
637	14 µg/L	B	4B	17	Tubercles Present	2	0
638					Fatpad Present	2	0
639					Color Bars Present	2	0
640					Territorial Agression	2	0
641					Bloated	0	0
642	14 µg/L	B	4B	18	Tubercles Present	2	0
643					Fatpad Present	2	0
644					Color Bars Present	2	0
645					Territorial Agression	2	0
646					Bloated	0	0
647	14 µg/L	B	4B	19	Tubercles Present	2	0
648					Fatpad Present	2	0
649					Color Bars Present	2	0
650					Territorial Agression	2	0
651					Bloated	0	0
652	14 µg/L	B	4B	20	Tubercles Present	2	0
653					Fatpad Present	2	0
654					Color Bars Present	2	0
655					Territorial Agression	2	0
656					Bloated	0	0
657	14 µg/L	B	4B	21	Tubercles Present	2	0
658					Fatpad Present	2	0
659					Color Bars Present	2	0
660					Territorial Agression	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
661					Bloated	0	0
662	14 µg/L	C	4C	0	Tubercles Present	2	0
663					Fatpad Present	2	0
664					Color Bars Present	2	0
665					Territorial Agression	2	0
666					Bloated	0	0
667	14 µg/L	C	4C	1	Tubercles Present	2	0
668					Fatpad Present	2	0
669					Color Bars Present	2	0
670					Territorial Agression	2	0
671					Bloated	0	0
672	14 µg/L	C	4C	2	Tubercles Present	2	0
673					Fatpad Present	2	0
674					Color Bars Present	2	0
675					Territorial Agression	2	0
676					Bloated	0	0
677	14 µg/L	C	4C	3	Tubercles Present	2	0
678					Fatpad Present	2	0
679					Color Bars Present	2	0
680					Territorial Agression	2	0
681					Bloated	0	0
682	14 µg/L	C	4C	4	Tubercles Present	2	0
683					Fatpad Present	2	0
684					Color Bars Present	2	0
685					Territorial Agression	2	0
686					Bloated	0	0
687	14 µg/L	C	4C	5	Tubercles Present	2	0
688					Fatpad Present	2	0
689					Color Bars Present	2	0
690					Territorial Agression	2	0
691					Bloated	0	0
692	14 µg/L	C	4C	6	Tubercles Present	2	0
693					Fatpad Present	2	0
694					Color Bars Present	2	0
695					Territorial Agression	2	0
696					Bloated	0	0
697	14 µg/L	C	4C	7	Tubercles Present	2	0
698					Fatpad Present	2	0
699					Color Bars Present	2	0
700					Territorial Agression	2	0
701					Bloated	0	0
702	14 µg/L	C	4C	8	Tubercles Present	2	0
703					Fatpad Present	2	0
704					Color Bars Present	2	0
705					Territorial Agression	2	0
706					Bloated	0	0
707	14 µg/L	C	4C	9	Tubercles Present	2	0
708					Fatpad Present	2	0
709					Color Bars Present	2	0
710					Territorial Agression	2	0
711					Bloated	0	0
712	14 µg/L	C	4C	10	Tubercles Present	2	0
713					Fatpad Present	2	0
714					Color Bars Present	2	0
715					Territorial Agression	2	0
716					Bloated	0	0
717	14 µg/L	C	4C	11	Tubercles Present	2	0
718					Fatpad Present	2	0
719					Color Bars Present	2	0
720					Territorial Agression	2	0
721					Bloated	0	0
722	14 µg/L	C	4C	12	Tubercles Present	2	0
723					Fatpad Present	2	0
724					Color Bars Present	2	0
725					Territorial Agression	2	0
726					Bloated	0	0
727	14 µg/L	C	4C	13	Tubercles Present	2	0
728					Fatpad Present	2	0
729					Color Bars Present	2	0
730					Territorial Agression	2	0
731					Bloated	0	0
732	14 µg/L	C	4C	14	Tubercles Present	2	0
733					Fatpad Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
734					Color Bars Present	2	0
735					Territorial Agression	2	0
736					Bloated	0	0
737	14 µg/L	C	4C	15	Tubercles Present	2	0
738					Fatpad Present	2	0
739					Color Bars Present	2	0
740					Territorial Agression	2	0
741					Bloated	0	0
742	14 µg/L	C	4C	16	Tubercles Present	2	0
743					Fatpad Present	2	0
744					Color Bars Present	2	0
745					Territorial Agression	2	0
746					Bloated	0	0
747	14 µg/L	C	4C	17	Tubercles Present	2	0
748					Fatpad Present	2	0
749					Color Bars Present	2	0
750					Territorial Agression	2	0
751					Bloated	0	0
752	14 µg/L	C	4C	18	Tubercles Present	2	0
753					Fatpad Present	2	0
754					Color Bars Present	2	0
755					Territorial Agression	2	0
756					Bloated	0	0
757	14 µg/L	C	4C	19	Tubercles Present	2	0
758					Fatpad Present	2	0
759					Color Bars Present	2	0
760					Territorial Agression	2	0
761					Bloated	0	0
762	14 µg/L	C	4C	20	Tubercles Present	2	0
763					Fatpad Present	2	0
764					Color Bars Present	2	0
765					Territorial Agression	2	0
766					Bloated	0	0
767	14 µg/L	C	4C	21	Tubercles Present	2	0
768					Fatpad Present	2	0
769					Color Bars Present	2	0
770					Territorial Agression	2	0
771					Bloated	0	0
772	14 µg/L	D	4D	0	Tubercles Present	2	0
773					Fatpad Present	2	0
774					Color Bars Present	2	0
775					Territorial Agression	2	0
776					Bloated	0	0
777	14 µg/L	D	4D	1	Tubercles Present	2	0
778					Fatpad Present	2	0
779					Color Bars Present	2	0
780					Territorial Agression	2	0
781					Bloated	0	0
782	14 µg/L	D	4D	2	Tubercles Present	2	0
783					Fatpad Present	2	0
784					Color Bars Present	2	0
785					Territorial Agression	2	0
786					Bloated	0	0
787	14 µg/L	D	4D	3	Tubercles Present	2	0
788					Fatpad Present	2	0
789					Color Bars Present	2	0
790					Territorial Agression	2	0
791					Bloated	0	0
792	14 µg/L	D	4D	4	Tubercles Present	2	0
793					Fatpad Present	2	0
794					Color Bars Present	2	0
795					Territorial Agression	2	0
796					Bloated	0	0
797	14 µg/L	D	4D	5	Tubercles Present	2	0
798					Fatpad Present	2	0
799					Color Bars Present	2	0
800					Territorial Agression	2	0
801					Bloated	0	0
802	14 µg/L	D	4D	6	Tubercles Present	2	0
803					Fatpad Present	2	0
804					Color Bars Present	2	0
805					Territorial Agression	2	0
806					Bloated	0	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
807	14 µg/L	D	4D	7	Tubercles Present	2	0
808					Fatpad Present	2	0
809					Color Bars Present	2	1
810					Territorial Agression	2	0
811					Bloated	0	0
812	14 µg/L	D	4D	8	Tubercles Present	2	0
813					Fatpad Present	2	0
814					Color Bars Present	2	1
815					Territorial Agression	2	0
816					Bloated	0	0
817	14 µg/L	D	4D	9	Tubercles Present	2	0
818					Fatpad Present	2	0
819					Color Bars Present	2	0
820					Territorial Agression	2	0
821					Bloated	0	0
822	14 µg/L	D	4D	10	Tubercles Present	2	0
823					Fatpad Present	2	0
824					Color Bars Present	2	0
825					Territorial Agression	2	0
826					Bloated	0	0
827	14 µg/L	D	4D	11	Tubercles Present	2	0
828					Fatpad Present	2	0
829					Color Bars Present	2	0
830					Territorial Agression	2	0
831					Bloated	0	0
832	14 µg/L	D	4D	12	Tubercles Present	2	0
833					Fatpad Present	2	0
834					Color Bars Present	2	0
835					Territorial Agression	2	0
836					Bloated	0	0
837	14 µg/L	D	4D	13	Tubercles Present	2	0
838					Fatpad Present	2	0
839					Color Bars Present	2	0
840					Territorial Agression	2	0
841					Bloated	0	0
842	14 µg/L	D	4D	14	Tubercles Present	2	0
843					Fatpad Present	2	0
844					Color Bars Present	2	0
845					Territorial Agression	2	0
846					Bloated	0	0
847	14 µg/L	D	4D	15	Tubercles Present	2	0
848					Fatpad Present	2	0
849					Color Bars Present	2	0
850					Territorial Agression	2	0
851					Bloated	0	0
852	14 µg/L	D	4D	16	Tubercles Present	2	0
853					Fatpad Present	2	0
854					Color Bars Present	2	0
855					Territorial Agression	2	0
856					Bloated	0	0
857	14 µg/L	D	4D	17	Tubercles Present	2	0
858					Fatpad Present	2	0
859					Color Bars Present	2	0
860					Territorial Agression	2	0
861					Bloated	0	0
862	14 µg/L	D	4D	18	Tubercles Present	2	0
863					Fatpad Present	2	0
864					Color Bars Present	2	0
865					Territorial Agression	2	0
866					Bloated	0	0
867	14 µg/L	D	4D	19	Tubercles Present	2	0
868					Fatpad Present	2	0
869					Color Bars Present	2	0
870					Territorial Agression	2	0
871					Bloated	0	0
872	14 µg/L	D	4D	20	Tubercles Present	2	0
873					Fatpad Present	2	0
874					Color Bars Present	2	0
875					Territorial Agression	2	0
876					Bloated	0	0
877	14 µg/L	D	4D	21	Tubercles Present	2	0
878					Fatpad Present	2	0
879					Color Bars Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
880					Territorial Agression	2	0
881					Bloated	0	0
882	35 µg/L	A	3A	0	Tubercles Present	2	0
883					Fatpad Present	2	0
884					Color Bars Present	2	0
885					Territorial Agression	2	0
886					Bloated	0	0
887	35 µg/L	A	3A	1	Tubercles Present	2	0
888					Fatpad Present	2	0
889					Color Bars Present	2	0
890					Territorial Agression	2	0
891					Bloated	0	0
892	35 µg/L	A	3A	2	Tubercles Present	2	0
893					Fatpad Present	2	0
894					Color Bars Present	2	0
895					Territorial Agression	2	0
896					Bloated	0	0
897	35 µg/L	A	3A	3	Tubercles Present	2	0
898					Fatpad Present	2	0
899					Color Bars Present	2	0
900					Territorial Agression	2	0
901					Bloated	0	0
902	35 µg/L	A	3A	4	Tubercles Present	2	0
903					Fatpad Present	2	0
904					Color Bars Present	2	0
905					Territorial Agression	2	0
906					Bloated	0	0
907	35 µg/L	A	3A	5	Tubercles Present	2	0
908					Fatpad Present	2	0
909					Color Bars Present	2	0
910					Territorial Agression	2	0
911					Bloated	0	0
912	35 µg/L	A	3A	6	Tubercles Present	2	0
913					Fatpad Present	2	0
914					Color Bars Present	2	0
915					Territorial Agression	2	0
916					Bloated	0	0
917	35 µg/L	A	3A	7	Tubercles Present	2	0
918					Fatpad Present	2	0
919					Color Bars Present	2	0
920					Territorial Agression	2	0
921					Bloated	0	0
922	35 µg/L	A	3A	8	Tubercles Present	2	0
923					Fatpad Present	2	0
924					Color Bars Present	2	0
925					Territorial Agression	2	0
926					Bloated	0	0
927	35 µg/L	A	3A	9	Tubercles Present	2	0
928					Fatpad Present	2	0
929					Color Bars Present	2	0
930					Territorial Agression	2	0
931					Bloated	0	0
932	35 µg/L	A	3A	10	Tubercles Present	2	0
933					Fatpad Present	2	0
934					Color Bars Present	2	0
935					Territorial Agression	2	0
936					Bloated	0	0
937	35 µg/L	A	3A	11	Tubercles Present	2	0
938					Fatpad Present	2	0
939					Color Bars Present	2	0
940					Territorial Agression	2	0
941					Bloated	0	0
942	35 µg/L	A	3A	12	Tubercles Present	2	0
943					Fatpad Present	2	0
944					Color Bars Present	2	0
945					Territorial Agression	2	0
946					Bloated	0	0
947	35 µg/L	A	3A	13	Tubercles Present	2	0
948					Fatpad Present	2	0
949					Color Bars Present	2	0
950					Territorial Agression	2	0
951					Bloated	0	0
952	35 µg/L	A	3A	14	Tubercles Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
953					Fatpad Present	2	0
954					Color Bars Present	2	0
955					Territorial Agression	2	0
956					Bloated	0	0
957	35 µg/L	A	3A	15	Tubercles Present	2	0
958					Fatpad Present	2	0
959					Color Bars Present	2	0
960					Territorial Agression	2	0
961					Bloated	0	0
962	35 µg/L	A	3A	16	Tubercles Present	2	0
963					Fatpad Present	2	0
964					Color Bars Present	2	0
965					Territorial Agression	2	0
966					Bloated	0	0
967	35 µg/L	A	3A	17	Tubercles Present	2	0
968					Fatpad Present	2	0
969					Color Bars Present	2	0
970					Territorial Agression	2	0
971					Bloated	0	0
972	35 µg/L	A	3A	18	Tubercles Present	2	0
973					Fatpad Present	2	0
974					Color Bars Present	2	0
975					Territorial Agression	2	0
976					Bloated	0	0
977	35 µg/L	A	3A	19	Tubercles Present	2	0
978					Fatpad Present	2	0
979					Color Bars Present	2	0
980					Territorial Agression	2	0
981					Bloated	0	0
982	35 µg/L	A	3A	20	Tubercles Present	2	0
983					Fatpad Present	2	0
984					Color Bars Present	2	0
985					Territorial Agression	2	0
986					Bloated	0	0
987	35 µg/L	A	3A	21	Tubercles Present	2	0
988					Fatpad Present	2	0
989					Color Bars Present	2	0
990					Territorial Agression	2	0
991					Bloated	0	0
992	35 µg/L	B	3B	0	Tubercles Present	2	0
993					Fatpad Present	2	0
994					Color Bars Present	2	0
995					Territorial Agression	2	0
996					Bloated	0	0
997	35 µg/L	B	3B	1	Tubercles Present	0	0
998					Fatpad Present	2	0
999					Color Bars Present	1	0
1000					Territorial Agression	2	0
1001					Bloated	0	0
1002	35 µg/L	B	3B	2	Tubercles Present	2	0
1003					Fatpad Present	2	0
1004					Color Bars Present	2	0
1005					Territorial Agression	2	0
1006					Bloated	0	0
1007	35 µg/L	B	3B	3	Tubercles Present	2	0
1008					Fatpad Present	2	0
1009					Color Bars Present	2	0
1010					Territorial Agression	2	0
1011					Bloated	0	0
1012	35 µg/L	B	3B	4	Tubercles Present	2	0
1013					Fatpad Present	2	0
1014					Color Bars Present	2	0
1015					Territorial Agression	2	0
1016					Bloated	0	0
1017	35 µg/L	B	3B	5	Tubercles Present	2	0
1018					Fatpad Present	2	0
1019					Color Bars Present	2	0
1020					Territorial Agression	2	0
1021					Bloated	0	0
1022	35 µg/L	B	3B	6	Tubercles Present	2	0
1023					Fatpad Present	2	0
1024					Color Bars Present	2	0
1025					Territorial Agression	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1026					Bloated	0	0
1027	35 µg/L	B	3B	7	Tubercles Present	2	0
1028					Fatpad Present	2	0
1029					Color Bars Present	2	0
1030					Territorial Agression	2	0
1031					Bloated	0	0
1032	35 µg/L	B	3B	8	Tubercles Present	2	0
1033					Fatpad Present	2	0
1034					Color Bars Present	2	0
1035					Territorial Agression	2	0
1036					Bloated	0	0
1037	35 µg/L	B	3B	9	Tubercles Present	2	0
1038					Fatpad Present	2	0
1039					Color Bars Present	2	0
1040					Territorial Agression	2	0
1041					Bloated	0	0
1042	35 µg/L	B	3B	10	Tubercles Present	2	0
1043					Fatpad Present	2	0
1044					Color Bars Present	2	0
1045					Territorial Agression	2	0
1046					Bloated	0	0
1047	35 µg/L	B	3B	11	Tubercles Present	2	0
1048					Fatpad Present	2	0
1049					Color Bars Present	2	0
1050					Territorial Agression	2	0
1051					Bloated	0	0
1052	35 µg/L	B	3B	12	Tubercles Present	2	0
1053					Fatpad Present	2	0
1054					Color Bars Present	2	0
1055					Territorial Agression	2	0
1056					Bloated	0	0
1057	35 µg/L	B	3B	13	Tubercles Present	2	0
1058					Fatpad Present	2	0
1059					Color Bars Present	2	0
1060					Territorial Agression	2	0
1061					Bloated	0	0
1062	35 µg/L	B	3B	14	Tubercles Present	2	0
1063					Fatpad Present	2	0
1064					Color Bars Present	2	0
1065					Territorial Agression	2	0
1066					Bloated	0	0
1067	35 µg/L	B	3B	15	Tubercles Present	2	0
1068					Fatpad Present	2	0
1069					Color Bars Present	2	0
1070					Territorial Agression	2	0
1071					Bloated	0	0
1072	35 µg/L	B	3B	16	Tubercles Present	2	0
1073					Fatpad Present	2	0
1074					Color Bars Present	2	0
1075					Territorial Agression	2	0
1076					Bloated	0	0
1077	35 µg/L	B	3B	17	Tubercles Present	2	0
1078					Fatpad Present	2	0
1079					Color Bars Present	2	0
1080					Territorial Agression	2	0
1081					Bloated	0	0
1082	35 µg/L	B	3B	18	Tubercles Present	2	0
1083					Fatpad Present	2	0
1084					Color Bars Present	2	0
1085					Territorial Agression	2	0
1086					Bloated	0	0
1087	35 µg/L	B	3B	19	Tubercles Present	2	0
1088					Fatpad Present	2	0
1089					Color Bars Present	2	0
1090					Territorial Agression	2	0
1091					Bloated	0	0
1092	35 µg/L	B	3B	20	Tubercles Present	2	0
1093					Fatpad Present	2	0
1094					Color Bars Present	2	0
1095					Territorial Agression	2	0
1096					Bloated	0	0
1097	35 µg/L	B	3B	21	Tubercles Present	2	0
1098					Fatpad Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1099					Color Bars Present	2	0
1100					Territorial Agression	2	0
1101					Bloated	0	0
1102	35 µg/L	C	3C	0	Tubercles Present	2	0
1103					Fatpad Present	2	0
1104					Color Bars Present	2	0
1105					Territorial Agression	2	0
1106					Bloated	0	0
1107	35 µg/L	C	3C	1	Tubercles Present	2	0
1108					Fatpad Present	2	0
1109					Color Bars Present	2	0
1110					Territorial Agression	2	0
1111					Bloated	0	0
1112	35 µg/L	C	3C	2	Tubercles Present	2	0
1113					Fatpad Present	2	0
1114					Color Bars Present	2	0
1115					Territorial Agression	2	0
1116					Bloated	0	0
1117	35 µg/L	C	3C	3	Tubercles Present	2	0
1118					Fatpad Present	2	0
1119					Color Bars Present	2	0
1120					Territorial Agression	2	0
1121					Bloated	0	0
1122	35 µg/L	C	3C	4	Tubercles Present	2	0
1123					Fatpad Present	2	0
1124					Color Bars Present	2	0
1125					Territorial Agression	2	0
1126					Bloated	0	0
1127	35 µg/L	C	3C	5	Tubercles Present	2	0
1128					Fatpad Present	2	0
1129					Color Bars Present	2	0
1130					Territorial Agression	2	0
1131					Bloated	0	0
1132	35 µg/L	C	3C	6	Tubercles Present	2	0
1133					Fatpad Present	2	0
1134					Color Bars Present	2	0
1135					Territorial Agression	2	0
1136					Bloated	0	0
1137	35 µg/L	C	3C	7	Tubercles Present	2	0
1138					Fatpad Present	2	0
1139					Color Bars Present	2	0
1140					Territorial Agression	2	0
1141					Bloated	0	0
1142	35 µg/L	C	3C	8	Tubercles Present	2	0
1143					Fatpad Present	2	0
1144					Color Bars Present	2	0
1145					Territorial Agression	2	0
1146					Bloated	0	0
1147	35 µg/L	C	3C	9	Tubercles Present	2	0
1148					Fatpad Present	2	0
1149					Color Bars Present	2	0
1150					Territorial Agression	2	0
1151					Bloated	0	0
1152	35 µg/L	C	3C	10	Tubercles Present	2	0
1153					Fatpad Present	2	0
1154					Color Bars Present	2	0
1155					Territorial Agression	2	0
1156					Bloated	0	0
1157	35 µg/L	C	3C	11	Tubercles Present	2	0
1158					Fatpad Present	2	0
1159					Color Bars Present	2	0
1160					Territorial Agression	2	0
1161					Bloated	0	0
1162	35 µg/L	C	3C	12	Tubercles Present	2	0
1163					Fatpad Present	2	0
1164					Color Bars Present	2	0
1165					Territorial Agression	2	0
1166					Bloated	0	0
1167	35 µg/L	C	3C	13	Tubercles Present	2	0
1168					Fatpad Present	2	0
1169					Color Bars Present	2	0
1170					Territorial Agression	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1171					Bloated	0	0
1172	35 µg/L	C	3C	14	Tubercles Present	2	0
1173					Fatpad Present	2	0
1174					Color Bars Present	2	0
1175					Territorial Agression	2	0
1176					Bloated	0	0
1177	35 µg/L	C	3C	15	Tubercles Present	2	0
1178					Fatpad Present	2	0
1179					Color Bars Present	2	0
1180					Territorial Agression	2	0
1181					Bloated	0	0
1182	35 µg/L	C	3C	16	Tubercles Present	2	0
1183					Fatpad Present	2	0
1184					Color Bars Present	2	0
1185					Territorial Agression	2	0
1186					Bloated	0	0
1187	35 µg/L	C	3C	17	Tubercles Present	2	0
1188					Fatpad Present	2	0
1189					Color Bars Present	2	0
1190					Territorial Agression	2	0
1191					Bloated	0	0
1192	35 µg/L	C	3C	18	Tubercles Present	2	0
1193					Fatpad Present	2	0
1194					Color Bars Present	2	0
1195					Territorial Agression	2	0
1196					Bloated	0	0
1197	35 µg/L	C	3C	19	Tubercles Present	2	0
1198					Fatpad Present	2	0
1199					Color Bars Present	2	0
1200					Territorial Agression	2	0
1201					Bloated	0	0
1202	35 µg/L	C	3C	20	Tubercles Present	2	0
1203					Fatpad Present	2	0
1204					Color Bars Present	2	0
1205					Territorial Agression	2	0
1206					Bloated	0	0
1207	35 µg/L	C	3C	21	Tubercles Present	2	0
1208					Fatpad Present	2	0
1209					Color Bars Present	2	0
1210					Territorial Agression	2	0
1211					Bloated	0	0
1212	35 µg/L	D	3D	0	Tubercles Present	2	0
1213					Fatpad Present	2	0
1214					Color Bars Present	2	0
1215					Territorial Agression	2	0
1216					Bloated	0	0
1217	35 µg/L	D	3D	1	Tubercles Present	0	0
1218					Fatpad Present	2	0
1219					Color Bars Present	1	0
1220					Territorial Agression	2	0
1221					Bloated	0	0
1222	35 µg/L	D	3D	2	Tubercles Present	2	0
1223					Fatpad Present	2	0
1224					Color Bars Present	2	0
1225					Territorial Agression	2	0
1226					Bloated	0	0
1227	35 µg/L	D	3D	3	Tubercles Present	2	0
1228					Fatpad Present	2	0
1229					Color Bars Present	2	0
1230					Territorial Agression	2	0
1231					Bloated	0	0
1232	35 µg/L	D	3D	4	Tubercles Present	2	0
1233					Fatpad Present	2	0
1234					Color Bars Present	2	0
1235					Territorial Agression	2	0
1236					Bloated	0	0
1237	35 µg/L	D	3D	5	Tubercles Present	2	0
1238					Fatpad Present	2	0
1239					Color Bars Present	2	0
1240					Territorial Agression	2	0
1241					Bloated	0	0
1242	35 µg/L	D	3D	6	Tubercles Present	2	0
1243					Fatpad Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1244					Color Bars Present	2	0
1245					Territorial Agression	2	0
1246					Bloated	0	0
1247	35 µg/L	D	3D	7	Tubercles Present	2	0
1248					Fatpad Present	2	0
1249					Color Bars Present	2	0
1250					Territorial Agression	2	0
1251					Bloated	0	0
1252	35 µg/L	D	3D	8	Tubercles Present	2	0
1253					Fatpad Present	2	0
1254					Color Bars Present	2	0
1255					Territorial Agression	2	0
1256					Bloated	0	0
1257	35 µg/L	D	3D	9	Tubercles Present	2	0
1258					Fatpad Present	2	0
1259					Color Bars Present	2	0
1260					Territorial Agression	2	0
1261					Bloated	0	0
1262	35 µg/L	D	3D	10	Tubercles Present	2	0
1263					Fatpad Present	2	0
1264					Color Bars Present	2	0
1265					Territorial Agression	2	0
1266					Bloated	0	0
1267	35 µg/L	D	3D	11	Tubercles Present	2	0
1268					Fatpad Present	2	0
1269					Color Bars Present	2	0
1270					Territorial Agression	2	0
1271					Bloated	0	0
1272	35 µg/L	D	3D	12	Tubercles Present	2	0
1273					Fatpad Present	2	0
1274					Color Bars Present	2	0
1275					Territorial Agression	2	0
1276					Bloated	0	0
1277	35 µg/L	D	3D	13	Tubercles Present	2	0
1278					Fatpad Present	2	0
1279					Color Bars Present	2	0
1280					Territorial Agression	2	0
1281					Bloated	0	0
1282	35 µg/L	D	3D	14	Tubercles Present	2	0
1283					Fatpad Present	2	0
1284					Color Bars Present	2	0
1285					Territorial Agression	2	0
1286					Bloated	0	0
1287	35 µg/L	D	3D	15	Tubercles Present	2	0
1288					Fatpad Present	2	0
1289					Color Bars Present	2	0
1290					Territorial Agression	2	0
1291					Bloated	0	0
1292	35 µg/L	D	3D	16	Tubercles Present	2	0
1293					Fatpad Present	2	0
1294					Color Bars Present	2	0
1295					Territorial Agression	2	0
1296					Bloated	0	0
1297	35 µg/L	D	3D	17	Tubercles Present	2	0
1298					Fatpad Present	2	0
1299					Color Bars Present	2	0
1300					Territorial Agression	2	0
1301					Bloated	0	0
1302	35 µg/L	D	3D	18	Tubercles Present	2	0
1303					Fatpad Present	2	0
1304					Color Bars Present	2	0
1305					Territorial Agression	2	0
1306					Bloated	0	0
1307	35 µg/L	D	3D	19	Tubercles Present	2	0
1308					Fatpad Present	2	0
1309					Color Bars Present	2	0
1310					Territorial Agression	2	0
1311					Bloated	0	0
1312	35 µg/L	D	3D	20	Tubercles Present	2	0
1313					Fatpad Present	2	0
1314					Color Bars Present	2	0
1315					Territorial Agression	2	0
1316					Bloated	0	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1317	35 µg/L	D	3D	21	Tubercles Present	2	0
1318					Fatpad Present	2	0
1319					Color Bars Present	2	0
1320					Territorial Agression	2	0
1321					Bloated	0	0
1322	88 µg/L	A	2A	0	Tubercles Present	2	0
1323					Fatpad Present	2	0
1324					Color Bars Present	2	0
1325					Territorial Agression	2	0
1326					Bloated	0	0
1327	88 µg/L	A	2A	1	Tubercles Present	2	0
1328					Fatpad Present	2	0
1329					Color Bars Present	2	0
1330					Territorial Agression	2	0
1331					Bloated	0	0
1332	88 µg/L	A	2A	2	Tubercles Present	2	0
1333					Fatpad Present	2	0
1334					Color Bars Present	2	0
1335					Territorial Agression	2	0
1336					Bloated	0	0
1337	88 µg/L	A	2A	3	Tubercles Present	2	0
1338					Fatpad Present	2	0
1339					Color Bars Present	2	0
1340					Territorial Agression	2	0
1341					Bloated	0	0
1342	88 µg/L	A	2A	4	Tubercles Present	2	0
1343					Fatpad Present	2	0
1344					Color Bars Present	2	0
1345					Territorial Agression	2	0
1346					Bloated	0	0
1347	88 µg/L	A	2A	5	Tubercles Present	2	0
1348					Fatpad Present	2	0
1349					Color Bars Present	2	0
1350					Territorial Agression	2	0
1351					Bloated	0	0
1352	88 µg/L	A	2A	6	Tubercles Present	2	0
1353					Fatpad Present	2	0
1354					Color Bars Present	2	0
1355					Territorial Agression	2	0
1356					Bloated	0	0
1357	88 µg/L	A	2A	7	Tubercles Present	2	0
1358					Fatpad Present	2	0
1359					Color Bars Present	2	2
1360					Territorial Agression	2	0
1361					Bloated	0	0
1362	88 µg/L	A	2A	8	Tubercles Present	2	0
1363					Fatpad Present	2	0
1364					Color Bars Present	2	2
1365					Territorial Agression	2	0
1366					Bloated	0	0
1367	88 µg/L	A	2A	9	Tubercles Present	2	0
1368					Fatpad Present	2	0
1369					Color Bars Present	2	1
1370					Territorial Agression	2	0
1371					Bloated	0	0
1372	88 µg/L	A	2A	10	Tubercles Present	2	0
1373					Fatpad Present	2	0
1374					Color Bars Present	2	1
1375					Territorial Agression	2	0
1376					Bloated	0	0
1377	88 µg/L	A	2A	11	Tubercles Present	2	0
1378					Fatpad Present	2	0
1379					Color Bars Present	2	1
1380					Territorial Agression	2	0
1381					Bloated	0	0
1382	88 µg/L	A	2A	12	Tubercles Present	2	0
1383					Fatpad Present	2	0
1384					Color Bars Present	2	1
1385					Territorial Agression	2	0
1386					Bloated	0	0
1387	88 µg/L	A	2A	13	Tubercles Present	2	0
1388					Fatpad Present	2	0
1389					Color Bars Present	2	1

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1390					Territorial Agression	2	0
1391					Bloated	0	0
1392	88 µg/L	A	2A	14	Tubercles Present	2	0
1393					Fatpad Present	2	0
1394					Color Bars Present	2	1
1395					Territorial Agression	2	0
1396					Bloated	0	0
1397	88 µg/L	A	2A	15	Tubercles Present	2	0
1398					Fatpad Present	2	0
1399					Color Bars Present	2	1
1400					Territorial Agression	2	0
1401					Bloated	0	0
1402	88 µg/L	A	2A	16	Tubercles Present	2	0
1403					Fatpad Present	2	0
1404					Color Bars Present	2	1
1405					Territorial Agression	2	0
1406					Bloated	0	0
1407	88 µg/L	A	2A	17	Tubercles Present	2	0
1408					Fatpad Present	2	0
1409					Color Bars Present	2	1
1410					Territorial Agression	2	0
1411					Bloated	0	0
1412	88 µg/L	A	2A	18	Tubercles Present	2	0
1413					Fatpad Present	2	0
1414					Color Bars Present	2	1
1415					Territorial Agression	2	0
1416					Bloated	0	0
1417	88 µg/L	A	2A	19	Tubercles Present	2	0
1418					Fatpad Present	2	0
1419					Color Bars Present	2	1
1420					Territorial Agression	2	0
1421					Bloated	0	0
1422	88 µg/L	A	2A	20	Tubercles Present	2	0
1423					Fatpad Present	2	0
1424					Color Bars Present	2	1
1425					Territorial Agression	2	0
1426					Bloated	0	0
1427	88 µg/L	A	2A	21	Tubercles Present	2	0
1428					Fatpad Present	2	0
1429					Color Bars Present	2	0
1430					Territorial Agression	2	0
1431					Bloated	0	0
1432	88 µg/L	B	2B	0	Tubercles Present	2	0
1433					Fatpad Present	2	0
1434					Color Bars Present	2	0
1435					Territorial Agression	2	0
1436					Bloated	0	0
1437	88 µg/L	B	2B	1	Tubercles Present	2	0
1438					Fatpad Present	2	0
1439					Color Bars Present	2	0
1440					Territorial Agression	2	0
1441					Bloated	0	0
1442	88 µg/L	B	2B	2	Tubercles Present	2	0
1443					Fatpad Present	2	0
1444					Color Bars Present	2	0
1445					Territorial Agression	2	0
1446					Bloated	0	0
1447	88 µg/L	B	2B	3	Tubercles Present	2	0
1448					Fatpad Present	2	0
1449					Color Bars Present	2	0
1450					Territorial Agression	2	0
1451					Bloated	0	0
1452	88 µg/L	B	2B	4	Tubercles Present	2	0
1453					Fatpad Present	2	0
1454					Color Bars Present	2	0
1455					Territorial Agression	2	0
1456					Bloated	0	0
1457	88 µg/L	B	2B	5	Tubercles Present	2	0
1458					Fatpad Present	2	0
1459					Color Bars Present	2	0
1460					Territorial Agression	2	0
1461					Bloated	0	0
1462	88 µg/L	B	2B	6	Tubercles Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1463					Fatpad Present	2	0
1464					Color Bars Present	2	0
1465					Territorial Agression	2	0
1466					Bloated	0	0
1467	88 µg/L	B	2B	7	Tubercles Present	2	0
1468					Fatpad Present	2	0
1469					Color Bars Present	2	0
1470					Territorial Agression	2	0
1471					Bloated	0	0
1472	88 µg/L	B	2B	8	Tubercles Present	2	0
1473					Fatpad Present	2	0
1474					Color Bars Present	2	0
1475					Territorial Agression	2	0
1476					Bloated	0	0
1477	88 µg/L	B	2B	9	Tubercles Present	2	0
1478					Fatpad Present	2	0
1479					Color Bars Present	2	0
1480					Territorial Agression	2	0
1481					Bloated	0	0
1482	88 µg/L	B	2B	10	Tubercles Present	2	0
1483					Fatpad Present	2	0
1484					Color Bars Present	2	0
1485					Territorial Agression	2	0
1486					Bloated	0	0
1487	88 µg/L	B	2B	11	Tubercles Present	2	0
1488					Fatpad Present	2	0
1489					Color Bars Present	2	0
1490					Territorial Agression	2	0
1491					Bloated	0	0
1492	88 µg/L	B	2B	12	Tubercles Present	2	0
1493					Fatpad Present	2	0
1494					Color Bars Present	2	0
1495					Territorial Agression	2	0
1496					Bloated	0	0
1497	88 µg/L	B	2B	13	Tubercles Present	2	0
1498					Fatpad Present	2	0
1499					Color Bars Present	2	0
1500					Territorial Agression	2	0
1501					Bloated	0	0
1502	88 µg/L	B	2B	14	Tubercles Present	2	0
1503					Fatpad Present	2	0
1504					Color Bars Present	2	0
1505					Territorial Agression	2	0
1506					Bloated	0	0
1507	88 µg/L	B	2B	15	Tubercles Present	2	0
1508					Fatpad Present	2	0
1509					Color Bars Present	2	0
1510					Territorial Agression	2	0
1511					Bloated	0	0
1512	88 µg/L	B	2B	16	Tubercles Present	2	0
1513					Fatpad Present	2	0
1514					Color Bars Present	2	0
1515					Territorial Agression	2	0
1516					Bloated	0	0
1517	88 µg/L	B	2B	17	Tubercles Present	2	0
1518					Fatpad Present	2	0
1519					Color Bars Present	2	0
1520					Territorial Agression	2	0
1521					Bloated	0	0
1522	88 µg/L	B	2B	18	Tubercles Present	2	0
1523					Fatpad Present	2	0
1524					Color Bars Present	2	0
1525					Territorial Agression	2	0
1526					Bloated	0	0
1527	88 µg/L	B	2B	19	Tubercles Present	2	0
1528					Fatpad Present	2	0
1529					Color Bars Present	2	0
1530					Territorial Agression	2	0
1531					Bloated	0	0
1532	88 µg/L	B	2B	20	Tubercles Present	2	0
1533					Fatpad Present	2	0
1534					Color Bars Present	2	0
1535					Territorial Agression	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1536					Bloated	0	0
1537	88 µg/L	B	2B	21	Tubercles Present	2	0
1538					Fatpad Present	2	0
1539					Color Bars Present	2	0
1540					Territorial Agression	2	0
1541					Bloated	0	0
1542	88 µg/L	C	2C	0	Tubercles Present	2	0
1543					Fatpad Present	2	0
1544					Color Bars Present	2	0
1545					Territorial Agression	2	0
1546					Bloated	0	0
1547	88 µg/L	C	2C	1	Tubercles Present	2	0
1548					Fatpad Present	2	0
1549					Color Bars Present	2	0
1550					Territorial Agression	2	0
1551					Bloated	0	0
1552	88 µg/L	C	2C	2	Tubercles Present	2	0
1553					Fatpad Present	2	0
1554					Color Bars Present	2	0
1555					Territorial Agression	2	0
1556					Bloated	0	0
1557	88 µg/L	C	2C	3	Tubercles Present	2	0
1558					Fatpad Present	2	0
1559					Color Bars Present	2	0
1560					Territorial Agression	2	0
1561					Bloated	0	0
1562	88 µg/L	C	2C	4	Tubercles Present	2	0
1563					Fatpad Present	2	0
1564					Color Bars Present	2	0
1565					Territorial Agression	2	0
1566					Bloated	0	0
1567	88 µg/L	C	2C	5	Tubercles Present	2	0
1568					Fatpad Present	2	0
1569					Color Bars Present	2	0
1570					Territorial Agression	2	0
1571					Bloated	0	0
1572	88 µg/L	C	2C	6	Tubercles Present	2	0
1573					Fatpad Present	2	0
1574					Color Bars Present	2	0
1575					Territorial Agression	2	0
1576					Bloated	0	0
1577	88 µg/L	C	2C	7	Tubercles Present	2	0
1578					Fatpad Present	2	0
1579					Color Bars Present	2	0
1580					Territorial Agression	2	0
1581					Bloated	0	0
1582	88 µg/L	C	2C	8	Tubercles Present	2	0
1583					Fatpad Present	2	0
1584					Color Bars Present	2	0
1585					Territorial Agression	2	0
1586					Bloated	0	0
1587	88 µg/L	C	2C	9	Tubercles Present	2	0
1588					Fatpad Present	2	0
1589					Color Bars Present	2	0
1590					Territorial Agression	2	0
1591					Bloated	0	0
1592	88 µg/L	C	2C	10	Tubercles Present	2	0
1593					Fatpad Present	2	0
1594					Color Bars Present	2	0
1595					Territorial Agression	2	0
1596					Bloated	0	0
1597	88 µg/L	C	2C	11	Tubercles Present	2	0
1598					Fatpad Present	2	0
1599					Color Bars Present	2	0
1600					Territorial Agression	2	0
1601					Bloated	0	0
1602	88 µg/L	C	2C	12	Tubercles Present	2	0
1603					Fatpad Present	2	0
1604					Color Bars Present	2	0
1605					Territorial Agression	2	0
1606					Bloated	0	0
1607	88 µg/L	C	2C	13	Tubercles Present	2	0
1608					Fatpad Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1609					Color Bars Present	2	0
1610					Territorial Agression	2	0
1611					Bloated	0	0
1612	88 µg/L	C	2C	14	Tubercles Present	2	0
1613					Fatpad Present	2	0
1614					Color Bars Present	2	0
1615					Territorial Agression	2	0
1616					Bloated	0	0
1617	88 µg/L	C	2C	15	Tubercles Present	2	0
1618					Fatpad Present	2	0
1619					Color Bars Present	2	0
1620					Territorial Agression	2	0
1621					Bloated	0	0
1622	88 µg/L	C	2C	16	Tubercles Present	2	0
1623					Fatpad Present	2	0
1624					Color Bars Present	2	0
1625					Territorial Agression	2	0
1626					Bloated	0	0
1627	88 µg/L	C	2C	17	Tubercles Present	2	0
1628					Fatpad Present	2	0
1629					Color Bars Present	2	0
1630					Territorial Agression	2	0
1631					Bloated	0	0
1632	88 µg/L	C	2C	18	Tubercles Present	2	0
1633					Fatpad Present	2	0
1634					Color Bars Present	2	0
1635					Territorial Agression	2	0
1636					Bloated	0	0
1637	88 µg/L	C	2C	19	Tubercles Present	2	0
1638					Fatpad Present	2	0
1639					Color Bars Present	2	0
1640					Territorial Agression	2	0
1641					Bloated	0	0
1642	88 µg/L	C	2C	20	Tubercles Present	2	0
1643					Fatpad Present	2	0
1644					Color Bars Present	2	0
1645					Territorial Agression	2	0
1646					Bloated	0	0
1647	88 µg/L	C	2C	21	Tubercles Present	2	0
1648					Fatpad Present	2	0
1649					Color Bars Present	2	0
1650					Territorial Agression	2	0
1651					Bloated	0	0
1652	88 µg/L	D	2D	0	Tubercles Present	2	0
1653					Fatpad Present	2	0
1654					Color Bars Present	2	0
1655					Territorial Agression	2	0
1656					Bloated	0	0
1657	88 µg/L	D	2D	1	Tubercles Present	2	0
1658					Fatpad Present	2	0
1659					Color Bars Present	2	0
1660					Territorial Agression	2	0
1661					Bloated	0	0
1662	88 µg/L	D	2D	2	Tubercles Present	2	0
1663					Fatpad Present	2	0
1664					Color Bars Present	2	0
1665					Territorial Agression	2	0
1666					Bloated	0	0
1667	88 µg/L	D	2D	3	Tubercles Present	2	0
1668					Fatpad Present	2	0
1669					Color Bars Present	2	0
1670					Territorial Agression	2	0
1671					Bloated	0	0
1672	88 µg/L	D	2D	4	Tubercles Present	2	0
1673					Fatpad Present	2	0
1674					Color Bars Present	2	0
1675					Territorial Agression	2	0
1676					Bloated	0	0
1677	88 µg/L	D	2D	5	Tubercles Present	2	0
1678					Fatpad Present	2	0
1679					Color Bars Present	2	0
1680					Territorial Agression	2	0
1681					Bloated	0	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1682	88 µg/L	D	2D	6	Tubercles Present	2	0
1683					Fatpad Present	2	0
1684					Color Bars Present	2	0
1685					Territorial Agression	2	0
1686					Bloated	0	0
1687	88 µg/L	D	2D	7	Tubercles Present	2	0
1688					Fatpad Present	2	0
1689					Color Bars Present	2	0
1690					Territorial Agression	2	0
1691					Bloated	0	0
1692	88 µg/L	D	2D	8	Tubercles Present	2	0
1693					Fatpad Present	2	0
1694					Color Bars Present	2	0
1695					Territorial Agression	2	0
1696					Bloated	0	0
1697	88 µg/L	D	2D	9	Tubercles Present	2	0
1698					Fatpad Present	2	0
1699					Color Bars Present	2	0
1700					Territorial Agression	2	0
1701					Bloated	0	0
1702	88 µg/L	D	2D	10	Tubercles Present	2	0
1703					Fatpad Present	2	0
1704					Color Bars Present	2	0
1705					Territorial Agression	2	0
1706					Bloated	0	0
1707	88 µg/L	D	2D	11	Tubercles Present	2	0
1708					Fatpad Present	2	0
1709					Color Bars Present	2	0
1710					Territorial Agression	2	0
1711					Bloated	0	0
1712	88 µg/L	D	2D	12	Tubercles Present	2	0
1713					Fatpad Present	2	0
1714					Color Bars Present	2	0
1715					Territorial Agression	2	0
1716					Bloated	0	0
1717	88 µg/L	D	2D	13	Tubercles Present	2	0
1718					Fatpad Present	2	0
1719					Color Bars Present	2	0
1720					Territorial Agression	2	0
1721					Bloated	0	0
1722	88 µg/L	D	2D	14	Tubercles Present	2	0
1723					Fatpad Present	2	0
1724					Color Bars Present	2	0
1725					Territorial Agression	2	0
1726					Bloated	0	0
1727	88 µg/L	D	2D	15	Tubercles Present	2	0
1728					Fatpad Present	2	0
1729					Color Bars Present	2	0
1730					Territorial Agression	2	0
1731					Bloated	0	0
1732	88 µg/L	D	2D	16	Tubercles Present	2	0
1733					Fatpad Present	2	0
1734					Color Bars Present	2	0
1735					Territorial Agression	2	0
1736					Bloated	0	0
1737	88 µg/L	D	2D	17	Tubercles Present	2	0
1738					Fatpad Present	2	0
1739					Color Bars Present	2	0
1740					Territorial Agression	2	0
1741					Bloated	0	0
1742	88 µg/L	D	2D	18	Tubercles Present	2	0
1743					Fatpad Present	2	0
1744					Color Bars Present	2	0
1745					Territorial Agression	2	0
1746					Bloated	0	0
1747	88 µg/L	D	2D	19	Tubercles Present	2	0
1748					Fatpad Present	2	0
1749					Color Bars Present	2	0
1750					Territorial Agression	2	0
1751					Bloated	0	0
1752	88 µg/L	D	2D	20	Tubercles Present	2	0
1753					Fatpad Present	2	0
1754					Color Bars Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1755					Territorial Agression	2	0
1756					Bloated	0	0
1757	88 µg/L	D	2D	21	Tubercles Present	2	0
1758					Fatpad Present	2	0
1759					Color Bars Present	2	0
1760					Territorial Agression	2	0
1761					Bloated	0	0
1762	220 µg/L	A	1A	0	Tubercles Present	2	0
1763					Fatpad Present	2	0
1764					Color Bars Present	2	0
1765					Territorial Agression	2	0
1766					Bloated	0	0
1767	220 µg/L	A	1A	1	Tubercles Present	2	0
1768					Fatpad Present	2	0
1769					Color Bars Present	2	0
1770					Territorial Agression	2	0
1771					Bloated	0	0
1772	220 µg/L	A	1A	2	Tubercles Present	2	0
1773					Fatpad Present	2	0
1774					Color Bars Present	2	0
1775					Territorial Agression	2	0
1776					Bloated	0	0
1777	220 µg/L	A	1A	3	Tubercles Present	2	0
1778					Fatpad Present	2	0
1779					Color Bars Present	2	0
1780					Territorial Agression	2	0
1781					Bloated	0	0
1782	220 µg/L	A	1A	4	Tubercles Present	2	0
1783					Fatpad Present	2	0
1784					Color Bars Present	2	0
1785					Territorial Agression	2	0
1786					Bloated	0	0
1787	220 µg/L	A	1A	5	Tubercles Present	2	0
1788					Fatpad Present	2	0
1789					Color Bars Present	2	0
1790					Territorial Agression	2	0
1791					Bloated	0	0
1792	220 µg/L	A	1A	6	Tubercles Present	2	0
1793					Fatpad Present	2	0
1794					Color Bars Present	2	0
1795					Territorial Agression	2	0
1796					Bloated	0	0
1797	220 µg/L	A	1A	7	Tubercles Present	2	0
1798					Fatpad Present	2	0
1799					Color Bars Present	2	0
1800					Territorial Agression	2	0
1801					Bloated	0	0
1802	220 µg/L	A	1A	8	Tubercles Present	2	0
1803					Fatpad Present	2	0
1804					Color Bars Present	2	0
1805					Territorial Agression	2	0
1806					Bloated	0	0
1807	220 µg/L	A	1A	9	Tubercles Present	2	0
1808					Fatpad Present	2	0
1809					Color Bars Present	2	0
1810					Territorial Agression	2	0
1811					Bloated	0	0
1812	220 µg/L	A	1A	10	Tubercles Present	2	0
1813					Fatpad Present	2	0
1814					Color Bars Present	2	0
1815					Territorial Agression	2	0
1816					Bloated	0	0
1817	220 µg/L	A	1A	11	Tubercles Present	2	0
1818					Fatpad Present	2	0
1819					Color Bars Present	2	0
1820					Territorial Agression	2	0
1821					Bloated	0	0
1822	220 µg/L	A	1A	12	Tubercles Present	2	0
1823					Fatpad Present	2	0
1824					Color Bars Present	2	0
1825					Territorial Agression	2	0
1826					Bloated	0	0
1827	220 µg/L	A	1A	13	Tubercles Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1828					Fatpad Present	2	0
1829					Color Bars Present	2	0
1830					Territorial Agression	2	0
1831					Bloated	0	0
1832	220 µg/L	A	1A	14	Tubercles Present	2	0
1833					Fatpad Present	2	0
1834					Color Bars Present	2	0
1835					Territorial Agression	2	0
1836					Bloated	0	0
1837	220 µg/L	A	1A	15	Tubercles Present	2	0
1838					Fatpad Present	2	0
1839					Color Bars Present	2	0
1840					Territorial Agression	2	0
1841					Bloated	0	0
1842	220 µg/L	A	1A	16	Tubercles Present	2	0
1843					Fatpad Present	2	0
1844					Color Bars Present	2	0
1845					Territorial Agression	2	0
1846					Bloated	0	0
1847	220 µg/L	A	1A	17	Tubercles Present	2	0
1848					Fatpad Present	2	0
1849					Color Bars Present	2	0
1850					Territorial Agression	2	0
1851					Bloated	0	0
1852	220 µg/L	A	1A	18	Tubercles Present	2	0
1853					Fatpad Present	2	0
1854					Color Bars Present	2	0
1855					Territorial Agression	2	0
1856					Bloated	0	0
1857	220 µg/L	A	1A	19	Tubercles Present	2	0
1858					Fatpad Present	2	0
1859					Color Bars Present	2	0
1860					Territorial Agression	2	0
1861					Bloated	0	0
1862	220 µg/L	A	1A	20	Tubercles Present	2	0
1863					Fatpad Present	2	0
1864					Color Bars Present	2	0
1865					Territorial Agression	2	0
1866					Bloated	0	0
1867	220 µg/L	A	1A	21	Tubercles Present	2	0
1868					Fatpad Present	2	0
1869					Color Bars Present	2	0
1870					Territorial Agression	2	0
1871					Bloated	0	0
1872	220 µg/L	B	1B	0	Tubercles Present	2	0
1873					Fatpad Present	2	0
1874					Color Bars Present	2	0
1875					Territorial Agression	2	0
1876					Bloated	0	0
1877	220 µg/L	B	1B	1	Tubercles Present	2	0
1878					Fatpad Present	2	0
1879					Color Bars Present	2	0
1880					Territorial Agression	2	0
1881					Bloated	0	0
1882	220 µg/L	B	1B	2	Tubercles Present	2	0
1883					Fatpad Present	2	0
1884					Color Bars Present	2	0
1885					Territorial Agression	2	0
1886					Bloated	0	0
1887	220 µg/L	B	1B	3	Tubercles Present	2	0
1888					Fatpad Present	2	0
1889					Color Bars Present	2	0
1890					Territorial Agression	2	0
1891					Bloated	0	0
1892	220 µg/L	B	1B	4	Tubercles Present	2	0
1893					Fatpad Present	2	0
1894					Color Bars Present	2	0
1895					Territorial Agression	2	0
1896					Bloated	0	0
1897	220 µg/L	B	1B	5	Tubercles Present	2	0
1898					Fatpad Present	2	0
1899					Color Bars Present	2	0
1900					Territorial Agression	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1901					Bloated	0	0
1902	220 µg/L	B	1B	6	Tubercles Present	2	0
1903					Fatpad Present	2	0
1904					Color Bars Present	2	0
1905					Territorial Agression	2	0
1906					Bloated	0	0
1907	220 µg/L	B	1B	7	Tubercles Present	2	0
1908					Fatpad Present	2	0
1909					Color Bars Present	2	0
1910					Territorial Agression	2	0
1911					Bloated	0	0
1912	220 µg/L	B	1B	8	Tubercles Present	2	0
1913					Fatpad Present	2	0
1914					Color Bars Present	2	0
1915					Territorial Agression	2	0
1916					Bloated	0	0
1917	220 µg/L	B	1B	9	Tubercles Present	2	0
1918					Fatpad Present	2	0
1919					Color Bars Present	2	0
1920					Territorial Agression	2	0
1921					Bloated	0	0
1922	220 µg/L	B	1B	10	Tubercles Present	2	0
1923					Fatpad Present	2	0
1924					Color Bars Present	2	0
1925					Territorial Agression	2	0
1926					Bloated	0	0
1927	220 µg/L	B	1B	11	Tubercles Present	2	0
1928					Fatpad Present	2	0
1929					Color Bars Present	2	0
1930					Territorial Agression	2	0
1931					Bloated	0	0
1932	220 µg/L	B	1B	12	Tubercles Present	2	0
1933					Fatpad Present	2	0
1934					Color Bars Present	2	0
1935					Territorial Agression	2	0
1936					Bloated	0	0
1937	220 µg/L	B	1B	13	Tubercles Present	2	0
1938					Fatpad Present	2	0
1939					Color Bars Present	2	0
1940					Territorial Agression	2	0
1941					Bloated	0	0
1942	220 µg/L	B	1B	14	Tubercles Present	2	0
1943					Fatpad Present	2	0
1944					Color Bars Present	2	0
1945					Territorial Agression	2	0
1946					Bloated	0	0
1947	220 µg/L	B	1B	15	Tubercles Present	2	0
1948					Fatpad Present	2	0
1949					Color Bars Present	2	0
1950					Territorial Agression	2	0
1951					Bloated	0	0
1952	220 µg/L	B	1B	16	Tubercles Present	2	0
1953					Fatpad Present	2	0
1954					Color Bars Present	2	0
1955					Territorial Agression	2	0
1956					Bloated	0	0
1957	220 µg/L	B	1B	17	Tubercles Present	2	0
1958					Fatpad Present	2	0
1959					Color Bars Present	2	0
1960					Territorial Agression	2	0
1961					Bloated	0	0
1962	220 µg/L	B	1B	18	Tubercles Present	2	0
1963					Fatpad Present	2	0
1964					Color Bars Present	2	0
1965					Territorial Agression	2	0
1966					Bloated	0	0
1967	220 µg/L	B	1B	19	Tubercles Present	2	0
1968					Fatpad Present	2	0
1969					Color Bars Present	2	0
1970					Territorial Agression	2	0
1971					Bloated	0	0
1972	220 µg/L	B	1B	20	Tubercles Present	2	0
1973					Fatpad Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
1974					Color Bars Present	2	0
1975					Territorial Agression	2	0
1976					Bloated	0	0
1977	220 µg/L	B	1B	21	Tubercles Present	2	0
1978					Fatpad Present	2	0
1979					Color Bars Present	2	0
1980					Territorial Agression	2	0
1981					Bloated	0	0
1982	220 µg/L	C	1C	0	Tubercles Present	2	0
1983					Fatpad Present	2	0
1984					Color Bars Present	2	0
1985					Territorial Agression	2	0
1986					Bloated	0	0
1987	220 µg/L	C	1C	1	Tubercles Present	2	0
1988					Fatpad Present	2	0
1989					Color Bars Present	2	0
1990					Territorial Agression	2	0
1991					Bloated	0	0
1992	220 µg/L	C	1C	2	Tubercles Present	2	0
1993					Fatpad Present	2	0
1994					Color Bars Present	2	0
1995					Territorial Agression	2	0
1996					Bloated	0	0
1997	220 µg/L	C	1C	3	Tubercles Present	2	0
1998					Fatpad Present	2	0
1999					Color Bars Present	2	0
2000					Territorial Agression	2	0
2001					Bloated	0	0
2002	220 µg/L	C	1C	4	Tubercles Present	2	0
2003					Fatpad Present	2	0
2004					Color Bars Present	2	0
2005					Territorial Agression	2	0
2006					Bloated	0	0
2007	220 µg/L	C	1C	5	Tubercles Present	2	0
2008					Fatpad Present	2	0
2009					Color Bars Present	2	0
2010					Territorial Agression	2	0
2011					Bloated	0	0
2012	220 µg/L	C	1C	6	Tubercles Present	2	0
2013					Fatpad Present	2	0
2014					Color Bars Present	2	0
2015					Territorial Agression	2	0
2016					Bloated	0	0
2017	220 µg/L	C	1C	7	Tubercles Present	2	0
2018					Fatpad Present	2	0
2019					Color Bars Present	2	0
2020					Territorial Agression	2	0
2021					Bloated	0	0
2022	220 µg/L	C	1C	8	Tubercles Present	2	0
2023					Fatpad Present	2	0
2024					Color Bars Present	2	0
2025					Territorial Agression	2	0
2026					Bloated	0	0
2027	220 µg/L	C	1C	9	Tubercles Present	2	0
2028					Fatpad Present	2	0
2029					Color Bars Present	2	0
2030					Territorial Agression	2	0
2031					Bloated	0	0
2032	220 µg/L	C	1C	10	Tubercles Present	2	0
2033					Fatpad Present	2	0
2034					Color Bars Present	2	0
2035					Territorial Agression	2	0
2036					Bloated	0	0
2037	220 µg/L	C	1C	11	Tubercles Present	2	0
2038					Fatpad Present	2	0
2039					Color Bars Present	2	0
2040					Territorial Agression	2	0
2041					Bloated	0	0
2042	220 µg/L	C	1C	12	Tubercles Present	2	0
2043					Fatpad Present	2	0
2044					Color Bars Present	2	0
2045					Territorial Agression	2	0
2046					Bloated	0	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
2047	220 µg/L	C	1C	13	Tubercles Present	2	0
2048					Fatpad Present	2	0
2049					Color Bars Present	2	0
2050					Territorial Agression	2	0
2051					Bloated	0	0
2052	220 µg/L	C	1C	14	Tubercles Present	2	0
2053					Fatpad Present	2	0
2054					Color Bars Present	2	0
2055					Territorial Agression	2	0
2056					Bloated	0	0
2057	220 µg/L	C	1C	15	Tubercles Present	2	0
2058					Fatpad Present	2	0
2059					Color Bars Present	2	0
2060					Territorial Agression	2	0
2061					Bloated	0	0
2062	220 µg/L	C	1C	16	Tubercles Present	2	0
2063					Fatpad Present	2	0
2064					Color Bars Present	2	0
2065					Territorial Agression	2	0
2066					Bloated	0	0
2067	220 µg/L	C	1C	17	Tubercles Present	2	0
2068					Fatpad Present	2	0
2069					Color Bars Present	2	0
2070					Territorial Agression	2	0
2071					Bloated	0	0
2072	220 µg/L	C	1C	18	Tubercles Present	2	0
2073					Fatpad Present	2	0
2074					Color Bars Present	2	0
2075					Territorial Agression	2	0
2076					Bloated	0	0
2077	220 µg/L	C	1C	19	Tubercles Present	2	0
2078					Fatpad Present	2	0
2079					Color Bars Present	2	0
2080					Territorial Agression	2	0
2081					Bloated	0	0
2082	220 µg/L	C	1C	20	Tubercles Present	2	0
2083					Fatpad Present	2	0
2084					Color Bars Present	2	0
2085					Territorial Agression	2	0
2086					Bloated	0	0
2087	220 µg/L	C	1C	21	Tubercles Present	2	0
2088					Fatpad Present	2	0
2089					Color Bars Present	2	0
2090					Territorial Agression	2	0
2091					Bloated	0	0
2092	220 µg/L	D	1D	0	Tubercles Present	2	0
2093					Fatpad Present	2	0
2094					Color Bars Present	2	0
2095					Territorial Agression	2	0
2096					Bloated	0	0
2097	220 µg/L	D	1D	1	Tubercles Present	2	0
2098					Fatpad Present	2	0
2099					Color Bars Present	2	0
2100					Territorial Agression	2	0
2101					Bloated	0	0
2102	220 µg/L	D	1D	2	Tubercles Present	2	0
2103					Fatpad Present	2	0
2104					Color Bars Present	2	0
2105					Territorial Agression	2	0
2106					Bloated	0	0
2107	220 µg/L	D	1D	3	Tubercles Present	2	0
2108					Fatpad Present	2	0
2109					Color Bars Present	2	0
2110					Territorial Agression	2	0
2111					Bloated	0	0
2112	220 µg/L	D	1D	4	Tubercles Present	2	0
2113					Fatpad Present	2	0
2114					Color Bars Present	2	0
2115					Territorial Agression	2	0
2116					Bloated	0	0
2117	220 µg/L	D	1D	5	Tubercles Present	2	0
2118					Fatpad Present	2	0
2119					Color Bars Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
2120					Territorial Agression	2	0
2121					Bloated	0	0
2122	220 µg/L	D	1D	6	Tubercles Present	2	0
2123					Fatpad Present	2	0
2124					Color Bars Present	2	0
2125					Territorial Agression	2	0
2126					Bloated	0	0
2127	220 µg/L	D	1D	7	Tubercles Present	2	0
2128					Fatpad Present	2	0
2129					Color Bars Present	2	0
2130					Territorial Agression	2	0
2131					Bloated	0	0
2132	220 µg/L	D	1D	8	Tubercles Present	2	0
2133					Fatpad Present	2	0
2134					Color Bars Present	2	0
2135					Territorial Agression	2	0
2136					Bloated	0	0
2137	220 µg/L	D	1D	9	Tubercles Present	2	0
2138					Fatpad Present	2	0
2139					Color Bars Present	2	0
2140					Territorial Agression	2	0
2141					Bloated	0	0
2142	220 µg/L	D	1D	10	Tubercles Present	2	0
2143					Fatpad Present	2	0
2144					Color Bars Present	2	0
2145					Territorial Agression	2	0
2146					Bloated	0	0
2147	220 µg/L	D	1D	11	Tubercles Present	2	0
2148					Fatpad Present	2	0
2149					Color Bars Present	2	0
2150					Territorial Agression	2	0
2151					Bloated	0	0
2152	220 µg/L	D	1D	12	Tubercles Present	2	0
2153					Fatpad Present	2	0
2154					Color Bars Present	2	0
2155					Territorial Agression	2	0
2156					Bloated	0	0
2157	220 µg/L	D	1D	13	Tubercles Present	2	0
2158					Fatpad Present	2	0
2159					Color Bars Present	2	0
2160					Territorial Agression	2	0
2161					Bloated	0	0
2162	220 µg/L	D	1D	14	Tubercles Present	2	0
2163					Fatpad Present	2	0
2164					Color Bars Present	2	0
2165					Territorial Agression	2	0
2166					Bloated	0	0
2167	220 µg/L	D	1D	15	Tubercles Present	2	0
2168					Fatpad Present	2	0
2169					Color Bars Present	2	0
2170					Territorial Agression	2	0
2171					Bloated	0	0
2172	220 µg/L	D	1D	16	Tubercles Present	2	0
2173					Fatpad Present	2	0
2174					Color Bars Present	2	0
2175					Territorial Agression	2	0
2176					Bloated	0	0
2177	220 µg/L	D	1D	17	Tubercles Present	2	0
2178					Fatpad Present	2	0
2179					Color Bars Present	2	0
2180					Territorial Agression	2	0
2181					Bloated	0	0
2182	220 µg/L	D	1D	18	Tubercles Present	2	0
2183					Fatpad Present	2	0
2184					Color Bars Present	2	0
2185					Territorial Agression	2	0
2186					Bloated	0	0
2187	220 µg/L	D	1D	19	Tubercles Present	2	0
2188					Fatpad Present	2	0
2189					Color Bars Present	2	0
2190					Territorial Agression	2	0
2191					Bloated	0	0
2192	220 µg/L	D	1D	20	Tubercles Present	2	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Clinical Signs (Daily)

	Name of Treatment Group	Replicate ID	Tank ID	Test Day (0 to 21)	Secondary Sex Characteristics and Other Clinical Signs	Number of Males Affected	Number of Females Affected
2193					Fatpad Present	2	0
2194					Color Bars Present	2	0
2195					Territorial Agression	2	0
2196					Bloated	0	0
2197	220 µg/L	D	1D	21	Tubercles Present	2	0
2198					Fatpad Present	2	0
2199					Color Bars Present	2	0
2200					Territorial Agression	2	0
2201					Bloated	0	0

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Individual Observations (Day21)

	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Body Weight (g)	Body Length (mm)	Secondary Sex Characteristics and Other Clinical Signs
	Control	A	5A	5AM1	M	4.5377	51.41	
	Control	A	5A	5AM2	M	4.1920	48.63	
	Control	A	5A	5AF1	F	2.1604	45.46	
	Control	A	5A	5AF2	F	2.4371	47.37	
	Control	A	5A	5AF3	F	2.8440	50.26	
	Control	A	5A	5AF4	F	1.8435	41.57	
	Control	B	5B	5BM1	M	4.9410	58.88	
	Control	B	5B	5BM2	M	5.2699	57.93	
	Control	B	5B	5BF1	F	2.4587	43.83	
	Control	B	5B	5BF2	F	2.2568	40.92	
	Control	B	5B	5BF3	F	2.2036	47.17	
	Control	B	5B	5BF4	F	2.6235	49.71	
	Control	C	5C	5CM1	M	5.0419	59.76	
	Control	C	5C	5CM2	M	4.1054	53.11	
	Control	C	5C	5CF1	F	1.9731	40.37	
	Control	C	5C	5CF2	F	1.7432	42.34	
	Control	C	5C	5CF3	F	2.4997	47.65	
	Control	C	5C	5CF4	F	2.2606	45.01	
	Control	D	5D	5DM1	M	3.4066	47.65	
	Control	D	5D	5DM2	M	3.3671	49.77	
	Control	D	5D	5DF1	F	2.3741	47.81	
	Control	D	5D	5DF2	F	1.9845	43.16	
	Control	D	5D	5DF3	F	2.5674	45.15	
	Control	D	5D	5DF4	F	1.8327	43.83	
	14 µg/L	A	4A	4AM1	M	4.1621	55.76	
	14 µg/L	A	4A	4AM2	M	3.9609	53.74	
	14 µg/L	A	4A	4AF1	F	2.3406	49.99	
	14 µg/L	A	4A	4AF2	F	1.7958	41.19	
	14 µg/L	A	4A	4AF3	F	1.6376	44.11	
	14 µg/L	A	4A	4AF4	F	NA	NA	
	14 µg/L	B	4B	4BM1	M	3.9205	49.56	
	14 µg/L	B	4B	4BM2	M	4.9282	54.29	
	14 µg/L	B	4B	4BF1	F	2.1113	44.95	
	14 µg/L	B	4B	4BF2	F	1.9412	43.80	
	14 µg/L	B	4B	4BF3	F	2.4012	49.51	
	14 µg/L	B	4B	4BF4	F	NA	NA	
	14 µg/L	C	4C	4CM1	M	4.3830	58.23	
	14 µg/L	C	4C	4CM2	M	3.8984	52.70	
	14 µg/L	C	4C	4CF1	F	2.5041	46.09	
	14 µg/L	C	4C	4CF2	F	2.5120	48.01	
	14 µg/L	C	4C	4CF3	F	2.6631	46.99	
	14 µg/L	C	4C	4CF4	F	2.8492	48.38	
	14 µg/L	D	4D	4DM1	M	4.7729	55.44	
	14 µg/L	D	4D	4DM2	M	3.6240	46.48	
	14 µg/L	D	4D	4DF1	F	2.1854	45.77	
	14 µg/L	D	4D	4DF2	F	2.5644	44.89	
	14 µg/L	D	4D	4DF3	F	2.4140	45.68	
	14 µg/L	D	4D	4DF4	F	2.7817	47.82	
	35 µg/L	A	3A	3AM1	M	4.5971	54.90	
	35 µg/L	A	3A	3AM2	M	4.6594	57.78	
	35 µg/L	A	3A	3AF1	F	2.4145	47.83	
	35 µg/L	A	3A	3AF2	F	2.8164	48.53	
	35 µg/L	A	3A	3AF3	F	2.4505	44.86	

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Individual Observations (Day21)

	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Body Weight (g)	Body Length (mm)	Secondary Sex Characteristics and Other Clinical Signs
55	35 µg/L	A	3A	3AF4	F	NA	NA	
56	35 µg/L	B	3B	3BM1	M	5.6141	61.33	
57	35 µg/L	B	3B	3BM2	M	4.1750	54.23	
58	35 µg/L	B	3B	3BF1	F	2.6944	45.81	
59	35 µg/L	B	3B	3BF2	F	2.2435	44.86	
60	35 µg/L	B	3B	3BF3	F	2.0859	45.58	
61	35 µg/L	B	3B	3BF4	F	1.9435	43.76	
62	35 µg/L	C	3C	3CM1	M	5.3589	57.00	
63	35 µg/L	C	3C	3CM2	M	4.4235	52.68	
64	35 µg/L	C	3C	3CF1	F	2.3914	47.04	
65	35 µg/L	C	3C	3CF2	F	1.9548	48.31	
66	35 µg/L	C	3C	3CF3	F	2.5294	46.96	
67	35 µg/L	C	3C	3CF4	F	2.1693	46.06	
68	35 µg/L	D	3D	3DM1	M	4.0024	54.06	
69	35 µg/L	D	3D	3DM2	M	4.1880	52.67	
70	35 µg/L	D	3D	3DF1	F	2.9943	51.11	
71	35 µg/L	D	3D	3DF2	F	2.5638	46.33	
72	35 µg/L	D	3D	3DF3	F	2.6335	47.96	
73	35 µg/L	D	3D	3DF4	F	2.3644	45.49	
74	88 µg/L	A	2A	2AM1	M	4.1689	50.79	
75	88 µg/L	A	2A	2AM2	M	3.8348	49.36	
76	88 µg/L	A	2A	2AF1	F	2.3670	46.16	
77	88 µg/L	A	2A	2AF2	F	2.4651	47.11	
78	88 µg/L	A	2A	2AF3	F	2.3602	45.17	
79	88 µg/L	A	2A	2AF4	F	NA	NA	
80	88 µg/L	B	2B	2BM1	M	3.7593	48.31	
81	88 µg/L	B	2B	2BM2	M	4.6320	51.11	
82	88 µg/L	B	2B	2BF1	F	2.5697	49.02	
83	88 µg/L	B	2B	2BF2	F	2.3529	46.83	
84	88 µg/L	B	2B	2BF3	F	2.1494	44.70	
85	88 µg/L	B	2B	2BF4	F	2.1557	44.50	
86	88 µg/L	C	2C	2CM1	M	4.6935	54.89	
87	88 µg/L	C	2C	2CM2	M	4.2325	56.41	
88	88 µg/L	C	2C	2CF1	F	1.8338	45.20	
89	88 µg/L	C	2C	2CF2	F	2.3384	47.81	
90	88 µg/L	C	2C	2CF3	F	1.8674	43.77	
91	88 µg/L	C	2C	2CF4	F	2.1651	45.13	
92	88 µg/L	D	2D	2DM1	M	3.7342	54.74	
93	88 µg/L	D	2D	2DM2	M	3.8848	50.64	
94	88 µg/L	D	2D	2DF1	F	2.1732	43.42	
95	88 µg/L	D	2D	2DF2	F	2.4197	45.43	
96	88 µg/L	D	2D	2DF3	F	2.4475	46.93	
97	88 µg/L	D	2D	2DF4	F	2.2989	46.22	
98	220 µg/L	A	1A	1AM1	M	4.3592	53.51	
99	220 µg/L	A	1A	1AM2	M	3.7273	49.78	
100	220 µg/L	A	1A	1AF1	F	2.1602	46.26	
101	220 µg/L	A	1A	1AF2	F	2.4051	46.42	
102	220 µg/L	A	1A	1AF3	F	2.0396	44.55	
103	220 µg/L	A	1A	1AF4	F	2.3674	46.23	
104	220 µg/L	B	1B	1BM1	M	3.6827	50.17	
105	220 µg/L	B	1B	1BM2	M	3.7027	50.12	
106	220 µg/L	B	1B	1BF1	F	2.4362	45.60	
107	220 µg/L	B	1B	1BF2	F	2.0507	44.22	

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Individual Observations (Day21)

	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Body Weight (g)	Body Length (mm)	Secondary Sex Characteristics and Other Clinical Signs
108	220 µg/L	B	1B	1BF3	F	2.5791	46.78	
109	220 µg/L	B	1B	1BF4	F	2.0051	44.39	
110	220 µg/L	C	1C	1CM1	M	4.7427	57.02	
111	220 µg/L	C	1C	1CM2	M	4.2773	51.11	
112	220 µg/L	C	1C	1CF1	F	2.6389	47.08	
113	220 µg/L	C	1C	1CF2	F	2.2358	47.36	
114	220 µg/L	C	1C	1CF3	F	2.6148	45.99	
115	220 µg/L	C	1C	1CF4	F	1.9874	44.47	
116	220 µg/L	D	1D	1DM1	M	4.0646	51.10	
117	220 µg/L	D	1D	1DM2	M	3.9018	52.33	
118	220 µg/L	D	1D	1DF1	F	2.3165	47.57	
119	220 µg/L	D	1D	1DF2	F	2.1282	43.91	
120	220 µg/L	D	1D	1DF3	F	2.7065	46.55	
121	220 µg/L	D	1D	1DF4	F	3.0139	51.94	
122								
123								

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Nuptial Tubercle Scores (Day21)

	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Mapping Region (A-F)	Rating (1-3)	Number of Tubercles of Specified Rating
	Control	A	5A	5AM1	M	A	1	2
	Control	A	5A	5AM1	M	B	1	2
	Control	A	5A	5AM1	M	C	1	6
	Control	A	5A	5AM1	M	C	2	1
	Control	A	5A	5AM1	M	D	1	4
	Control	A	5A	5AM1	M	D	2	1
	Control	A	5A	5AM1	M	E	1	1
	Control	A	5A	5AM1	M	F	1	2
	Control	A	5A	5AM2	M	A	1	1
	Control	A	5A	5AM2	M	B	1	2
	Control	A	5A	5AM2	M	B	2	1
	Control	A	5A	5AM2	M	C	1	6
	Control	A	5A	5AM2	M	D	1	8
	Control	A	5A	5AM2	M	D	2	1
	Control	A	5A	5AM2	M	E	1	2
	Control	A	5A	5AM2	M	F	1	2
	Control	B	5B	5BM1	M	A	1	1
	Control	B	5B	5BM1	M	B	1	2
	Control	B	5B	5BM1	M	C	1	4
	Control	B	5B	5BM1	M	D	1	7
	Control	B	5B	5BM1	M	D	2	1
	Control	B	5B	5BM1	M	E	1	2
	Control	B	5B	5BM1	M	F	1	2
	Control	B	5B	5BM2	M	A	1	2
	Control	B	5B	5BM2	M	B	1	2
	Control	B	5B	5BM2	M	C	1	5
	Control	B	5B	5BM2	M	D	1	4
	Control	B	5B	5BM2	M	D	2	2
	Control	B	5B	5BM2	M	E	1	2
	Control	B	5B	5BM2	M	F	1	2
	Control	C	5C	5CM1	M	A	1	2
	Control	C	5C	5CM1	M	B	1	4
	Control	C	5C	5CM1	M	C	1	5
	Control	C	5C	5CM1	M	D	1	5
	Control	C	5C	5CM1	M	E	1	2
	Control	C	5C	5CM1	M	F	1	2
	Control	C	5C	5CM2	M	A	1	2
	Control	C	5C	5CM2	M	B	1	2
	Control	C	5C	5CM2	M	C	1	4
	Control	C	5C	5CM2	M	C	2	1
	Control	C	5C	5CM2	M	C	3	2
	Control	C	5C	5CM2	M	D	1	4
	Control	C	5C	5CM2	M	D	2	1
	Control	C	5C	5CM2	M	D	3	1
	Control	C	5C	5CM2	M	E	1	2
	Control	C	5C	5CM2	M	F	1	2
	Control	D	5D	5DM1	M	A	1	2
	Control	D	5D	5DM1	M	B	1	3
	Control	D	5D	5DM1	M	C	1	8
	Control	D	5D	5DM1	M	D	1	6
	Control	D	5D	5DM1	M	E	1	2

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx

Nuptial Tubercle Scores (Day21)

	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Mapping Region (A-F)	Rating (1-3)	Number of Tubercles of Specified Rating
53	Control	D	5D	5DM1	M	F	1	2
54	Control	D	5D	5DM2	M	A	1	2
55	Control	D	5D	5DM2	M	B	1	2
56	Control	D	5D	5DM2	M	C	1	5
57	Control	D	5D	5DM2	M	D	1	6
58	Control	D	5D	5DM2	M	E	1	1
59	Control	D	5D	5DM2	M	F	NA	NA
60	14 µg/L	A	4A	4AM1	M	A	1	1
61	14 µg/L	A	4A	4AM1	M	B	1	1
62	14 µg/L	A	4A	4AM1	M	C	1	7
63	14 µg/L	A	4A	4AM1	M	D	1	4
64	14 µg/L	A	4A	4AM1	M	D	2	1
65	14 µg/L	A	4A	4AM1	M	E	1	2
66	14 µg/L	A	4A	4AM1	M	F	NA	NA
67	14 µg/L	A	4A	4AM2	M	A	1	2
68	14 µg/L	A	4A	4AM2	M	B	1	1
69	14 µg/L	A	4A	4AM2	M	C	1	6
70	14 µg/L	A	4A	4AM2	M	C	2	1
71	14 µg/L	A	4A	4AM2	M	D	1	5
72	14 µg/L	A	4A	4AM2	M	E	1	2
73	14 µg/L	A	4A	4AM2	M	F	1	1
74	14 µg/L	B	4B	4BM1	M	A	1	2
75	14 µg/L	B	4B	4BM1	M	B	1	2
76	14 µg/L	B	4B	4BM1	M	C	1	4
77	14 µg/L	B	4B	4BM1	M	C	2	2
78	14 µg/L	B	4B	4BM1	M	D	1	5
79	14 µg/L	B	4B	4BM1	M	D	2	1
80	14 µg/L	B	4B	4BM1	M	E	1	2
81	14 µg/L	B	4B	4BM1	M	F	1	2
82	14 µg/L	B	4B	4BM2	M	A	1	2
83	14 µg/L	B	4B	4BM2	M	B	1	3
84	14 µg/L	B	4B	4BM2	M	C	1	5
85	14 µg/L	B	4B	4BM2	M	C	2	3
86	14 µg/L	B	4B	4BM2	M	D	1	4
87	14 µg/L	B	4B	4BM2	M	E	1	2
88	14 µg/L	B	4B	4BM2	M	F	1	3
89	14 µg/L	C	4C	4CM1	M	A	1	2
90	14 µg/L	C	4C	4CM1	M	B	1	2
91	14 µg/L	C	4C	4CM1	M	C	1	5
92	14 µg/L	C	4C	4CM1	M	D	1	5
93	14 µg/L	C	4C	4CM1	M	E	NA	NA
94	14 µg/L	C	4C	4CM1	M	F	NA	NA
95	14 µg/L	C	4C	4CM2	M	A	1	2
96	14 µg/L	C	4C	4CM2	M	B	1	1
97	14 µg/L	C	4C	4CM2	M	C	1	4
98	14 µg/L	C	4C	4CM2	M	D	1	6
99	14 µg/L	C	4C	4CM2	M	E	1	1
100	14 µg/L	C	4C	4CM2	M	F	NA	NA
101	14 µg/L	D	4D	4DM1	M	A	1	2
102	14 µg/L	D	4D	4DM1	M	B	2	2
103	14 µg/L	D	4D	4DM1	M	C	2	4

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Nuptial Tubercle Scores (Day21)

	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Mapping Region (A-F)	Rating (1-3)	Number of Tubercles of Specified Rating
104	14 µg/L	D	4D	4DM1	M	C	3	1
105	14 µg/L	D	4D	4DM1	M	D	1	5
106	14 µg/L	D	4D	4DM1	M	D	2	1
107	14 µg/L	D	4D	4DM1	M	D	3	1
108	14 µg/L	D	4D	4DM1	M	E	1	2
109	14 µg/L	D	4D	4DM1	M	F	NA	NA
110	14 µg/L	D	4D	4DM2	M	A	1	2
111	14 µg/L	D	4D	4DM2	M	B	1	2
112	14 µg/L	D	4D	4DM2	M	C	1	7
113	14 µg/L	D	4D	4DM2	M	D	1	5
114	14 µg/L	D	4D	4DM2	M	E	1	2
115	14 µg/L	D	4D	4DM2	M	F	1	2
116	35 µg/L	A	3A	3AM1	M	A	NA	NA
117	35 µg/L	A	3A	3AM1	M	B	1	2
118	35 µg/L	A	3A	3AM1	M	C	1	4
119	35 µg/L	A	3A	3AM1	M	C	2	1
120	35 µg/L	A	3A	3AM1	M	D	1	5
121	35 µg/L	A	3A	3AM1	M	E	1	2
122	35 µg/L	A	3A	3AM1	M	F	1	3
123	35 µg/L	A	3A	3AM2	M	A	1	2
124	35 µg/L	A	3A	3AM2	M	B	1	2
125	35 µg/L	A	3A	3AM2	M	C	1	5
126	35 µg/L	A	3A	3AM2	M	D	1	3
127	35 µg/L	A	3A	3AM2	M	E	1	2
128	35 µg/L	A	3A	3AM2	M	F	1	1
129	35 µg/L	B	3B	3BM1	M	A	1	2
130	35 µg/L	B	3B	3BM1	M	B	1	2
131	35 µg/L	B	3B	3BM1	M	C	1	3
132	35 µg/L	B	3B	3BM1	M	C	2	1
133	35 µg/L	B	3B	3BM1	M	D	1	4
134	35 µg/L	B	3B	3BM1	M	D	2	1
135	35 µg/L	B	3B	3BM1	M	E	1	2
136	35 µg/L	B	3B	3BM1	M	F	1	2
137	35 µg/L	B	3B	3BM2	M	A	NA	NA
138	35 µg/L	B	3B	3BM2	M	B	1	1
139	35 µg/L	B	3B	3BM2	M	C	1	5
140	35 µg/L	B	3B	3BM2	M	D	1	5
141	35 µg/L	B	3B	3BM2	M	E	1	2
142	35 µg/L	B	3B	3BM2	M	F	1	1
143	35 µg/L	C	3C	3CM1	M	A	NA	NA
144	35 µg/L	C	3C	3CM1	M	B	NA	NA
145	35 µg/L	C	3C	3CM1	M	C	1	3
146	35 µg/L	C	3C	3CM1	M	D	1	4
147	35 µg/L	C	3C	3CM1	M	E	1	1
148	35 µg/L	C	3C	3CM1	M	F	NA	NA
149	35 µg/L	C	3C	3CM2	M	A	1	1
150	35 µg/L	C	3C	3CM2	M	B	1	3
151	35 µg/L	C	3C	3CM2	M	C	1	7
152	35 µg/L	C	3C	3CM2	M	D	1	6
153	35 µg/L	C	3C	3CM2	M	E	1	2
154	35 µg/L	C	3C	3CM2	M	F	NA	NA

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Nuptial Tubercle Scores (Day21)

	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Mapping Region (A-F)	Rating (1-3)	Number of Tubercles of Specified Rating
155	35 µg/L	D	3D	3DM1	M	A	NA	NA
156	35 µg/L	D	3D	3DM1	M	B	1	3
157	35 µg/L	D	3D	3DM1	M	C	1	6
158	35 µg/L	D	3D	3DM1	M	D	1	5
159	35 µg/L	D	3D	3DM1	M	E	1	1
160	35 µg/L	D	3D	3DM1	M	F	1	1
161	35 µg/L	D	3D	3DM2	M	A	NA	NA
162	35 µg/L	D	3D	3DM2	M	B	1	2
163	35 µg/L	D	3D	3DM2	M	C	1	3
164	35 µg/L	D	3D	3DM2	M	C	2	2
165	35 µg/L	D	3D	3DM2	M	D	1	10
166	35 µg/L	D	3D	3DM2	M	E	1	1
167	35 µg/L	D	3D	3DM2	M	F	NA	NA
168	88 µg/L	A	2A	2AM1	M	A	NA	NA
169	88 µg/L	A	2A	2AM1	M	B	1	2
170	88 µg/L	A	2A	2AM1	M	C	1	4
171	88 µg/L	A	2A	2AM1	M	D	1	5
172	88 µg/L	A	2A	2AM1	M	E	NA	NA
173	88 µg/L	A	2A	2AM1	M	F	NA	NA
174	88 µg/L	A	2A	2AM2	M	A	1	2
175	88 µg/L	A	2A	2AM2	M	B	1	4
176	88 µg/L	A	2A	2AM2	M	C	1	6
177	88 µg/L	A	2A	2AM2	M	C	2	1
178	88 µg/L	A	2A	2AM2	M	D	1	6
179	88 µg/L	A	2A	2AM2	M	E	1	2
180	88 µg/L	A	2A	2AM2	M	F	1	2
181	88 µg/L	B	2B	2BM1	M	A	NA	NA
182	88 µg/L	B	2B	2BM1	M	B	1	4
183	88 µg/L	B	2B	2BM1	M	C	1	5
184	88 µg/L	B	2B	2BM1	M	D	1	6
185	88 µg/L	B	2B	2BM1	M	D	2	1
186	88 µg/L	B	2B	2BM1	M	E	1	2
187	88 µg/L	B	2B	2BM1	M	F	1	4
188	88 µg/L	B	2B	2BM2	M	A	1	2
189	88 µg/L	B	2B	2BM2	M	B	1	6
190	88 µg/L	B	2B	2BM2	M	C	2	3
191	88 µg/L	B	2B	2BM2	M	D	1	7
192	88 µg/L	B	2B	2BM2	M	E	1	1
193	88 µg/L	B	2B	2BM2	M	F	1	1
194	88 µg/L	C	2C	2CM1	M	A	1	2
195	88 µg/L	C	2C	2CM1	M	B	1	2
196	88 µg/L	C	2C	2CM1	M	C	1	4
197	88 µg/L	C	2C	2CM1	M	D	1	6
198	88 µg/L	C	2C	2CM1	M	D	2	2
199	88 µg/L	C	2C	2CM1	M	E	1	1
200	88 µg/L	C	2C	2CM1	M	F	1	4
201	88 µg/L	C	2C	2CM2	M	A	NA	NA
202	88 µg/L	C	2C	2CM2	M	B	1	1
203	88 µg/L	C	2C	2CM2	M	C	1	3
204	88 µg/L	C	2C	2CM2	M	D	1	5
205	88 µg/L	C	2C	2CM2	M	E	1	2

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Nuptial Tubercle Scores (Day21)

	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Mapping Region (A-F)	Rating (1-3)	Number of Tubercles of Specified Rating
206	88 µg/L	C	2C	2CM2	M	F	1	3
207	88 µg/L	D	2D	2DM1	M	A	1	1
208	88 µg/L	D	2D	2DM1	M	B	1	2
209	88 µg/L	D	2D	2DM1	M	C	1	2
210	88 µg/L	D	2D	2DM1	M	C	2	2
211	88 µg/L	D	2D	2DM1	M	D	1	3
212	88 µg/L	D	2D	2DM1	M	D	2	2
213	88 µg/L	D	2D	2DM1	M	E	1	2
214	88 µg/L	D	2D	2DM1	M	F	NA	NA
215	88 µg/L	D	2D	2DM2	M	A	NA	NA
216	88 µg/L	D	2D	2DM2	M	B	NA	NA
217	88 µg/L	D	2D	2DM2	M	C	1	4
218	88 µg/L	D	2D	2DM2	M	D	1	2
219	88 µg/L	D	2D	2DM2	M	E	NA	NA
220	88 µg/L	D	2D	2DM2	M	F	NA	NA
221	220 µg/L	A	1A	1AM1	M	A	1	2
222	220 µg/L	A	1A	1AM1	M	B	1	4
223	220 µg/L	A	1A	1AM1	M	C	1	2
224	220 µg/L	A	1A	1AM1	M	C	3	2
225	220 µg/L	A	1A	1AM1	M	D	1	4
226	220 µg/L	A	1A	1AM1	M	D	2	1
227	220 µg/L	A	1A	1AM1	M	E	1	2
228	220 µg/L	A	1A	1AM1	M	F	NA	NA
229	220 µg/L	A	1A	1AM2	M	A	1	2
230	220 µg/L	A	1A	1AM2	M	B	1	2
231	220 µg/L	A	1A	1AM2	M	C	1	3
232	220 µg/L	A	1A	1AM2	M	C	2	2
233	220 µg/L	A	1A	1AM2	M	D	1	4
234	220 µg/L	A	1A	1AM2	M	D	2	1
235	220 µg/L	A	1A	1AM2	M	E	1	2
236	220 µg/L	A	1A	1AM2	M	F	1	2
237	220 µg/L	B	1B	1BM1	M	A	NA	NA
238	220 µg/L	B	1B	1BM1	M	B	1	3
239	220 µg/L	B	1B	1BM1	M	C	1	3
240	220 µg/L	B	1B	1BM1	M	C	2	1
241	220 µg/L	B	1B	1BM1	M	D	1	5
242	220 µg/L	B	1B	1BM1	M	E	1	2
243	220 µg/L	B	1B	1BM1	M	F	1	2
244	220 µg/L	B	1B	1BM2	M	A	1	2
245	220 µg/L	B	1B	1BM2	M	B	1	2
246	220 µg/L	B	1B	1BM2	M	C	1	3
247	220 µg/L	B	1B	1BM2	M	D	1	4
248	220 µg/L	B	1B	1BM2	M	D	2	2
249	220 µg/L	B	1B	1BM2	M	E	1	2
250	220 µg/L	B	1B	1BM2	M	F	NA	NA
251	220 µg/L	C	1C	1CM1	M	A	1	2
252	220 µg/L	C	1C	1CM1	M	B	2	2
253	220 µg/L	C	1C	1CM1	M	C	1	3
254	220 µg/L	C	1C	1CM1	M	C	2	2
255	220 µg/L	C	1C	1CM1	M	D	1	5
256	220 µg/L	C	1C	1CM1	M	D	3	1

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Nuptial Tubercle Scores (Day21)

	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Mapping Region (A-F)	Rating (1-3)	Number of Tubercles of Specified Rating
257	220 µg/L	C	1C	1CM1	M	E	1	2
258	220 µg/L	C	1C	1CM1	M	F	NA	NA
259	220 µg/L	C	1C	1CM2	M	A	NA	NA
260	220 µg/L	C	1C	1CM2	M	B	1	2
261	220 µg/L	C	1C	1CM2	M	C	1	6
262	220 µg/L	C	1C	1CM2	M	D	1	5
263	220 µg/L	C	1C	1CM2	M	E	1	3
264	220 µg/L	C	1C	1CM2	M	F	NA	NA
265	220 µg/L	D	1D	1DM1	M	A	1	1
266	220 µg/L	D	1D	1DM1	M	B	1	2
267	220 µg/L	D	1D	1DM1	M	C	1	7
268	220 µg/L	D	1D	1DM1	M	D	1	6
269	220 µg/L	D	1D	1DM1	M	D	2	1
270	220 µg/L	D	1D	1DM1	M	E	NA	NA
271	220 µg/L	D	1D	1DM1	M	F	NA	NA
272	220 µg/L	D	1D	1DM2	M	A	NA	NA
273	220 µg/L	D	1D	1DM2	M	B	1	2
274	220 µg/L	D	1D	1DM2	M	C	1	6
275	220 µg/L	D	1D	1DM2	M	D	1	4
276	220 µg/L	D	1D	1DM2	M	E	1	3
277	220 µg/L	D	1D	1DM2	M	F	1	2
278								
279								
280	ed rating 1-3 for entire row on tubercle map.							
281	mined and found not to have any tubercles							
282								

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Left Testis Weight (mg)	Right Testis Weight (mg)
Control	A	5A	5AM1	NA	NA
Control	A	5A	5AM2	NA	NA
Control	B	5B	5BM1	NA	NA
Control	B	5B	5BM2	NA	NA
Control	C	5C	5CM1	NA	NA
Control	C	5C	5CM2	NA	NA
Control	D	5D	5DM1	NA	NA
Control	D	5D	5DM2	NA	NA
14 µg/L	A	4A	4AM1	NA	NA
14 µg/L	A	4A	4AM2	NA	NA
14 µg/L	B	4B	4BM1	NA	NA
14 µg/L	B	4B	4BM2	NA	NA
14 µg/L	C	4C	4CM1	NA	NA
14 µg/L	C	4C	4CM2	NA	NA
14 µg/L	D	4D	4DM1	NA	NA
14 µg/L	D	4D	4DM2	NA	NA
35 µg/L	A	3A	3AM1	NA	NA
35 µg/L	A	3A	3AM2	NA	NA
35 µg/L	B	3B	3BM1	NA	NA
35 µg/L	B	3B	3BM2	NA	NA
35 µg/L	C	3C	3CM1	NA	NA
35 µg/L	C	3C	3CM2	NA	NA
35 µg/L	D	3D	3DM1	NA	NA
35 µg/L	D	3D	3DM2	NA	NA
88 µg/L	A	2A	2AM1	NA	NA
88 µg/L	A	2A	2AM2	NA	NA
88 µg/L	B	2B	2BM1	NA	NA
88 µg/L	B	2B	2BM2	NA	NA
88 µg/L	C	2C	2CM1	NA	NA
88 µg/L	C	2C	2CM2	NA	NA
88 µg/L	D	2D	2DM1	NA	NA
88 µg/L	D	2D	2DM2	NA	NA
220 µg/L	A	1A	1AM1	NA	NA
220 µg/L	A	1A	1AM2	NA	NA
220 µg/L	B	1B	1BM1	NA	NA
220 µg/L	B	1B	1BM2	NA	NA
220 µg/L	C	1C	1CM1	NA	NA
220 µg/L	C	1C	1CM2	NA	NA
220 µg/L	D	1D	1DM1	NA	NA
220 µg/L	D	1D	1DM2	NA	NA

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Total Teste Weight (g)	GSI (%)
Control	A	5A	5AM1	0.0744	1.63959715274258
Control	A	5A	5AM2	0.0287	0.684637404580153
Control	B	5B	5BM1	0.0288	0.58287795992714
Control	B	5B	5BM2	0.0563	1.06833146739027
Control	C	5C	5CM1	0.0373	0.739800472044269
Control	C	5C	5CM2	0.0552	1.34457056559653
Control	D	5D	5DM1	0.0561	1.64680326425175
Control	D	5D	5DM2	0.0548	1.62751329036857
14 µg/L	A	4A	4AM1	0.0554	1.33105884048918
14 µg/L	A	4A	4AM2	0.0803	2.02731702390871
14 µg/L	B	4B	4BM1	0.0521	1.32891212855503
14 µg/L	B	4B	4BM2	0.071	1.44068828375472
14 µg/L	C	4C	4CM1	0.0634	1.44649783253479
14 µg/L	C	4C	4CM2	0.0445	1.14149394623435
14 µg/L	D	4D	4DM1	0.0974	2.04068805128957
14 µg/L	D	4D	4DM2	0.0653	1.80187637969095
35 µg/L	A	3A	3AM1	0.036	0.783102390637576
35 µg/L	A	3A	3AM2	0.0694	1.48946216251019
35 µg/L	B	3B	3BM1	0.1019	1.81507276322118
35 µg/L	B	3B	3BM2	0.0679	1.62634730538922
35 µg/L	C	3C	3CM1	0.0722	1.347291421747
35 µg/L	C	3C	3CM2	0.0678	1.53272295693455
35 µg/L	D	3D	3DM1	0.0342	0.854487307615431
35 µg/L	D	3D	3DM2	0.0533	1.27268385864374
88 µg/L	A	2A	2AM1	0.038	0.911511429873588
88 µg/L	A	2A	2AM2	0.0664	1.73151142171691
88 µg/L	B	2B	2BM1	0.048	1.27683345303647
88 µg/L	B	2B	2BM2	0.0865	1.86744386873921
88 µg/L	C	2C	2CM1	0.0686	1.46159582401193
88 µg/L	C	2C	2CM2	0.067	1.58298877731837
88 µg/L	D	2D	2DM1	0.0416	1.11402710085159
88 µg/L	D	2D	2DM2	0.0827	2.1288097199341
220 µg/L	A	1A	1AM1	0.0918	2.10589098917233
220 µg/L	A	1A	1AM2	0.0603	1.61779304053873
220 µg/L	B	1B	1BM1	0.0694	1.88448692535368
220 µg/L	B	1B	1BM2	0.0982	2.65211872417425
220 µg/L	C	1C	1CM1	0.1022	2.1548906740886
220 µg/L	C	1C	1CM2	0.1001	2.34026137984242
220 µg/L	D	1D	1DM1	0.0704	1.7320277518083
220 µg/L	D	1D	1DM2	0.0773	1.9811369111692

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Gonadal Staging Overall	Slide Number/Section ID
Control	A	5A	5AM1	1.5	
Control	A	5A	5AM2	1.5	
Control	B	5B	5BM1	2	
Control	B	5B	5BM2	1.5	
Control	C	5C	5CM1	1.5	
Control	C	5C	5CM2	2	
Control	D	5D	5DM1	2	
Control	D	5D	5DM2	1.5	
14 µg/L	A	4A	4AM1	1.5	
14 µg/L	A	4A	4AM2	3	
14 µg/L	B	4B	4BM1	2	
14 µg/L	B	4B	4BM2	1.5	
14 µg/L	C	4C	4CM1	2	
14 µg/L	C	4C	4CM2	1.5	
14 µg/L	D	4D	4DM1	2.5	
14 µg/L	D	4D	4DM2	1.5	
35 µg/L	A	3A	3AM1	1.5	
35 µg/L	A	3A	3AM2	1.5	
35 µg/L	B	3B	3BM1	2.5	
35 µg/L	B	3B	3BM2	2	
35 µg/L	C	3C	3CM1	2.5	
35 µg/L	C	3C	3CM2	2	
35 µg/L	D	3D	3DM1	1.5	
35 µg/L	D	3D	3DM2	1.5	
88 µg/L	A	2A	2AM1	2	
88 µg/L	A	2A	2AM2	2.5	
88 µg/L	B	2B	2BM1	1.5	
88 µg/L	B	2B	2BM2	2.5	
88 µg/L	C	2C	2CM1	3	
88 µg/L	C	2C	2CM2	1.5	
88 µg/L	D	2D	2DM1	1.5	
88 µg/L	D	2D	2DM2	2	
220 µg/L	A	1A	1AM1	2	
220 µg/L	A	1A	1AM2	2	
220 µg/L	B	1B	1BM1	1.5	
220 µg/L	B	1B	1BM2	2.5	
220 µg/L	C	1C	1CM1	2	
220 µg/L	C	1C	1CM2	3	
220 µg/L	D	1D	1DM1	2	
220 µg/L	D	1D	1DM2	2	

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Indicate Left or Right Testis	Gonadal Staging by Section
Control	A	5A	5AM1		
Control	A	5A	5AM2		
Control	B	5B	5BM1		
Control	B	5B	5BM2		
Control	C	5C	5CM1		
Control	C	5C	5CM2		
Control	D	5D	5DM1		
Control	D	5D	5DM2		
14 µg/L	A	4A	4AM1		
14 µg/L	A	4A	4AM2		
14 µg/L	B	4B	4BM1		
14 µg/L	B	4B	4BM2		
14 µg/L	C	4C	4CM1		
14 µg/L	C	4C	4CM2		
14 µg/L	D	4D	4DM1		
14 µg/L	D	4D	4DM2		
35 µg/L	A	3A	3AM1		
35 µg/L	A	3A	3AM2		
35 µg/L	B	3B	3BM1		
35 µg/L	B	3B	3BM2		
35 µg/L	C	3C	3CM1		
35 µg/L	C	3C	3CM2		
35 µg/L	D	3D	3DM1		
35 µg/L	D	3D	3DM2		
88 µg/L	A	2A	2AM1		
88 µg/L	A	2A	2AM2		
88 µg/L	B	2B	2BM1		
88 µg/L	B	2B	2BM2		
88 µg/L	C	2C	2CM1		
88 µg/L	C	2C	2CM2		
88 µg/L	D	2D	2DM1		
88 µg/L	D	2D	2DM2		
220 µg/L	A	1A	1AM1		
220 µg/L	A	1A	1AM2		
220 µg/L	B	1B	1BM1		
220 µg/L	B	1B	1BM2		
220 µg/L	C	1C	1CM1		
220 µg/L	C	1C	1CM2		
220 µg/L	D	1D	1DM1		
220 µg/L	D	1D	1DM2		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Cellular Infiltrate, Macrophages, Perifollicular	Follicle Numbers, Increased
Control	A	5A	5AM1		
Control	A	5A	5AM2		
Control	B	5B	5BM1		
Control	B	5B	5BM2		
Control	C	5C	5CM1		
Control	C	5C	5CM2		
Control	D	5D	5DM1		
Control	D	5D	5DM2		
14 µg/L	A	4A	4AM1		
14 µg/L	A	4A	4AM2		
14 µg/L	B	4B	4BM1		
14 µg/L	B	4B	4BM2		
14 µg/L	C	4C	4CM1		
14 µg/L	C	4C	4CM2		
14 µg/L	D	4D	4DM1		
14 µg/L	D	4D	4DM2		
35 µg/L	A	3A	3AM1		
35 µg/L	A	3A	3AM2		
35 µg/L	B	3B	3BM1		
35 µg/L	B	3B	3BM2		
35 µg/L	C	3C	3CM1		
35 µg/L	C	3C	3CM2		
35 µg/L	D	3D	3DM1		
35 µg/L	D	3D	3DM2		
88 µg/L	A	2A	2AM1		
88 µg/L	A	2A	2AM2		
88 µg/L	B	2B	2BM1		
88 µg/L	B	2B	2BM2		
88 µg/L	C	2C	2CM1		
88 µg/L	C	2C	2CM2		
88 µg/L	D	2D	2DM1		
88 µg/L	D	2D	2DM2		
220 µg/L	A	1A	1AM1		
220 µg/L	A	1A	1AM2		
220 µg/L	B	1B	1BM1		
220 µg/L	B	1B	1BM2		
220 µg/L	C	1C	1CM1		
220 µg/L	C	1C	1CM2		
220 µg/L	D	1D	1DM1		
220 µg/L	D	1D	1DM2		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Follicular Cells, Hypertrophy/Hyperplasia	Phenotype
Control	A	5A	5AM1		1
Control	A	5A	5AM2		2
Control	B	5B	5BM1		1
Control	B	5B	5BM2		1
Control	C	5C	5CM1		1
Control	C	5C	5CM2		1
Control	D	5D	5DM1		1
Control	D	5D	5DM2		1
14 µg/L	A	4A	4AM1		1
14 µg/L	A	4A	4AM2		1
14 µg/L	B	4B	4BM1		1
14 µg/L	B	4B	4BM2		1
14 µg/L	C	4C	4CM1		1
14 µg/L	C	4C	4CM2		1
14 µg/L	D	4D	4DM1		1
14 µg/L	D	4D	4DM2		1
35 µg/L	A	3A	3AM1		1
35 µg/L	A	3A	3AM2		1
35 µg/L	B	3B	3BM1		1
35 µg/L	B	3B	3BM2		1
35 µg/L	C	3C	3CM1		1
35 µg/L	C	3C	3CM2		1
35 µg/L	D	3D	3DM1		1
35 µg/L	D	3D	3DM2		1
88 µg/L	A	2A	2AM1		1
88 µg/L	A	2A	2AM2		1
88 µg/L	B	2B	2BM1		1
88 µg/L	B	2B	2BM2		1
88 µg/L	C	2C	2CM1		1
88 µg/L	C	2C	2CM2		1
88 µg/L	D	2D	2DM1		1
88 µg/L	D	2D	2DM2		1
220 µg/L	A	1A	1AM1		1
220 µg/L	A	1A	1AM2		1
220 µg/L	B	1B	1BM1		1
220 µg/L	B	1B	1BM2		1
220 µg/L	C	1C	1CM1		1
220 µg/L	C	1C	1CM2		1
220 µg/L	D	1D	1DM1		1
220 µg/L	D	1D	1DM2		1

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Collecting Ducts, Histiocytes, Intraluminal	Histiocytes, Intraluminal
Control	A	5A	5AM1		
Control	A	5A	5AM2		
Control	B	5B	5BM1		
Control	B	5B	5BM2		
Control	C	5C	5CM1		
Control	C	5C	5CM2		
Control	D	5D	5DM1		
Control	D	5D	5DM2		
14 µg/L	A	4A	4AM1		
14 µg/L	A	4A	4AM2		
14 µg/L	B	4B	4BM1		
14 µg/L	B	4B	4BM2		
14 µg/L	C	4C	4CM1		
14 µg/L	C	4C	4CM2		
14 µg/L	D	4D	4DM1		
14 µg/L	D	4D	4DM2		
35 µg/L	A	3A	3AM1		
35 µg/L	A	3A	3AM2		
35 µg/L	B	3B	3BM1		
35 µg/L	B	3B	3BM2		
35 µg/L	C	3C	3CM1		
35 µg/L	C	3C	3CM2		
35 µg/L	D	3D	3DM1		
35 µg/L	D	3D	3DM2		
88 µg/L	A	2A	2AM1		
88 µg/L	A	2A	2AM2		
88 µg/L	B	2B	2BM1		
88 µg/L	B	2B	2BM2		
88 µg/L	C	2C	2CM1		
88 µg/L	C	2C	2CM2		
88 µg/L	D	2D	2DM1		
88 µg/L	D	2D	2DM2		
220 µg/L	A	1A	1AM1		
220 µg/L	A	1A	1AM2		
220 µg/L	B	1B	1BM1		
220 µg/L	B	1B	1BM2		
220 µg/L	C	1C	1CM1		
220 µg/L	C	1C	1CM2		
220 µg/L	D	1D	1DM1		
220 µg/L	D	1D	1DM2		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Mineralization	Mineralization, Collecting Ducts
Control	A	5A	5AM1		
Control	A	5A	5AM2		
Control	B	5B	5BM1		
Control	B	5B	5BM2	2	
Control	C	5C	5CM1		
Control	C	5C	5CM2		
Control	D	5D	5DM1		
Control	D	5D	5DM2		
14 µg/L	A	4A	4AM1		
14 µg/L	A	4A	4AM2		
14 µg/L	B	4B	4BM1	1	
14 µg/L	B	4B	4BM2		
14 µg/L	C	4C	4CM1		
14 µg/L	C	4C	4CM2		
14 µg/L	D	4D	4DM1		
14 µg/L	D	4D	4DM2		
35 µg/L	A	3A	3AM1		
35 µg/L	A	3A	3AM2		
35 µg/L	B	3B	3BM1		
35 µg/L	B	3B	3BM2		
35 µg/L	C	3C	3CM1		
35 µg/L	C	3C	3CM2		
35 µg/L	D	3D	3DM1		
35 µg/L	D	3D	3DM2		
88 µg/L	A	2A	2AM1		
88 µg/L	A	2A	2AM2		
88 µg/L	B	2B	2BM1		
88 µg/L	B	2B	2BM2		
88 µg/L	C	2C	2CM1		
88 µg/L	C	2C	2CM2		
88 µg/L	D	2D	2DM1		
88 µg/L	D	2D	2DM2		
220 µg/L	A	1A	1AM1		
220 µg/L	A	1A	1AM2	1	
220 µg/L	B	1B	1BM1	1	
220 µg/L	B	1B	1BM2		
220 µg/L	C	1C	1CM1		
220 µg/L	C	1C	1CM2		
220 µg/L	D	1D	1DM1		
220 µg/L	D	1D	1DM2		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Severity Scoring Method	Increased Proportion of Spermatogonia (Spermatogonia, Increased)
Control	A	5A	5AM1	1	
Control	A	5A	5AM2	1	
Control	B	5B	5BM1	1	
Control	B	5B	5BM2	1	
Control	C	5C	5CM1	1	
Control	C	5C	5CM2	1	
Control	D	5D	5DM1	1	
Control	D	5D	5DM2	1	
14 µg/L	A	4A	4AM1	1	
14 µg/L	A	4A	4AM2	1	
14 µg/L	B	4B	4BM1	1	
14 µg/L	B	4B	4BM2	1	
14 µg/L	C	4C	4CM1	1	
14 µg/L	C	4C	4CM2	1	
14 µg/L	D	4D	4DM1	1	
14 µg/L	D	4D	4DM2	1	
35 µg/L	A	3A	3AM1	1	
35 µg/L	A	3A	3AM2	1	
35 µg/L	B	3B	3BM1	1	
35 µg/L	B	3B	3BM2	1	
35 µg/L	C	3C	3CM1	1	
35 µg/L	C	3C	3CM2	1	
35 µg/L	D	3D	3DM1	1	
35 µg/L	D	3D	3DM2	1	
88 µg/L	A	2A	2AM1	1	
88 µg/L	A	2A	2AM2	1	
88 µg/L	B	2B	2BM1	1	
88 µg/L	B	2B	2BM2	1	
88 µg/L	C	2C	2CM1	1	
88 µg/L	C	2C	2CM2	1	
88 µg/L	D	2D	2DM1	1	
88 µg/L	D	2D	2DM2	1	
220 µg/L	A	1A	1AM1	1	
220 µg/L	A	1A	1AM2	1	
220 µg/L	B	1B	1BM1	1	
220 µg/L	B	1B	1BM2	1	
220 µg/L	C	1C	1CM1	1	
220 µg/L	C	1C	1CM2	1	
220 µg/L	D	1D	1DM1	1	
220 µg/L	D	1D	1DM2	1	

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Presence of Testis-ova	Increased Testicular Degeneration (Testicular Degeneration)
Control	A	5A	5AM1		1
Control	A	5A	5AM2		
Control	B	5B	5BM1		
Control	B	5B	5BM2		
Control	C	5C	5CM1		
Control	C	5C	5CM2		1
Control	D	5D	5DM1		
Control	D	5D	5DM2		
14 µg/L	A	4A	4AM1		
14 µg/L	A	4A	4AM2		
14 µg/L	B	4B	4BM1		1
14 µg/L	B	4B	4BM2		
14 µg/L	C	4C	4CM1		
14 µg/L	C	4C	4CM2		1
14 µg/L	D	4D	4DM1		
14 µg/L	D	4D	4DM2		
35 µg/L	A	3A	3AM1		
35 µg/L	A	3A	3AM2		1
35 µg/L	B	3B	3BM1		
35 µg/L	B	3B	3BM2		
35 µg/L	C	3C	3CM1		
35 µg/L	C	3C	3CM2		
35 µg/L	D	3D	3DM1		
35 µg/L	D	3D	3DM2		
88 µg/L	A	2A	2AM1		
88 µg/L	A	2A	2AM2		
88 µg/L	B	2B	2BM1		
88 µg/L	B	2B	2BM2		
88 µg/L	C	2C	2CM1		
88 µg/L	C	2C	2CM2		1
88 µg/L	D	2D	2DM1		
88 µg/L	D	2D	2DM2		
220 µg/L	A	1A	1AM1		
220 µg/L	A	1A	1AM2		
220 µg/L	B	1B	1BM1		
220 µg/L	B	1B	1BM2		
220 µg/L	C	1C	1CM1		
220 µg/L	C	1C	1CM2		2
220 µg/L	D	1D	1DM1		
220 µg/L	D	1D	1DM2		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Interstitial Cell Hypertrophy/ Hyperplasia (Interstitial Cells, Increased)
Control	A	5A	5AM1	
Control	A	5A	5AM2	
Control	B	5B	5BM1	2
Control	B	5B	5BM2	1
Control	C	5C	5CM1	1
Control	C	5C	5CM2	
Control	D	5D	5DM1	
Control	D	5D	5DM2	2
14 µg/L	A	4A	4AM1	
14 µg/L	A	4A	4AM2	
14 µg/L	B	4B	4BM1	
14 µg/L	B	4B	4BM2	
14 µg/L	C	4C	4CM1	
14 µg/L	C	4C	4CM2	
14 µg/L	D	4D	4DM1	
14 µg/L	D	4D	4DM2	
35 µg/L	A	3A	3AM1	
35 µg/L	A	3A	3AM2	
35 µg/L	B	3B	3BM1	2
35 µg/L	B	3B	3BM2	
35 µg/L	C	3C	3CM1	
35 µg/L	C	3C	3CM2	
35 µg/L	D	3D	3DM1	
35 µg/L	D	3D	3DM2	
88 µg/L	A	2A	2AM1	
88 µg/L	A	2A	2AM2	
88 µg/L	B	2B	2BM1	
88 µg/L	B	2B	2BM2	
88 µg/L	C	2C	2CM1	
88 µg/L	C	2C	2CM2	2
88 µg/L	D	2D	2DM1	
88 µg/L	D	2D	2DM2	
220 µg/L	A	1A	1AM1	
220 µg/L	A	1A	1AM2	
220 µg/L	B	1B	1BM1	
220 µg/L	B	1B	1BM2	
220 µg/L	C	1C	1CM1	
220 µg/L	C	1C	1CM2	2
220 µg/L	D	1D	1DM1	
220 µg/L	D	1D	1DM2	

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Decreased Proportion of Spermatogonia	Increased Vascular or Interstitial Proteinaceous Fluid
Control	A	5A	5AM1		
Control	A	5A	5AM2		
Control	B	5B	5BM1		
Control	B	5B	5BM2		
Control	C	5C	5CM1		
Control	C	5C	5CM2		
Control	D	5D	5DM1		
Control	D	5D	5DM2		
14 µg/L	A	4A	4AM1		
14 µg/L	A	4A	4AM2		
14 µg/L	B	4B	4BM1		
14 µg/L	B	4B	4BM2		
14 µg/L	C	4C	4CM1		
14 µg/L	C	4C	4CM2		
14 µg/L	D	4D	4DM1		
14 µg/L	D	4D	4DM2		
35 µg/L	A	3A	3AM1		
35 µg/L	A	3A	3AM2		
35 µg/L	B	3B	3BM1		
35 µg/L	B	3B	3BM2		
35 µg/L	C	3C	3CM1		
35 µg/L	C	3C	3CM2		
35 µg/L	D	3D	3DM1		
35 µg/L	D	3D	3DM2		
88 µg/L	A	2A	2AM1		
88 µg/L	A	2A	2AM2		
88 µg/L	B	2B	2BM1		
88 µg/L	B	2B	2BM2		
88 µg/L	C	2C	2CM1		
88 µg/L	C	2C	2CM2		
88 µg/L	D	2D	2DM1		
88 µg/L	D	2D	2DM2		
220 µg/L	A	1A	1AM1		
220 µg/L	A	1A	1AM2		
220 µg/L	B	1B	1BM1		
220 µg/L	B	1B	1BM2		
220 µg/L	C	1C	1CM1		
220 µg/L	C	1C	1CM2		
220 µg/L	D	1D	1DM1		
220 µg/L	D	1D	1DM2		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Asynchronous Gonad Development
Control	A	5A	5AM1	
Control	A	5A	5AM2	
Control	B	5B	5BM1	
Control	B	5B	5BM2	
Control	C	5C	5CM1	
Control	C	5C	5CM2	
Control	D	5D	5DM1	
Control	D	5D	5DM2	
14 µg/L	A	4A	4AM1	
14 µg/L	A	4A	4AM2	
14 µg/L	B	4B	4BM1	
14 µg/L	B	4B	4BM2	
14 µg/L	C	4C	4CM1	
14 µg/L	C	4C	4CM2	
14 µg/L	D	4D	4DM1	
14 µg/L	D	4D	4DM2	
35 µg/L	A	3A	3AM1	
35 µg/L	A	3A	3AM2	
35 µg/L	B	3B	3BM1	
35 µg/L	B	3B	3BM2	
35 µg/L	C	3C	3CM1	
35 µg/L	C	3C	3CM2	
35 µg/L	D	3D	3DM1	
35 µg/L	D	3D	3DM2	
88 µg/L	A	2A	2AM1	
88 µg/L	A	2A	2AM2	
88 µg/L	B	2B	2BM1	
88 µg/L	B	2B	2BM2	
88 µg/L	C	2C	2CM1	
88 µg/L	C	2C	2CM2	
88 µg/L	D	2D	2DM1	
88 µg/L	D	2D	2DM2	
220 µg/L	A	1A	1AM1	
220 µg/L	A	1A	1AM2	
220 µg/L	B	1B	1BM1	
220 µg/L	B	1B	1BM2	
220 µg/L	C	1C	1CM1	
220 µg/L	C	1C	1CM2	
220 µg/L	D	1D	1DM1	
220 µg/L	D	1D	1DM2	

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Altered Proportions of Spermatocytes or Spermatids
Control	A	5A	5AM1	
Control	A	5A	5AM2	
Control	B	5B	5BM1	
Control	B	5B	5BM2	
Control	C	5C	5CM1	
Control	C	5C	5CM2	
Control	D	5D	5DM1	
Control	D	5D	5DM2	
14 µg/L	A	4A	4AM1	
14 µg/L	A	4A	4AM2	
14 µg/L	B	4B	4BM1	
14 µg/L	B	4B	4BM2	
14 µg/L	C	4C	4CM1	
14 µg/L	C	4C	4CM2	
14 µg/L	D	4D	4DM1	
14 µg/L	D	4D	4DM2	
35 µg/L	A	3A	3AM1	
35 µg/L	A	3A	3AM2	
35 µg/L	B	3B	3BM1	
35 µg/L	B	3B	3BM2	
35 µg/L	C	3C	3CM1	
35 µg/L	C	3C	3CM2	
35 µg/L	D	3D	3DM1	
35 µg/L	D	3D	3DM2	
88 µg/L	A	2A	2AM1	
88 µg/L	A	2A	2AM2	
88 µg/L	B	2B	2BM1	
88 µg/L	B	2B	2BM2	
88 µg/L	C	2C	2CM1	
88 µg/L	C	2C	2CM2	
88 µg/L	D	2D	2DM1	
88 µg/L	D	2D	2DM2	
220 µg/L	A	1A	1AM1	
220 µg/L	A	1A	1AM2	
220 µg/L	B	1B	1BM1	
220 µg/L	B	1B	1BM2	
220 µg/L	C	1C	1CM1	
220 µg/L	C	1C	1CM2	
220 µg/L	D	1D	1DM1	
220 µg/L	D	1D	1DM2	

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Granulomatous Inflammation (Inflammation, Granulomatous)	Comments
Control	A	5A	5AM1		
Control	A	5A	5AM2		
Control	B	5B	5BM1		
Control	B	5B	5BM2		
Control	C	5C	5CM1		
Control	C	5C	5CM2		
Control	D	5D	5DM1		
Control	D	5D	5DM2		
14 µg/L	A	4A	4AM1		
14 µg/L	A	4A	4AM2		
14 µg/L	B	4B	4BM1		
14 µg/L	B	4B	4BM2		
14 µg/L	C	4C	4CM1		
14 µg/L	C	4C	4CM2		
14 µg/L	D	4D	4DM1		
14 µg/L	D	4D	4DM2		
35 µg/L	A	3A	3AM1		
35 µg/L	A	3A	3AM2		
35 µg/L	B	3B	3BM1		
35 µg/L	B	3B	3BM2		
35 µg/L	C	3C	3CM1		
35 µg/L	C	3C	3CM2		
35 µg/L	D	3D	3DM1		
35 µg/L	D	3D	3DM2		
88 µg/L	A	2A	2AM1		
88 µg/L	A	2A	2AM2		
88 µg/L	B	2B	2BM1		
88 µg/L	B	2B	2BM2		
88 µg/L	C	2C	2CM1		
88 µg/L	C	2C	2CM2		
88 µg/L	D	2D	2DM1		
88 µg/L	D	2D	2DM2		
220 µg/L	A	1A	1AM1		
220 µg/L	A	1A	1AM2		
220 µg/L	B	1B	1BM1		
220 µg/L	B	1B	1BM2		
220 µg/L	C	1C	1CM1		
220 µg/L	C	1C	1CM2	2	
220 µg/L	D	1D	1DM1		
220 µg/L	D	1D	1DM2		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Not remarkable	Collecting Ducts, Mineralization
Control	A	5A	5AM1	X	
Control	A	5A	5AM2		2
Control	B	5B	5BM1	X	
Control	B	5B	5BM2		2
Control	C	5C	5CM1	X	
Control	C	5C	5CM2		2
Control	D	5D	5DM1	X	
Control	D	5D	5DM2		
14 µg/L	A	4A	4AM1		
14 µg/L	A	4A	4AM2		
14 µg/L	B	4B	4BM1		1
14 µg/L	B	4B	4BM2		
14 µg/L	C	4C	4CM1		
14 µg/L	C	4C	4CM2		
14 µg/L	D	4D	4DM1		2
14 µg/L	D	4D	4DM2		
35 µg/L	A	3A	3AM1		
35 µg/L	A	3A	3AM2		
35 µg/L	B	3B	3BM1		1
35 µg/L	B	3B	3BM2		
35 µg/L	C	3C	3CM1		
35 µg/L	C	3C	3CM2		
35 µg/L	D	3D	3DM1		
35 µg/L	D	3D	3DM2		1
88 µg/L	A	2A	2AM1		
88 µg/L	A	2A	2AM2		
88 µg/L	B	2B	2BM1		
88 µg/L	B	2B	2BM2		
88 µg/L	C	2C	2CM1		
88 µg/L	C	2C	2CM2		
88 µg/L	D	2D	2DM1		
88 µg/L	D	2D	2DM2		
220 µg/L	A	1A	1AM1	X	1
220 µg/L	A	1A	1AM2		1
220 µg/L	B	1B	1BM1		1
220 µg/L	B	1B	1BM2	X	
220 µg/L	C	1C	1CM1	X	1
220 µg/L	C	1C	1CM2		
220 µg/L	D	1D	1DM1		2
220 µg/L	D	1D	1DM2	X	

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Collecting Ducts, Oocyte	Proteinaceous Fluid, Intravascular/Interstitial
Control	A	5A	5AM1		
Control	A	5A	5AM2	2	
Control	B	5B	5BM1		
Control	B	5B	5BM2		
Control	C	5C	5CM1		
Control	C	5C	5CM2		
Control	D	5D	5DM1		
Control	D	5D	5DM2		
14 µg/L	A	4A	4AM1		
14 µg/L	A	4A	4AM2		
14 µg/L	B	4B	4BM1		
14 µg/L	B	4B	4BM2		
14 µg/L	C	4C	4CM1		
14 µg/L	C	4C	4CM2		
14 µg/L	D	4D	4DM1		
14 µg/L	D	4D	4DM2		
35 µg/L	A	3A	3AM1		
35 µg/L	A	3A	3AM2		
35 µg/L	B	3B	3BM1		
35 µg/L	B	3B	3BM2		
35 µg/L	C	3C	3CM1		
35 µg/L	C	3C	3CM2		
35 µg/L	D	3D	3DM1		
35 µg/L	D	3D	3DM2		
88 µg/L	A	2A	2AM1		
88 µg/L	A	2A	2AM2		
88 µg/L	B	2B	2BM1		
88 µg/L	B	2B	2BM2		
88 µg/L	C	2C	2CM1		
88 µg/L	C	2C	2CM2		1
88 µg/L	D	2D	2DM1		1
88 µg/L	D	2D	2DM2		
220 µg/L	A	1A	1AM1		2
220 µg/L	A	1A	1AM2		2
220 µg/L	B	1B	1BM1		2
220 µg/L	B	1B	1BM2		2
220 µg/L	C	1C	1CM1		1
220 µg/L	C	1C	1CM2		1
220 µg/L	D	1D	1DM1		2
220 µg/L	D	1D	1DM2		2

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Interstitial Cells, Decreased
Control	A	5A	5AM1	
Control	A	5A	5AM2	
Control	B	5B	5BM1	
Control	B	5B	5BM2	
Control	C	5C	5CM1	
Control	C	5C	5CM2	
Control	D	5D	5DM1	
Control	D	5D	5DM2	
14 µg/L	A	4A	4AM1	
14 µg/L	A	4A	4AM2	
14 µg/L	B	4B	4BM1	
14 µg/L	B	4B	4BM2	
14 µg/L	C	4C	4CM1	
14 µg/L	C	4C	4CM2	
14 µg/L	D	4D	4DM1	
14 µg/L	D	4D	4DM2	
35 µg/L	A	3A	3AM1	
35 µg/L	A	3A	3AM2	
35 µg/L	B	3B	3BM1	
35 µg/L	B	3B	3BM2	
35 µg/L	C	3C	3CM1	
35 µg/L	C	3C	3CM2	
35 µg/L	D	3D	3DM1	
35 µg/L	D	3D	3DM2	
88 µg/L	A	2A	2AM1	
88 µg/L	A	2A	2AM2	
88 µg/L	B	2B	2BM1	
88 µg/L	B	2B	2BM2	
88 µg/L	C	2C	2CM1	
88 µg/L	C	2C	2CM2	
88 µg/L	D	2D	2DM1	
88 µg/L	D	2D	2DM2	
220 µg/L	A	1A	1AM1	1
220 µg/L	A	1A	1AM2	
220 µg/L	B	1B	1BM1	
220 µg/L	B	1B	1BM2	
220 µg/L	C	1C	1CM1	
220 µg/L	C	1C	1CM2	
220 µg/L	D	1D	1DM1	
220 µg/L	D	1D	1DM2	

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Left Ovary Weight (mg)	Right Ovary Weight (mg)	Total ovary weight (g)
Control	A	5A	5AF1	NA	NA	0.2044
Control	A	5A	5AF2	NA	NA	0.416
Control	A	5A	5AF3	NA	NA	0.4466
Control	A	5A	5AF4	NA	NA	0.2375
Control	B	5B	5BF1	NA	NA	0.3195
Control	B	5B	5BF2	NA	NA	0.5403
Control	B	5B	5BF3	NA	NA	0.2892
Control	B	5B	5BF4	NA	NA	0.4357
Control	C	5C	5CF1	NA	NA	0.3614
Control	C	5C	5CF2	NA	NA	0.1892
Control	C	5C	5CF3	NA	NA	0.2222
Control	C	5C	5CF4	NA	NA	0.3926
Control	D	5D	5DF1	NA	NA	0.2838
Control	D	5D	5DF2	NA	NA	0.3862
Control	D	5D	5DF3	NA	NA	0.5241
Control	D	5D	5DF4	NA	NA	0.2524
14 µg/L	A	4A	4AF1	NA	NA	0.2171
14 µg/L	A	4A	4AF2	NA	NA	0.2197
14 µg/L	A	4A	4AF3	NA	NA	0.1857
14 µg/L	A	4A	4AF4	NA	NA	NA
14 µg/L	B	4B	4BF1	NA	NA	0.269
14 µg/L	B	4B	4BF2	NA	NA	0.3299
14 µg/L	B	4B	4BF3	NA	NA	0.1923
14 µg/L	B	4B	4BF4	NA	NA	NA
14 µg/L	C	4C	4CF1	NA	NA	0.3809
14 µg/L	C	4C	4CF2	NA	NA	0.3582
14 µg/L	C	4C	4CF3	NA	NA	0.3497
14 µg/L	C	4C	4CF4	NA	NA	0.4713
14 µg/L	D	4D	4DF1	NA	NA	0.2632
14 µg/L	D	4D	4DF2	NA	NA	0.4231
14 µg/L	D	4D	4DF3	NA	NA	0.3706
14 µg/L	D	4D	4DF4	NA	NA	0.4504
35 µg/L	A	3A	3AF1	NA	NA	0.2368
35 µg/L	A	3A	3AF2	NA	NA	0.3012
35 µg/L	A	3A	3AF3	NA	NA	0.5682
35 µg/L	A	3A	3AF4	NA	NA	NA
35 µg/L	B	3B	3BF1	NA	NA	0.4758
35 µg/L	B	3B	3BF2	NA	NA	0.3663
35 µg/L	B	3B	3BF3	NA	NA	0.2935
35 µg/L	B	3B	3BF4	NA	NA	0.2638
35 µg/L	C	3C	3CF1	NA	NA	0.4469
35 µg/L	C	3C	3CF2	NA	NA	0.2342
35 µg/L	C	3C	3CF3	NA	NA	0.4614
35 µg/L	C	3C	3CF4	NA	NA	0.2522

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Left Ovary Weight (mg)	Right Ovary Weight (mg)	Total ovary weight (g)
35 µg/L	D	3D	3DF1	NA	NA	0.5077
35 µg/L	D	3D	3DF2	NA	NA	0.3415
35 µg/L	D	3D	3DF3	NA	NA	0.3666
35 µg/L	D	3D	3DF4	NA	NA	0.2727
88 µg/L	A	2A	2AF1	NA	NA	0.2696
88 µg/L	A	2A	2AF2	NA	NA	0.4406
88 µg/L	A	2A	2AF3	NA	NA	0.4627
88 µg/L	A	2A	2AF4	NA	NA	NA
88 µg/L	B	2B	2BF1	NA	NA	0.4313
88 µg/L	B	2B	2BF2	NA	NA	0.5533
88 µg/L	B	2B	2BF3	NA	NA	0.4544
88 µg/L	B	2B	2BF4	NA	NA	0.4701
88 µg/L	C	2C	2CF1	NA	NA	0.0398
88 µg/L	C	2C	2CF2	NA	NA	0.3467
88 µg/L	C	2C	2CF3	NA	NA	0.1938
88 µg/L	C	2C	2CF4	NA	NA	0.4527
88 µg/L	D	2D	2DF1	NA	NA	0.2671
88 µg/L	D	2D	2DF2	NA	NA	0.4156
88 µg/L	D	2D	2DF3	NA	NA	0.6135
88 µg/L	D	2D	2DF4	NA	NA	0.4258
220 µg/L	A	1A	1AF1	NA	NA	0.3427
220 µg/L	A	1A	1AF2	NA	NA	0.3526
220 µg/L	A	1A	1AF3	NA	NA	0.2865
220 µg/L	A	1A	1AF4	NA	NA	0.5629
220 µg/L	B	1B	1BF1	NA	NA	0.438
220 µg/L	B	1B	1BF2	NA	NA	0.4246
220 µg/L	B	1B	1BF3	NA	NA	0.5437
220 µg/L	B	1B	1BF4	NA	NA	0.398
220 µg/L	C	1C	1CF1	NA	NA	0.4931
220 µg/L	C	1C	1CF2	NA	NA	0.3416
220 µg/L	C	1C	1CF3	NA	NA	0.5432
220 µg/L	C	1C	1CF4	NA	NA	0.3903
220 µg/L	D	1D	1DF1	NA	NA	0.2271
220 µg/L	D	1D	1DF2	NA	NA	0.3902
220 µg/L	D	1D	1DF3	NA	NA	0.5565
220 µg/L	D	1D	1DF4	NA	NA	0.4282

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	GSI (%)	Gonadal Staging Overall
Control	A	5A	5AF1	9.4612108868728	2
Control	A	5A	5AF2	17.0694678101022	3
Control	A	5A	5AF3	15.7032348804501	3.5
Control	A	5A	5AF4	12.8831027935991	2
Control	B	5B	5BF1	12.9946719811282	2.5
Control	B	5B	5BF2	23.9409783764622	3.5
Control	B	5B	5BF3	13.1239789435469	4
Control	B	5B	5BF4	16.6075852868306	3.5
Control	C	5C	5CF1	18.3163549744058	3.5
Control	C	5C	5CF2	10.8536025699862	4
Control	C	5C	5CF3	8.88906668800256	4
Control	C	5C	5CF4	17.3670706891976	3
Control	D	5D	5DF1	11.9540036224253	2.5
Control	D	5D	5DF2	19.4608213655833	3.5
Control	D	5D	5DF3	20.4136480486095	3.5
Control	D	5D	5DF4	13.772030337753	2.5
14 µg/L	A	4A	4AF1	9.27539947022131	2.5
14 µg/L	A	4A	4AF2	12.2341017930727	3.5
14 µg/L	A	4A	4AF3	11.3397655105032	2
14 µg/L	A	4A	4AF4	NA	
14 µg/L	B	4B	4BF1	12.7409652820537	2.5
14 µg/L	B	4B	4BF2	16.9946424891819	3.5
14 µg/L	B	4B	4BF3	8.00849575212394	2
14 µg/L	B	4B	4BF4	NA	
14 µg/L	C	4C	4CF1	15.2110538716505	3
14 µg/L	C	4C	4CF2	14.2595541401274	3
14 µg/L	C	4C	4CF3	13.1313131313131	3.5
14 µg/L	C	4C	4CF4	16.5414853292152	4
14 µg/L	D	4D	4DF1	12.0435618193466	2.5
14 µg/L	D	4D	4DF2	16.4989861176104	3
14 µg/L	D	4D	4DF3	15.3521126760563	3.5
14 µg/L	D	4D	4DF4	16.1915375489808	3.5
35 µg/L	A	3A	3AF1	9.80741354317664	3
35 µg/L	A	3A	3AF2	10.6945036216447	4
35 µg/L	A	3A	3AF3	23.1871046725158	3.5
35 µg/L	A	3A	3AF4	NA	
35 µg/L	B	3B	3BF1	17.6588479809976	3.5
35 µg/L	B	3B	3BF2	16.3271673724092	3
35 µg/L	B	3B	3BF3	14.0706649407929	2.5
35 µg/L	B	3B	3BF4	13.5734499614098	2.5
35 µg/L	C	3C	3CF1	18.6877979426278	3.5
35 µg/L	C	3C	3CF2	11.9807652956824	2.5
35 µg/L	C	3C	3CF3	18.2414801929311	3.5
35 µg/L	C	3C	3CF4	11.6258700963444	2.5

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	GSI (%)	Gonadal Staging Overall
35 µg/L	D	3D	3DF1	16.9555488761981	4
35 µg/L	D	3D	3DF2	13.3200717684687	4
35 µg/L	D	3D	3DF3	13.920637934308	2.5
35 µg/L	D	3D	3DF4	11.5335814582981	3
88 µg/L	A	2A	2AF1	11.389945078158	2.5
88 µg/L	A	2A	2AF2	17.8735142590564	3.5
88 µg/L	A	2A	2AF3	19.6042708245064	3
88 µg/L	A	2A	2AF4	NA	
88 µg/L	B	2B	2BF1	16.7840603961552	3
88 µg/L	B	2B	2BF2	23.5156615240767	3.5
88 µg/L	B	2B	2BF3	21.140783474458	3.5
88 µg/L	B	2B	2BF4	21.807301572575	3.5
88 µg/L	C	2C	2CF1	2.17035663649253	1
88 µg/L	C	2C	2CF2	14.8263770099213	4
88 µg/L	C	2C	2CF3	10.37806575988	3.5
88 µg/L	C	2C	2CF4	20.9089649438825	3.5
88 µg/L	D	2D	2DF1	12.2906313270753	3
88 µg/L	D	2D	2DF2	17.1756829359011	3.5
88 µg/L	D	2D	2DF3	25.0663942798774	3.5
88 µg/L	D	2D	2DF4	18.5219017791118	3
220 µg/L	A	1A	1AF1	15.8642718266827	3
220 µg/L	A	1A	1AF2	14.6605130763794	3
220 µg/L	A	1A	1AF3	14.0468719356737	3
220 µg/L	A	1A	1AF4	23.7771394779083	3.5
220 µg/L	B	1B	1BF1	17.9788194729497	3.5
220 µg/L	B	1B	1BF2	20.7051250792412	3.5
220 µg/L	B	1B	1BF3	21.0809972471017	3.5
220 µg/L	B	1B	1BF4	19.8493840706199	3.5
220 µg/L	C	1C	1CF1	18.6858160597219	3.5
220 µg/L	C	1C	1CF2	15.278647463995	3
220 µg/L	C	1C	1CF3	20.7740553770843	3.5
220 µg/L	C	1C	1CF4	19.638723960954	3
220 µg/L	D	1D	1DF1	9.80358299158213	2.5
220 µg/L	D	1D	1DF2	18.3347429752843	3.5
220 µg/L	D	1D	1DF3	20.561610936634	3.5
220 µg/L	D	1D	1DF4	14.2075052257872	3.5

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Slide Number/ Section ID	Indicate Left or Right Ovary
Control	A	5A	5AF1		
Control	A	5A	5AF2		
Control	A	5A	5AF3		
Control	A	5A	5AF4		
Control	B	5B	5BF1		
Control	B	5B	5BF2		
Control	B	5B	5BF3		
Control	B	5B	5BF4		
Control	C	5C	5CF1		
Control	C	5C	5CF2		
Control	C	5C	5CF3		
Control	C	5C	5CF4		
Control	D	5D	5DF1		
Control	D	5D	5DF2		
Control	D	5D	5DF3		
Control	D	5D	5DF4		
14 µg/L	A	4A	4AF1		
14 µg/L	A	4A	4AF2		
14 µg/L	A	4A	4AF3		
14 µg/L	A	4A	4AF4		
14 µg/L	B	4B	4BF1		
14 µg/L	B	4B	4BF2		
14 µg/L	B	4B	4BF3		
14 µg/L	B	4B	4BF4		
14 µg/L	C	4C	4CF1		
14 µg/L	C	4C	4CF2		
14 µg/L	C	4C	4CF3		
14 µg/L	C	4C	4CF4		
14 µg/L	D	4D	4DF1		
14 µg/L	D	4D	4DF2		
14 µg/L	D	4D	4DF3		
14 µg/L	D	4D	4DF4		
35 µg/L	A	3A	3AF1		
35 µg/L	A	3A	3AF2		
35 µg/L	A	3A	3AF3		
35 µg/L	A	3A	3AF4		
35 µg/L	B	3B	3BF1		
35 µg/L	B	3B	3BF2		
35 µg/L	B	3B	3BF3		
35 µg/L	B	3B	3BF4		
35 µg/L	C	3C	3CF1		
35 µg/L	C	3C	3CF2		
35 µg/L	C	3C	3CF3		
35 µg/L	C	3C	3CF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Slide Number/ Section ID	Indicate Left or Right Ovary
35 µg/L	D	3D	3DF1		
35 µg/L	D	3D	3DF2		
35 µg/L	D	3D	3DF3		
35 µg/L	D	3D	3DF4		
88 µg/L	A	2A	2AF1		
88 µg/L	A	2A	2AF2		
88 µg/L	A	2A	2AF3		
88 µg/L	A	2A	2AF4		
88 µg/L	B	2B	2BF1		
88 µg/L	B	2B	2BF2		
88 µg/L	B	2B	2BF3		
88 µg/L	B	2B	2BF4		
88 µg/L	C	2C	2CF1		
88 µg/L	C	2C	2CF2		
88 µg/L	C	2C	2CF3		
88 µg/L	C	2C	2CF4		
88 µg/L	D	2D	2DF1		
88 µg/L	D	2D	2DF2		
88 µg/L	D	2D	2DF3		
88 µg/L	D	2D	2DF4		
220 µg/L	A	1A	1AF1		
220 µg/L	A	1A	1AF2		
220 µg/L	A	1A	1AF3		
220 µg/L	A	1A	1AF4		
220 µg/L	B	1B	1BF1		
220 µg/L	B	1B	1BF2		
220 µg/L	B	1B	1BF3		
220 µg/L	B	1B	1BF4		
220 µg/L	C	1C	1CF1		
220 µg/L	C	1C	1CF2		
220 µg/L	C	1C	1CF3		
220 µg/L	C	1C	1CF4		
220 µg/L	D	1D	1DF1		
220 µg/L	D	1D	1DF2		
220 µg/L	D	1D	1DF3		
220 µg/L	D	1D	1DF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Gonadal Staging by Section	Cellular Infiltrate, Macrophages, Perifollicular
Control	A	5A	5AF1		
Control	A	5A	5AF2		
Control	A	5A	5AF3		
Control	A	5A	5AF4		
Control	B	5B	5BF1		
Control	B	5B	5BF2		
Control	B	5B	5BF3		
Control	B	5B	5BF4		
Control	C	5C	5CF1		
Control	C	5C	5CF2		
Control	C	5C	5CF3		
Control	C	5C	5CF4		
Control	D	5D	5DF1		
Control	D	5D	5DF2		
Control	D	5D	5DF3		
Control	D	5D	5DF4		
14 µg/L	A	4A	4AF1		
14 µg/L	A	4A	4AF2		
14 µg/L	A	4A	4AF3		
14 µg/L	A	4A	4AF4		
14 µg/L	B	4B	4BF1		
14 µg/L	B	4B	4BF2		
14 µg/L	B	4B	4BF3		
14 µg/L	B	4B	4BF4		
14 µg/L	C	4C	4CF1		
14 µg/L	C	4C	4CF2		
14 µg/L	C	4C	4CF3		
14 µg/L	C	4C	4CF4		
14 µg/L	D	4D	4DF1		
14 µg/L	D	4D	4DF2		
14 µg/L	D	4D	4DF3		
14 µg/L	D	4D	4DF4		
35 µg/L	A	3A	3AF1		
35 µg/L	A	3A	3AF2		
35 µg/L	A	3A	3AF3		
35 µg/L	A	3A	3AF4		
35 µg/L	B	3B	3BF1		
35 µg/L	B	3B	3BF2		
35 µg/L	B	3B	3BF3		
35 µg/L	B	3B	3BF4		
35 µg/L	C	3C	3CF1		
35 µg/L	C	3C	3CF2		
35 µg/L	C	3C	3CF3		
35 µg/L	C	3C	3CF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Gonadal Staging by Section	Cellular Infiltrate, Macrophages, Perifollicular
35 µg/L	D	3D	3DF1		
35 µg/L	D	3D	3DF2		
35 µg/L	D	3D	3DF3		
35 µg/L	D	3D	3DF4		
88 µg/L	A	2A	2AF1		
88 µg/L	A	2A	2AF2		
88 µg/L	A	2A	2AF3		
88 µg/L	A	2A	2AF4		
88 µg/L	B	2B	2BF1		
88 µg/L	B	2B	2BF2		
88 µg/L	B	2B	2BF3		
88 µg/L	B	2B	2BF4		
88 µg/L	C	2C	2CF1		
88 µg/L	C	2C	2CF2		
88 µg/L	C	2C	2CF3		
88 µg/L	C	2C	2CF4		
88 µg/L	D	2D	2DF1		
88 µg/L	D	2D	2DF2		
88 µg/L	D	2D	2DF3		
88 µg/L	D	2D	2DF4		
220 µg/L	A	1A	1AF1		
220 µg/L	A	1A	1AF2		
220 µg/L	A	1A	1AF3		
220 µg/L	A	1A	1AF4		
220 µg/L	B	1B	1BF1		
220 µg/L	B	1B	1BF2		
220 µg/L	B	1B	1BF3		
220 µg/L	B	1B	1BF4		
220 µg/L	C	1C	1CF1		
220 µg/L	C	1C	1CF2		
220 µg/L	C	1C	1CF3		
220 µg/L	C	1C	1CF4		
220 µg/L	D	1D	1DF1		
220 µg/L	D	1D	1DF2		
220 µg/L	D	1D	1DF3		
220 µg/L	D	1D	1DF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Follicle Numbers, Increased	Follicular Cells, Hypertrophy/Hyperplasia
Control	A	5A	5AF1		
Control	A	5A	5AF2		
Control	A	5A	5AF3		
Control	A	5A	5AF4		
Control	B	5B	5BF1		
Control	B	5B	5BF2		
Control	B	5B	5BF3		
Control	B	5B	5BF4		
Control	C	5C	5CF1		
Control	C	5C	5CF2		
Control	C	5C	5CF3		
Control	C	5C	5CF4		
Control	D	5D	5DF1		
Control	D	5D	5DF2		
Control	D	5D	5DF3		
Control	D	5D	5DF4		
14 µg/L	A	4A	4AF1		
14 µg/L	A	4A	4AF2		
14 µg/L	A	4A	4AF3		
14 µg/L	A	4A	4AF4		
14 µg/L	B	4B	4BF1		
14 µg/L	B	4B	4BF2		
14 µg/L	B	4B	4BF3		
14 µg/L	B	4B	4BF4		
14 µg/L	C	4C	4CF1		
14 µg/L	C	4C	4CF2		
14 µg/L	C	4C	4CF3		
14 µg/L	C	4C	4CF4		
14 µg/L	D	4D	4DF1		
14 µg/L	D	4D	4DF2		
14 µg/L	D	4D	4DF3		
14 µg/L	D	4D	4DF4		
35 µg/L	A	3A	3AF1		
35 µg/L	A	3A	3AF2		
35 µg/L	A	3A	3AF3		
35 µg/L	A	3A	3AF4		
35 µg/L	B	3B	3BF1		
35 µg/L	B	3B	3BF2		
35 µg/L	B	3B	3BF3		
35 µg/L	B	3B	3BF4		
35 µg/L	C	3C	3CF1		
35 µg/L	C	3C	3CF2		
35 µg/L	C	3C	3CF3		
35 µg/L	C	3C	3CF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Follicle Numbers, Increased	Follicular Cells, Hypertrophy/Hyperplasia
35 µg/L	D	3D	3DF1		
35 µg/L	D	3D	3DF2		
35 µg/L	D	3D	3DF3		
35 µg/L	D	3D	3DF4		
88 µg/L	A	2A	2AF1		
88 µg/L	A	2A	2AF2		
88 µg/L	A	2A	2AF3		
88 µg/L	A	2A	2AF4		
88 µg/L	B	2B	2BF1		
88 µg/L	B	2B	2BF2		
88 µg/L	B	2B	2BF3		
88 µg/L	B	2B	2BF4		
88 µg/L	C	2C	2CF1		
88 µg/L	C	2C	2CF2		
88 µg/L	C	2C	2CF3		
88 µg/L	C	2C	2CF4		
88 µg/L	D	2D	2DF1		
88 µg/L	D	2D	2DF2		
88 µg/L	D	2D	2DF3		
88 µg/L	D	2D	2DF4		
220 µg/L	A	1A	1AF1		
220 µg/L	A	1A	1AF2		
220 µg/L	A	1A	1AF3		
220 µg/L	A	1A	1AF4		
220 µg/L	B	1B	1BF1		
220 µg/L	B	1B	1BF2		
220 µg/L	B	1B	1BF3		
220 µg/L	B	1B	1BF4		
220 µg/L	C	1C	1CF1		
220 µg/L	C	1C	1CF2		
220 µg/L	C	1C	1CF3		
220 µg/L	C	1C	1CF4		
220 µg/L	D	1D	1DF1		
220 µg/L	D	1D	1DF2		
220 µg/L	D	1D	1DF3		
220 µg/L	D	1D	1DF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Phenotype	Granulosa Cells, Hyperplasia	Ooplasm Dysgenesis
Control	A	5A	5AF1	5		
Control	A	5A	5AF2	5		
Control	A	5A	5AF3	5		
Control	A	5A	5AF4	5		
Control	B	5B	5BF1	5		
Control	B	5B	5BF2	5		
Control	B	5B	5BF3	5		
Control	B	5B	5BF4	5		
Control	C	5C	5CF1	5		
Control	C	5C	5CF2	5		
Control	C	5C	5CF3	5		
Control	C	5C	5CF4	5		
Control	D	5D	5DF1	5		
Control	D	5D	5DF2	5		
Control	D	5D	5DF3	5		
Control	D	5D	5DF4	5		2
14 µg/L	A	4A	4AF1	5		
14 µg/L	A	4A	4AF2	5		
14 µg/L	A	4A	4AF3	5		
14 µg/L	A	4A	4AF4			
14 µg/L	B	4B	4BF1	5		2
14 µg/L	B	4B	4BF2	5		
14 µg/L	B	4B	4BF3	5		
14 µg/L	B	4B	4BF4			
14 µg/L	C	4C	4CF1	5		
14 µg/L	C	4C	4CF2	5		
14 µg/L	C	4C	4CF3	5		
14 µg/L	C	4C	4CF4	5		
14 µg/L	D	4D	4DF1	5		
14 µg/L	D	4D	4DF2	5		
14 µg/L	D	4D	4DF3	5		
14 µg/L	D	4D	4DF4	5		
35 µg/L	A	3A	3AF1	5		
35 µg/L	A	3A	3AF2	5		
35 µg/L	A	3A	3AF3	5		
35 µg/L	A	3A	3AF4			
35 µg/L	B	3B	3BF1	5		
35 µg/L	B	3B	3BF2	5		
35 µg/L	B	3B	3BF3	5		
35 µg/L	B	3B	3BF4	5		1
35 µg/L	C	3C	3CF1	5		
35 µg/L	C	3C	3CF2	5		2
35 µg/L	C	3C	3CF3	5		
35 µg/L	C	3C	3CF4	5		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Phenotype	Granulosa Cells, Hyperplasia	Ooplasm Dysgenesis
35 µg/L	D	3D	3DF1	5		
35 µg/L	D	3D	3DF2	5		
35 µg/L	D	3D	3DF3	5		
35 µg/L	D	3D	3DF4	5		
88 µg/L	A	2A	2AF1	5		
88 µg/L	A	2A	2AF2	5		
88 µg/L	A	2A	2AF3	5		
88 µg/L	A	2A	2AF4			
88 µg/L	B	2B	2BF1	5		
88 µg/L	B	2B	2BF2	5		
88 µg/L	B	2B	2BF3	5		
88 µg/L	B	2B	2BF4	5		
88 µg/L	C	2C	2CF1	5		
88 µg/L	C	2C	2CF2	5		
88 µg/L	C	2C	2CF3	5		
88 µg/L	C	2C	2CF4	5		
88 µg/L	D	2D	2DF1	5		
88 µg/L	D	2D	2DF2	5		
88 µg/L	D	2D	2DF3	5		
88 µg/L	D	2D	2DF4	5		
220 µg/L	A	1A	1AF1	5		
220 µg/L	A	1A	1AF2	5		
220 µg/L	A	1A	1AF3	5		
220 µg/L	A	1A	1AF4	5		
220 µg/L	B	1B	1BF1	5		
220 µg/L	B	1B	1BF2	5		
220 µg/L	B	1B	1BF3	5		
220 µg/L	B	1B	1BF4	5		
220 µg/L	C	1C	1CF1	5		
220 µg/L	C	1C	1CF2	5		
220 µg/L	C	1C	1CF3	5		
220 µg/L	C	1C	1CF4	5		
220 µg/L	D	1D	1DF1	5		
220 µg/L	D	1D	1DF2	5		
220 µg/L	D	1D	1DF3	5		
220 µg/L	D	1D	1DF4	5		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Ovarian Cyst	Perinucleolar Oocytes, Increased
Control	A	5A	5AF1		
Control	A	5A	5AF2		
Control	A	5A	5AF3		
Control	A	5A	5AF4		
Control	B	5B	5BF1		
Control	B	5B	5BF2		
Control	B	5B	5BF3		
Control	B	5B	5BF4		
Control	C	5C	5CF1		
Control	C	5C	5CF2		
Control	C	5C	5CF3		
Control	C	5C	5CF4		
Control	D	5D	5DF1		
Control	D	5D	5DF2		
Control	D	5D	5DF3		
Control	D	5D	5DF4		
14 µg/L	A	4A	4AF1		
14 µg/L	A	4A	4AF2		
14 µg/L	A	4A	4AF3		
14 µg/L	A	4A	4AF4		
14 µg/L	B	4B	4BF1		
14 µg/L	B	4B	4BF2		
14 µg/L	B	4B	4BF3		
14 µg/L	B	4B	4BF4		
14 µg/L	C	4C	4CF1		
14 µg/L	C	4C	4CF2		
14 µg/L	C	4C	4CF3		
14 µg/L	C	4C	4CF4		
14 µg/L	D	4D	4DF1		
14 µg/L	D	4D	4DF2		
14 µg/L	D	4D	4DF3		
14 µg/L	D	4D	4DF4		
35 µg/L	A	3A	3AF1		
35 µg/L	A	3A	3AF2		
35 µg/L	A	3A	3AF3		
35 µg/L	A	3A	3AF4		
35 µg/L	B	3B	3BF1		
35 µg/L	B	3B	3BF2		
35 µg/L	B	3B	3BF3		
35 µg/L	B	3B	3BF4		
35 µg/L	C	3C	3CF1		
35 µg/L	C	3C	3CF2		
35 µg/L	C	3C	3CF3		
35 µg/L	C	3C	3CF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Ovarian Cyst	Perinucleolar Oocytes, Increased
35 µg/L	D	3D	3DF1		
35 µg/L	D	3D	3DF2		
35 µg/L	D	3D	3DF3		
35 µg/L	D	3D	3DF4		
88 µg/L	A	2A	2AF1		
88 µg/L	A	2A	2AF2		
88 µg/L	A	2A	2AF3		
88 µg/L	A	2A	2AF4		
88 µg/L	B	2B	2BF1		
88 µg/L	B	2B	2BF2		
88 µg/L	B	2B	2BF3		
88 µg/L	B	2B	2BF4		
88 µg/L	C	2C	2CF1		
88 µg/L	C	2C	2CF2		
88 µg/L	C	2C	2CF3		
88 µg/L	C	2C	2CF4		
88 µg/L	D	2D	2DF1		
88 µg/L	D	2D	2DF2		
88 µg/L	D	2D	2DF3		
88 µg/L	D	2D	2DF4		
220 µg/L	A	1A	1AF1		
220 µg/L	A	1A	1AF2		
220 µg/L	A	1A	1AF3		
220 µg/L	A	1A	1AF4		
220 µg/L	B	1B	1BF1		
220 µg/L	B	1B	1BF2		
220 µg/L	B	1B	1BF3		
220 µg/L	B	1B	1BF4		
220 µg/L	C	1C	1CF1		
220 µg/L	C	1C	1CF2		
220 µg/L	C	1C	1CF3		
220 µg/L	C	1C	1CF4		
220 µg/L	D	1D	1DF1		
220 µg/L	D	1D	1DF2		
220 µg/L	D	1D	1DF3		
220 µg/L	D	1D	1DF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Post-Ovulatory Follicles	Severity Scoring Method
Control	A	5A	5AF1	2	1
Control	A	5A	5AF2	2	1
Control	A	5A	5AF3		1
Control	A	5A	5AF4	3	1
Control	B	5B	5BF1	3	1
Control	B	5B	5BF2		1
Control	B	5B	5BF3	3	1
Control	B	5B	5BF4		1
Control	C	5C	5CF1		1
Control	C	5C	5CF2	3	1
Control	C	5C	5CF3	3	1
Control	C	5C	5CF4	1	1
Control	D	5D	5DF1	3	1
Control	D	5D	5DF2		1
Control	D	5D	5DF3		1
Control	D	5D	5DF4	3	1
14 µg/L	A	4A	4AF1	3	1
14 µg/L	A	4A	4AF2		1
14 µg/L	A	4A	4AF3	3	1
14 µg/L	A	4A	4AF4		
14 µg/L	B	4B	4BF1	3	1
14 µg/L	B	4B	4BF2		1
14 µg/L	B	4B	4BF3	3	1
14 µg/L	B	4B	4BF4		
14 µg/L	C	4C	4CF1	3	1
14 µg/L	C	4C	4CF2	2	1
14 µg/L	C	4C	4CF3		1
14 µg/L	C	4C	4CF4	3	1
14 µg/L	D	4D	4DF1	3	1
14 µg/L	D	4D	4DF2	1	1
14 µg/L	D	4D	4DF3		1
14 µg/L	D	4D	4DF4		1
35 µg/L	A	3A	3AF1	3	1
35 µg/L	A	3A	3AF2	3	1
35 µg/L	A	3A	3AF3		1
35 µg/L	A	3A	3AF4		
35 µg/L	B	3B	3BF1		1
35 µg/L	B	3B	3BF2	2	1
35 µg/L	B	3B	3BF3	3	1
35 µg/L	B	3B	3BF4	3	1
35 µg/L	C	3C	3CF1		1
35 µg/L	C	3C	3CF2	3	1
35 µg/L	C	3C	3CF3		1
35 µg/L	C	3C	3CF4	3	1

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Post-Ovulatory Follicles	Severity Scoring Method
35 µg/L	D	3D	3DF1	3	1
35 µg/L	D	3D	3DF2	3	1
35 µg/L	D	3D	3DF3	3	1
35 µg/L	D	3D	3DF4	1	1
88 µg/L	A	2A	2AF1	3	1
88 µg/L	A	2A	2AF2		1
88 µg/L	A	2A	2AF3		1
88 µg/L	A	2A	2AF4		
88 µg/L	B	2B	2BF1		1
88 µg/L	B	2B	2BF2		1
88 µg/L	B	2B	2BF3		1
88 µg/L	B	2B	2BF4	1	1
88 µg/L	C	2C	2CF1		1
88 µg/L	C	2C	2CF2	3	1
88 µg/L	C	2C	2CF3		1
88 µg/L	C	2C	2CF4		1
88 µg/L	D	2D	2DF1		1
88 µg/L	D	2D	2DF2		1
88 µg/L	D	2D	2DF3		1
88 µg/L	D	2D	2DF4		1
220 µg/L	A	1A	1AF1	1	1
220 µg/L	A	1A	1AF2	1	1
220 µg/L	A	1A	1AF3	3	1
220 µg/L	A	1A	1AF4		1
220 µg/L	B	1B	1BF1		1
220 µg/L	B	1B	1BF2		1
220 µg/L	B	1B	1BF3		1
220 µg/L	B	1B	1BF4	2	1
220 µg/L	C	1C	1CF1		1
220 µg/L	C	1C	1CF2	1	1
220 µg/L	C	1C	1CF3		1
220 µg/L	C	1C	1CF4	2	1
220 µg/L	D	1D	1DF1	3	1
220 µg/L	D	1D	1DF2		1
220 µg/L	D	1D	1DF3		1
220 µg/L	D	1D	1DF4		1

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Increased Oocyte Atresia (Oocyte Atresia)	Perifollicular Cell Hyperplasia/ Hypertrophy
Control	A	5A	5AF1		
Control	A	5A	5AF2		
Control	A	5A	5AF3		
Control	A	5A	5AF4		
Control	B	5B	5BF1		
Control	B	5B	5BF2		
Control	B	5B	5BF3		
Control	B	5B	5BF4		
Control	C	5C	5CF1		
Control	C	5C	5CF2		
Control	C	5C	5CF3		
Control	C	5C	5CF4		
Control	D	5D	5DF1		
Control	D	5D	5DF2		
Control	D	5D	5DF3		
Control	D	5D	5DF4		
14 µg/L	A	4A	4AF1		
14 µg/L	A	4A	4AF2		
14 µg/L	A	4A	4AF3		
14 µg/L	A	4A	4AF4		
14 µg/L	B	4B	4BF1		
14 µg/L	B	4B	4BF2		
14 µg/L	B	4B	4BF3		
14 µg/L	B	4B	4BF4		
14 µg/L	C	4C	4CF1		
14 µg/L	C	4C	4CF2		
14 µg/L	C	4C	4CF3		
14 µg/L	C	4C	4CF4		
14 µg/L	D	4D	4DF1		
14 µg/L	D	4D	4DF2		
14 µg/L	D	4D	4DF3		
14 µg/L	D	4D	4DF4		
35 µg/L	A	3A	3AF1		
35 µg/L	A	3A	3AF2		
35 µg/L	A	3A	3AF3		
35 µg/L	A	3A	3AF4		
35 µg/L	B	3B	3BF1		
35 µg/L	B	3B	3BF2		
35 µg/L	B	3B	3BF3		
35 µg/L	B	3B	3BF4		
35 µg/L	C	3C	3CF1		
35 µg/L	C	3C	3CF2		
35 µg/L	C	3C	3CF3		
35 µg/L	C	3C	3CF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Increased Oocyte Atresia (Oocyte Atresia)	Perifollicular Cell Hyperplasia/ Hypertrophy
35 µg/L	D	3D	3DF1		
35 µg/L	D	3D	3DF2		
35 µg/L	D	3D	3DF3		
35 µg/L	D	3D	3DF4		
88 µg/L	A	2A	2AF1		
88 µg/L	A	2A	2AF2		
88 µg/L	A	2A	2AF3		
88 µg/L	A	2A	2AF4		
88 µg/L	B	2B	2BF1		
88 µg/L	B	2B	2BF2		
88 µg/L	B	2B	2BF3		
88 µg/L	B	2B	2BF4		
88 µg/L	C	2C	2CF1		
88 µg/L	C	2C	2CF2		
88 µg/L	C	2C	2CF3		
88 µg/L	C	2C	2CF4		
88 µg/L	D	2D	2DF1		
88 µg/L	D	2D	2DF2		
88 µg/L	D	2D	2DF3		
88 µg/L	D	2D	2DF4		
220 µg/L	A	1A	1AF1		
220 µg/L	A	1A	1AF2		
220 µg/L	A	1A	1AF3		
220 µg/L	A	1A	1AF4		
220 µg/L	B	1B	1BF1		
220 µg/L	B	1B	1BF2		
220 µg/L	B	1B	1BF3		
220 µg/L	B	1B	1BF4		
220 µg/L	C	1C	1CF1		
220 µg/L	C	1C	1CF2		
220 µg/L	C	1C	1CF3		
220 µg/L	C	1C	1CF4		
220 µg/L	D	1D	1DF1		
220 µg/L	D	1D	1DF2		
220 µg/L	D	1D	1DF3		
220 µg/L	D	1D	1DF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Decreased Yolk Formation	Interstitial Fibrosis	Egg Debris in Oviduct
Control	A	5A	5AF1			
Control	A	5A	5AF2			
Control	A	5A	5AF3			
Control	A	5A	5AF4			
Control	B	5B	5BF1			
Control	B	5B	5BF2			
Control	B	5B	5BF3			
Control	B	5B	5BF4			
Control	C	5C	5CF1			
Control	C	5C	5CF2			
Control	C	5C	5CF3			
Control	C	5C	5CF4			
Control	D	5D	5DF1			
Control	D	5D	5DF2			
Control	D	5D	5DF3			
Control	D	5D	5DF4			
14 µg/L	A	4A	4AF1			
14 µg/L	A	4A	4AF2			
14 µg/L	A	4A	4AF3			
14 µg/L	A	4A	4AF4			
14 µg/L	B	4B	4BF1			
14 µg/L	B	4B	4BF2			
14 µg/L	B	4B	4BF3			
14 µg/L	B	4B	4BF4			
14 µg/L	C	4C	4CF1			
14 µg/L	C	4C	4CF2			
14 µg/L	C	4C	4CF3			
14 µg/L	C	4C	4CF4			
14 µg/L	D	4D	4DF1			
14 µg/L	D	4D	4DF2			
14 µg/L	D	4D	4DF3			
14 µg/L	D	4D	4DF4			
35 µg/L	A	3A	3AF1			
35 µg/L	A	3A	3AF2			
35 µg/L	A	3A	3AF3			
35 µg/L	A	3A	3AF4			
35 µg/L	B	3B	3BF1			
35 µg/L	B	3B	3BF2			
35 µg/L	B	3B	3BF3			
35 µg/L	B	3B	3BF4			
35 µg/L	C	3C	3CF1			
35 µg/L	C	3C	3CF2			
35 µg/L	C	3C	3CF3			
35 µg/L	C	3C	3CF4			

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Decreased Yolk Formation	Interstitial Fibrosis	Egg Debris in Oviduct
35 µg/L	D	3D	3DF1			
35 µg/L	D	3D	3DF2			
35 µg/L	D	3D	3DF3			
35 µg/L	D	3D	3DF4			
88 µg/L	A	2A	2AF1			
88 µg/L	A	2A	2AF2			
88 µg/L	A	2A	2AF3			
88 µg/L	A	2A	2AF4			
88 µg/L	B	2B	2BF1			
88 µg/L	B	2B	2BF2			
88 µg/L	B	2B	2BF3			
88 µg/L	B	2B	2BF4			
88 µg/L	C	2C	2CF1			
88 µg/L	C	2C	2CF2			
88 µg/L	C	2C	2CF3			
88 µg/L	C	2C	2CF4			
88 µg/L	D	2D	2DF1			
88 µg/L	D	2D	2DF2			
88 µg/L	D	2D	2DF3			
88 µg/L	D	2D	2DF4			
220 µg/L	A	1A	1AF1			
220 µg/L	A	1A	1AF2			
220 µg/L	A	1A	1AF3			
220 µg/L	A	1A	1AF4			
220 µg/L	B	1B	1BF1			
220 µg/L	B	1B	1BF2			
220 µg/L	B	1B	1BF3			
220 µg/L	B	1B	1BF4			
220 µg/L	C	1C	1CF1			
220 µg/L	C	1C	1CF2			
220 µg/L	C	1C	1CF3			
220 µg/L	C	1C	1CF4			
220 µg/L	D	1D	1DF1			
220 µg/L	D	1D	1DF2			
220 µg/L	D	1D	1DF3			
220 µg/L	D	1D	1DF4			

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Granulomatous Inflammation (Inflammation, Granulomatous)
Control	A	5A	5AF1	
Control	A	5A	5AF2	
Control	A	5A	5AF3	
Control	A	5A	5AF4	
Control	B	5B	5BF1	
Control	B	5B	5BF2	
Control	B	5B	5BF3	
Control	B	5B	5BF4	
Control	C	5C	5CF1	
Control	C	5C	5CF2	
Control	C	5C	5CF3	2
Control	C	5C	5CF4	
Control	D	5D	5DF1	
Control	D	5D	5DF2	
Control	D	5D	5DF3	
Control	D	5D	5DF4	
14 µg/L	A	4A	4AF1	
14 µg/L	A	4A	4AF2	
14 µg/L	A	4A	4AF3	
14 µg/L	A	4A	4AF4	
14 µg/L	B	4B	4BF1	
14 µg/L	B	4B	4BF2	
14 µg/L	B	4B	4BF3	
14 µg/L	B	4B	4BF4	
14 µg/L	C	4C	4CF1	
14 µg/L	C	4C	4CF2	
14 µg/L	C	4C	4CF3	
14 µg/L	C	4C	4CF4	
14 µg/L	D	4D	4DF1	
14 µg/L	D	4D	4DF2	
14 µg/L	D	4D	4DF3	
14 µg/L	D	4D	4DF4	
35 µg/L	A	3A	3AF1	
35 µg/L	A	3A	3AF2	
35 µg/L	A	3A	3AF3	
35 µg/L	A	3A	3AF4	
35 µg/L	B	3B	3BF1	
35 µg/L	B	3B	3BF2	
35 µg/L	B	3B	3BF3	
35 µg/L	B	3B	3BF4	
35 µg/L	C	3C	3CF1	
35 µg/L	C	3C	3CF2	
35 µg/L	C	3C	3CF3	
35 µg/L	C	3C	3CF4	

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Granulomatous Inflammation (Inflammation, Granulomatous)
35 µg/L	D	3D	3DF1	
35 µg/L	D	3D	3DF2	
35 µg/L	D	3D	3DF3	
35 µg/L	D	3D	3DF4	
88 µg/L	A	2A	2AF1	
88 µg/L	A	2A	2AF2	
88 µg/L	A	2A	2AF3	
88 µg/L	A	2A	2AF4	
88 µg/L	B	2B	2BF1	
88 µg/L	B	2B	2BF2	
88 µg/L	B	2B	2BF3	
88 µg/L	B	2B	2BF4	
88 µg/L	C	2C	2CF1	
88 µg/L	C	2C	2CF2	
88 µg/L	C	2C	2CF3	
88 µg/L	C	2C	2CF4	
88 µg/L	D	2D	2DF1	
88 µg/L	D	2D	2DF2	
88 µg/L	D	2D	2DF3	
88 µg/L	D	2D	2DF4	
220 µg/L	A	1A	1AF1	
220 µg/L	A	1A	1AF2	
220 µg/L	A	1A	1AF3	
220 µg/L	A	1A	1AF4	
220 µg/L	B	1B	1BF1	
220 µg/L	B	1B	1BF2	
220 µg/L	B	1B	1BF3	
220 µg/L	B	1B	1BF4	
220 µg/L	C	1C	1CF1	
220 µg/L	C	1C	1CF2	
220 µg/L	C	1C	1CF3	
220 µg/L	C	1C	1CF4	
220 µg/L	D	1D	1DF1	
220 µg/L	D	1D	1DF2	
220 µg/L	D	1D	1DF3	
220 µg/L	D	1D	1DF4	

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Decreased Post-ovulatory Follicles	Comments
Control	A	5A	5AF1		
Control	A	5A	5AF2		
Control	A	5A	5AF3		
Control	A	5A	5AF4		
Control	B	5B	5BF1		
Control	B	5B	5BF2		
Control	B	5B	5BF3		
Control	B	5B	5BF4		
Control	C	5C	5CF1		
Control	C	5C	5CF2		
Control	C	5C	5CF3		
Control	C	5C	5CF4		
Control	D	5D	5DF1		
Control	D	5D	5DF2		
Control	D	5D	5DF3		
Control	D	5D	5DF4		
14 µg/L	A	4A	4AF1		
14 µg/L	A	4A	4AF2		
14 µg/L	A	4A	4AF3		
14 µg/L	A	4A	4AF4		
14 µg/L	B	4B	4BF1		
14 µg/L	B	4B	4BF2		
14 µg/L	B	4B	4BF3		
14 µg/L	B	4B	4BF4		
14 µg/L	C	4C	4CF1		
14 µg/L	C	4C	4CF2		
14 µg/L	C	4C	4CF3		
14 µg/L	C	4C	4CF4		
14 µg/L	D	4D	4DF1		
14 µg/L	D	4D	4DF2		
14 µg/L	D	4D	4DF3		
14 µg/L	D	4D	4DF4		
35 µg/L	A	3A	3AF1		
35 µg/L	A	3A	3AF2		
35 µg/L	A	3A	3AF3		
35 µg/L	A	3A	3AF4		
35 µg/L	B	3B	3BF1		
35 µg/L	B	3B	3BF2		
35 µg/L	B	3B	3BF3		
35 µg/L	B	3B	3BF4		
35 µg/L	C	3C	3CF1		
35 µg/L	C	3C	3CF2		
35 µg/L	C	3C	3CF3		
35 µg/L	C	3C	3CF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Decreased Post-ovulatory Follicles	Comments
35 µg/L	D	3D	3DF1		
35 µg/L	D	3D	3DF2		
35 µg/L	D	3D	3DF3		
35 µg/L	D	3D	3DF4		
88 µg/L	A	2A	2AF1		
88 µg/L	A	2A	2AF2		
88 µg/L	A	2A	2AF3		
88 µg/L	A	2A	2AF4		
88 µg/L	B	2B	2BF1		
88 µg/L	B	2B	2BF2		
88 µg/L	B	2B	2BF3		
88 µg/L	B	2B	2BF4		
88 µg/L	C	2C	2CF1		
88 µg/L	C	2C	2CF2		
88 µg/L	C	2C	2CF3		
88 µg/L	C	2C	2CF4		
88 µg/L	D	2D	2DF1		
88 µg/L	D	2D	2DF2		
88 µg/L	D	2D	2DF3		
88 µg/L	D	2D	2DF4		
220 µg/L	A	1A	1AF1		
220 µg/L	A	1A	1AF2		
220 µg/L	A	1A	1AF3		
220 µg/L	A	1A	1AF4		
220 µg/L	B	1B	1BF1		
220 µg/L	B	1B	1BF2		
220 µg/L	B	1B	1BF3		
220 µg/L	B	1B	1BF4		
220 µg/L	C	1C	1CF1		
220 µg/L	C	1C	1CF2		
220 µg/L	C	1C	1CF3		
220 µg/L	C	1C	1CF4		
220 µg/L	D	1D	1DF1		
220 µg/L	D	1D	1DF2		
220 µg/L	D	1D	1DF3		
220 µg/L	D	1D	1DF4		

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Not remarkable	Infection, Fungi	Oocyte Atresia, Increased
Control	A	5A	5AF1	X		
Control	A	5A	5AF2			
Control	A	5A	5AF3			
Control	A	5A	5AF4			
Control	B	5B	5BF1	X		
Control	B	5B	5BF2			
Control	B	5B	5BF3			
Control	B	5B	5BF4			
Control	C	5C	5CF1			
Control	C	5C	5CF2			
Control	C	5C	5CF3	X	2	
Control	C	5C	5CF4			
Control	D	5D	5DF1	X		
Control	D	5D	5DF2			
Control	D	5D	5DF3			
Control	D	5D	5DF4			
14 µg/L	A	4A	4AF1			
14 µg/L	A	4A	4AF2			
14 µg/L	A	4A	4AF3			
14 µg/L	A	4A	4AF4			
14 µg/L	B	4B	4BF1			
14 µg/L	B	4B	4BF2			
14 µg/L	B	4B	4BF3			1
14 µg/L	B	4B	4BF4			
14 µg/L	C	4C	4CF1			
14 µg/L	C	4C	4CF2			
14 µg/L	C	4C	4CF3			
14 µg/L	C	4C	4CF4			
14 µg/L	D	4D	4DF1			
14 µg/L	D	4D	4DF2			
14 µg/L	D	4D	4DF3			
14 µg/L	D	4D	4DF4			
35 µg/L	A	3A	3AF1			
35 µg/L	A	3A	3AF2			1
35 µg/L	A	3A	3AF3			
35 µg/L	A	3A	3AF4			
35 µg/L	B	3B	3BF1			
35 µg/L	B	3B	3BF2			
35 µg/L	B	3B	3BF3			
35 µg/L	B	3B	3BF4			
35 µg/L	C	3C	3CF1			
35 µg/L	C	3C	3CF2			
35 µg/L	C	3C	3CF3			
35 µg/L	C	3C	3CF4			1

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Not remarkable	Infection, Fungi	Oocyte Atresia, Increased
35 µg/L	D	3D	3DF1			
35 µg/L	D	3D	3DF2			
35 µg/L	D	3D	3DF3			
35 µg/L	D	3D	3DF4			
88 µg/L	A	2A	2AF1			
88 µg/L	A	2A	2AF2			
88 µg/L	A	2A	2AF3			
88 µg/L	A	2A	2AF4			
88 µg/L	B	2B	2BF1			2
88 µg/L	B	2B	2BF2			
88 µg/L	B	2B	2BF3			
88 µg/L	B	2B	2BF4			
88 µg/L	C	2C	2CF1			1
88 µg/L	C	2C	2CF2			
88 µg/L	C	2C	2CF3			
88 µg/L	C	2C	2CF4			
88 µg/L	D	2D	2DF1			
88 µg/L	D	2D	2DF2			
88 µg/L	D	2D	2DF3			
88 µg/L	D	2D	2DF4			1
220 µg/L	A	1A	1AF1	X		
220 µg/L	A	1A	1AF2			1
220 µg/L	A	1A	1AF3			
220 µg/L	A	1A	1AF4			
220 µg/L	B	1B	1BF1			3
220 µg/L	B	1B	1BF2			
220 µg/L	B	1B	1BF3	X		
220 µg/L	B	1B	1BF4			2
220 µg/L	C	1C	1CF1			
220 µg/L	C	1C	1CF2	X		
220 µg/L	C	1C	1CF3			
220 µg/L	C	1C	1CF4			
220 µg/L	D	1D	1DF1			
220 µg/L	D	1D	1DF2	X		3
220 µg/L	D	1D	1DF3			
220 µg/L	D	1D	1DF4			2

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Proteinaceous Fluid, Intravascular/Interstitial
Control	A	5A	5AF1	
Control	A	5A	5AF2	
Control	A	5A	5AF3	
Control	A	5A	5AF4	
Control	B	5B	5BF1	
Control	B	5B	5BF2	
Control	B	5B	5BF3	
Control	B	5B	5BF4	
Control	C	5C	5CF1	
Control	C	5C	5CF2	
Control	C	5C	5CF3	
Control	C	5C	5CF4	
Control	D	5D	5DF1	
Control	D	5D	5DF2	
Control	D	5D	5DF3	
Control	D	5D	5DF4	
14 µg/L	A	4A	4AF1	
14 µg/L	A	4A	4AF2	
14 µg/L	A	4A	4AF3	
14 µg/L	A	4A	4AF4	
14 µg/L	B	4B	4BF1	
14 µg/L	B	4B	4BF2	
14 µg/L	B	4B	4BF3	
14 µg/L	B	4B	4BF4	
14 µg/L	C	4C	4CF1	
14 µg/L	C	4C	4CF2	
14 µg/L	C	4C	4CF3	
14 µg/L	C	4C	4CF4	
14 µg/L	D	4D	4DF1	
14 µg/L	D	4D	4DF2	
14 µg/L	D	4D	4DF3	
14 µg/L	D	4D	4DF4	
35 µg/L	A	3A	3AF1	
35 µg/L	A	3A	3AF2	
35 µg/L	A	3A	3AF3	
35 µg/L	A	3A	3AF4	
35 µg/L	B	3B	3BF1	
35 µg/L	B	3B	3BF2	
35 µg/L	B	3B	3BF3	
35 µg/L	B	3B	3BF4	
35 µg/L	C	3C	3CF1	
35 µg/L	C	3C	3CF2	
35 µg/L	C	3C	3CF3	
35 µg/L	C	3C	3CF4	

Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Proteinaceous Fluid, Intravascular/Interstitial
35 µg/L	D	3D	3DF1	
35 µg/L	D	3D	3DF2	
35 µg/L	D	3D	3DF3	
35 µg/L	D	3D	3DF4	
88 µg/L	A	2A	2AF1	
88 µg/L	A	2A	2AF2	
88 µg/L	A	2A	2AF3	
88 µg/L	A	2A	2AF4	
88 µg/L	B	2B	2BF1	
88 µg/L	B	2B	2BF2	
88 µg/L	B	2B	2BF3	
88 µg/L	B	2B	2BF4	
88 µg/L	C	2C	2CF1	
88 µg/L	C	2C	2CF2	
88 µg/L	C	2C	2CF3	
88 µg/L	C	2C	2CF4	
88 µg/L	D	2D	2DF1	
88 µg/L	D	2D	2DF2	
88 µg/L	D	2D	2DF3	
88 µg/L	D	2D	2DF4	
220 µg/L	A	1A	1AF1	
220 µg/L	A	1A	1AF2	
220 µg/L	A	1A	1AF3	
220 µg/L	A	1A	1AF4	
220 µg/L	B	1B	1BF1	2
220 µg/L	B	1B	1BF2	
220 µg/L	B	1B	1BF3	
220 µg/L	B	1B	1BF4	
220 µg/L	C	1C	1CF1	
220 µg/L	C	1C	1CF2	
220 µg/L	C	1C	1CF3	
220 µg/L	C	1C	1CF4	
220 µg/L	D	1D	1DF1	
220 µg/L	D	1D	1DF2	2
220 µg/L	D	1D	1DF3	
220 µg/L	D	1D	1DF4	

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Clinical Chemistry (Day21)

	A	B	C	D	E	F	G	H	I	J	K
1	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Vitellogenin (ng/mL plasma)	17-β estradiol (µg/L plasma)	Testosterone (µg/L plasma)	Vitellogenin LOQ (ng/mL plasma)	17β-estradiol LOQ (µg/L plasma)	Testosterone LOQ (µg/L plasma)
2	Control	A	5A	5AM1	M	74	2.80E-01	4.77E-01	9.91	2.00E-01	1.00E-01
3	Control	A	5A	5AM2	M	96	2.19E+00	3.34E-01	9.91	2.00E-01	1.00E-01
4	Control	A	5A	5AF1	F	1510784	1.05E+01	4.65E-01	9.91	2.00E-01	1.00E-01
5	Control	A	5A	5AF2	F	379911	2.67E+01	2.46E+00	9.91	2.00E-01	1.00E-01
6	Control	A	5A	5AF3	F	693397	9.33E+00	3.45E+00	9.91	2.00E-01	1.00E-01
7	Control	A	5A	5AF4	F	2315908	1.11E+01	5.75E-01	9.91	2.00E-01	1.00E-01
8	Control	B	5B	5BM1	M	239	9.67E-01	6.01E-01	2.00	2.00E-01	1.00E-01
9	Control	B	5B	5BM2	M	541	2.21E-01	2.77E+00	2.00	2.00E-01	1.00E-01
10	Control	B	5B	5BF1	F	1137663	1.27E+01	8.52E-01	2.00	2.00E-01	1.00E-01
11	Control	B	5B	5BF2	F	324195	7.25E+00	1.22E+00	2.00	2.00E-01	1.00E-01
12	Control	B	5B	5BF3	F	1624150	5.69E+00	7.14E-01	2.00	2.00E-01	1.00E-01
13	Control	B	5B	5BF4	F	3209687	2.55E+00	1.02E+00	2.00	2.00E-01	1.00E-01
14	Control	C	5C	5CM1	M	116	4.11E-01	1.31E+00	1.76	2.00E-01	1.00E-01
15	Control	C	5C	5CM2	M	83	1.07E+00	1.32E+00	1.76	2.00E-01	1.00E-01
16	Control	C	5C	5CF1	F	199267	1.09E+01	2.98E+00	1.76	2.00E-01	1.00E-01
17	Control	C	5C	5CF2	F	1432604	1.23E+00	2.43E-01	1.76	2.00E-01	1.00E-01
18	Control	C	5C	5CF3	F	1676660	3.22E+00	9.69E-01	1.76	2.00E-01	1.00E-01
19	Control	C	5C	5CF4	F	508603	3.11E+01	1.72E+00	1.76	2.00E-01	1.00E-01
20	Control	D	5D	5DM1	M	19	7.09E-01	8.46E-01	2.28	2.00E-01	1.00E-01
21	Control	D	5D	5DM2	M	141	1.13E+00	1.26E+00	2.28	2.00E-01	1.00E-01
22	Control	D	5D	5DF1	F	146378	1.02E+02	1.09E+02	2.28	2.00E-01	1.00E-01
23	Control	D	5D	5DF2	F	1982261	8.41E+00	1.78E+00	2.28	2.00E-01	1.00E-01
24	Control	D	5D	5DF3	F	7112278	9.94E+00	7.73E+00	2.28	2.00E-01	1.00E-01
25	Control	D	5D	5DF4	F	45976	5.96E+00	1.75E+00	2.28	2.00E-01	1.00E-01
26	14 µg/L	A	4A	4AM1	M	7457	3.58E-01	3.19E-01	9.91	2.00E-01	1.00E-01
27	14 µg/L	A	4A	4AM2	M	200663	2.95E-01	7.11E-01	9.91	2.00E-01	1.00E-01
28	14 µg/L	A	4A	4AF1	F	7224869	1.74E+01	1.07E+00	9.91	2.00E-01	1.00E-01
29	14 µg/L	A	4A	4AF2	F	2118787	3.54E+00	1.92E+00	9.91	2.00E-01	1.00E-01
30	14 µg/L	A	4A	4AF3	F	518388	9.69E+00	5.06E-01	9.91	2.00E-01	1.00E-01
31	14 µg/L	A	4A	4AF4	F	NS	NS	NS	NA	NA	NA
32	14 µg/L	B	4B	4BM1	M	105	7.32E-01	1.39E+00	8.19	2.00E-01	1.00E-01
33	14 µg/L	B	4B	4BM2	M	2205	2.90E-01	1.93E+00	8.19	2.00E-01	1.00E-01
34	14 µg/L	B	4B	4BF1	F	NR	3.77E+01	1.96E+00	8.19	2.00E-01	1.00E-01
35	14 µg/L	B	4B	4BF2	F	914438	8.04E+00	4.83E+00	8.19	2.00E-01	1.00E-01
36	14 µg/L	B	4B	4BF3	F	782500	4.62E+00	5.98E-01	8.19	2.00E-01	1.00E-01
37	14 µg/L	B	4B	4BF4	F	NS	NS	NS	NA	NA	NA
38	14 µg/L	C	4C	4CM1	M	16476	5.75E-02	1.70E+00	1.65	2.00E-01	1.00E-01
39	14 µg/L	C	4C	4CM2	M	50	7.33E-02	5.22E-01	1.65	2.00E-01	1.00E-01
40	14 µg/L	C	4C	4CF1	F	2999904	5.53E+01	1.63E+00	1.65	2.00E-01	1.00E-01
41	14 µg/L	C	4C	4CF2	F	5924987	1.69E+01	3.31E+00	1.65	2.00E-01	1.00E-01
42	14 µg/L	C	4C	4CF3	F	2757284	9.81E+00	5.22E+00	1.65	2.00E-01	1.00E-01
43	14 µg/L	C	4C	4CF4	F	3471635	7.76E+00	5.30E-01	1.65	2.00E-01	1.00E-01

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Clinical Chemistry (Day21)

	A	B	C	D	E	F	G	H	I	J	K
1	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Vitellogenin (ng/mL plasma)	17-β estradiol (µg/L plasma)	Testosterone (µg/L plasma)	Vitellogenin LOQ (ng/mL plasma)	17β-estradiol LOQ (µg/L plasma)	Testosterone LOQ (µg/L plasma)
44	14 µg/L	D	4D	4DM1	M	175	5.75E-02	1.19E+00	1.45	2.00E-01	1.00E-01
45	14 µg/L	D	4D	4DM2	M	5.12	1.95E-01	7.09E-01	1.45	2.00E-01	1.00E-01
46	14 µg/L	D	4D	4DF1	F	2031107	4.81E+00	3.53E+00	1.45	2.00E-01	1.00E-01
47	14 µg/L	D	4D	4DF2	F	2872179	1.07E+01	1.41E+00	1.45	2.00E-01	1.00E-01
48	14 µg/L	D	4D	4DF3	F	1601666	6.66E+00	3.64E+00	1.45	2.00E-01	1.00E-01
49	14 µg/L	D	4D	4DF4	F	7965098	1.88E+01	4.09E+00	1.45	2.00E-01	1.00E-01
50	35 µg/L	A	3A	3AM1	M	1380	4.24E-01	2.91E-01	4.58	1.25E-01	1.00E-01
51	35 µg/L	A	3A	3AM2	M	2126	3.91E-01	2.87E-01	4.58	1.25E-01	1.00E-01
52	35 µg/L	A	3A	3AF1	F	2526567	1.61E+00	5.83E-01	4.58	1.25E-01	1.00E-01
53	35 µg/L	A	3A	3AF2	F	1432	2.08E+00	2.91E-01	4.58	1.25E-01	1.00E-01
54	35 µg/L	A	3A	3AF3	F	226783	3.83E+00	1.53E+00	4.58	1.25E-01	1.00E-01
55	35 µg/L	A	3A	3AF4	F	NS	NA	NA	NA	1.25E-01	NA
56	35 µg/L	B	3B	3BM1	M	70721	1.13E-01	5.34E-01	1.76	1.25E-01	1.00E-01
57	35 µg/L	B	3B	3BM2	M	10686	6.87E-01	3.04E+00	1.76	1.25E-01	1.00E-01
58	35 µg/L	B	3B	3BF1	F	3424898	1.14E+01	5.32E+00	1.76	1.25E-01	1.00E-01
59	35 µg/L	B	3B	3BF2	F	359471	3.59E+00	1.39E+00	1.76	1.25E-01	1.00E-01
60	35 µg/L	B	3B	3BF3	F	1533921	8.32E+00	5.31E-01	1.76	1.25E-01	1.00E-01
61	35 µg/L	B	3B	3BF4	F	2512918	2.93E+00	5.17E-01	1.76	1.25E-01	1.00E-01
62	35 µg/L	C	3C	3CM1	M	1474	4.70E-01	3.36E+00	1.45	1.25E-01	1.00E-01
63	35 µg/L	C	3C	3CM2	M	16675	5.58E+00	9.20E+00	1.45	1.25E-01	1.00E-01
64	35 µg/L	C	3C	3CF1	F	494666	3.11E+00	2.96E+00	1.45	1.25E-01	1.00E-01
65	35 µg/L	C	3C	3CF2	F	42295354	3.39E+00	4.22E-01	1.45	1.25E-01	1.00E-01
66	35 µg/L	C	3C	3CF3	F	425426	3.62E+01	1.30E+01	1.45	1.25E-01	1.00E-01
67	35 µg/L	C	3C	3CF4	F	57076	1.69E+00	7.05E-01	1.45	1.25E-01	1.00E-01
68	35 µg/L	D	3D	3DM1	M	32	3.59E-02	1.01E+00	2.37	1.25E-01	1.00E-01
69	35 µg/L	D	3D	3DM2	M	72	5.52E-01	6.93E-01	2.37	1.25E-01	1.00E-01
70	35 µg/L	D	3D	3DF1	F	1564103	NR	NR	2.37	1.25E-01	NA
71	35 µg/L	D	3D	3DF2	F	8993854	2.64E+00	4.27E-01	2.37	1.25E-01	1.00E-01
72	35 µg/L	D	3D	3DF3	F	146452	5.32E+00	8.43E-01	2.37	1.25E-01	1.00E-01
73	35 µg/L	D	3D	3DF4	F	1506085	2.43E+01	9.95E-01	2.37	1.25E-01	1.00E-01
74	88 µg/L	A	2A	2AM1	M	5779529	5.75E-02	4.86E+00	4.58	2.00E-01	1.00E-01
75	88 µg/L	A	2A	2AM2	M	16311566	1.37E-01	3.37E-01	4.58	2.00E-01	1.00E-01
76	88 µg/L	A	2A	2AF1	F	196323	3.47E+00	4.17E-01	4.58	2.00E-01	1.00E-01
77	88 µg/L	A	2A	2AF2	F	7809050	1.10E+01	6.79E+00	4.58	2.00E-01	1.00E-01
78	88 µg/L	A	2A	2AF3	F	2164638	1.45E+01	4.08E+00	4.58	2.00E-01	1.00E-01
79	88 µg/L	A	2A	2AF4	F	NS	NS	NS	NA	NA	NA
80	88 µg/L	B	2B	2BM1	M	10716174	2.00E-01	1.00E-01	2.00	2.00E-01	1.00E-01
81	88 µg/L	B	2B	2BM2	M	4663628	1.43E-01	4.22E-01	2.00	2.00E-01	1.00E-01
82	88 µg/L	B	2B	2BF1	F	514875	1.83E+00	8.12E-01	2.00	2.00E-01	1.00E-01
83	88 µg/L	B	2B	2BF2	F	381775	3.63E+00	3.79E+00	2.00	2.00E-01	1.00E-01
84	88 µg/L	B	2B	2BF3	F	223828	1.92E+00	1.16E+00	2.00	2.00E-01	1.00E-01
85	88 µg/L	B	2B	2BF4	F	3099313	1.08E+00	1.17E+00	2.00	2.00E-01	1.00E-01

I:\Toxicology\1. Ecotox Study Data\Battelle\2-ethyl paraben\6120 FHM FSTRA\13784.6120 Biologica Data DESTS.xlsx Clinical Chemistry (Day21)

	A	B	C	D	E	F	G	H	I	J	K
1	Name of Treatment Group	Replicate ID	Tank ID	Day 21 Animal ID	Sex (M or F)	Vitellogenin (ng/mL plasma)	17-β estradiol (µg/L plasma)	Testosterone (µg/L plasma)	Vitellogenin LOQ (ng/mL plasma)	17β-estradiol LOQ (µg/L plasma)	Testosterone LOQ (µg/L plasma)
86	88 µg/L	C	2C	2CM1	M	5942258	1.64E-01	3.87E+00	2.28	2.00E-01	1.00E-01
87	88 µg/L	C	2C	2CM2	M	23557855	2.70E-01	1.18E-01	2.28	2.00E-01	1.00E-01
88	88 µg/L	C	2C	2CF1	F	413625	1.04E+00	1.00E-01	2.28	2.00E-01	1.00E-01
89	88 µg/L	C	2C	2CF2	F	626163	1.32E+00	2.99E-01	2.28	2.00E-01	1.00E-01
90	88 µg/L	C	2C	2CF3	F	1307521	3.55E+00	9.33E-01	2.28	2.00E-01	1.00E-01
91	88 µg/L	C	2C	2CF4	F	NR	7.64E+00	2.00E+00	2.28	2.00E-01	1.00E-01
92	88 µg/L	D	2D	2DM1	M	436751	1.08E+00	7.23E-01	10.41	2.00E-01	1.00E-01
93	88 µg/L	D	2D	2DM2	M	426055	1.38E+00	3.68E+00	10.41	2.00E-01	1.00E-01
94	88 µg/L	D	2D	2DF1	F	48035	1.89E+01	4.45E+00	10.41	2.00E-01	1.00E-01
95	88 µg/L	D	2D	2DF2	F	306698	6.24E+00	2.50E+00	10.41	2.00E-01	1.00E-01
96	88 µg/L	D	2D	2DF3	F	103562	2.23E+00	8.86E+00	10.41	2.00E-01	1.00E-01
97	88 µg/L	D	2D	2DF4	F	18811	2.80E+00	1.11E+00	10.41	2.00E-01	1.00E-01
98	220 µg/L	A	1A	1AM1	M	ADL	7.46E-01	8.93E-01	2.36	2.00E-01	1.00E-01
99	220 µg/L	A	1A	1AM2	M	24186650	6.70E-01	1.63E-01	2.36	2.00E-01	1.00E-01
100	220 µg/L	A	1A	1AF1	F	478327	2.62E+01	2.39E+00	2.36	2.00E-01	1.00E-01
101	220 µg/L	A	1A	1AF2	F	780451	8.04E+00	3.23E+00	2.36	2.00E-01	1.00E-01
102	220 µg/L	A	1A	1AF3	F	932641	3.57E+01	4.07E+00	2.36	2.00E-01	1.00E-01
103	220 µg/L	A	1A	1AF4	F	190132	6.02E+00	4.69E+00	2.36	2.00E-01	1.00E-01
104	220 µg/L	B	1B	1BM1	M	ADL	9.89E-01	2.20E-01	1.65	2.00E-01	1.00E-01
105	220 µg/L	B	1B	1BM2	M	ADL	4.24E+00	4.89E-01	1.65	2.00E-01	1.00E-01
106	220 µg/L	B	1B	1BF1	F	1060936	1.27E+01	4.68E+00	1.65	2.00E-01	1.00E-01
107	220 µg/L	B	1B	1BF2	F	944799	7.90E+00	3.49E+00	1.65	2.00E-01	1.00E-01
108	220 µg/L	B	1B	1BF3	F	1125236	7.91E+00	4.16E+00	1.65	2.00E-01	1.00E-01
109	220 µg/L	B	1B	1BF4	F	NR	9.48E+00	2.34E+00	1.65	2.00E-01	1.00E-01
110	220 µg/L	C	1C	1CM1	M	12354030	6.64E-01	1.59E+00	10.41	2.00E-01	1.00E-01
111	220 µg/L	C	1C	1CM2	M	14202274	4.81E+00	1.59E+01	10.41	2.00E-01	1.00E-01
112	220 µg/L	C	1C	1CF1	F	114243	3.64E+00	1.44E+00	10.41	2.00E-01	1.00E-01
113	220 µg/L	C	1C	1CF2	F	6482298	1.45E+01	2.00E+00	10.41	2.00E-01	1.00E-01
114	220 µg/L	C	1C	1CF3	F	NR	9.78E+00	3.23E+00	10.41	2.00E-01	1.00E-01
115	220 µg/L	C	1C	1CF4	F	NR	1.23E+00	2.43E-01	10.41	2.00E-01	1.00E-01
116	220 µg/L	D	1D	1DM1	M	ADL	1.29E+00	1.02E-01	8.19	2.00E-01	1.00E-01
117	220 µg/L	D	1D	1DM2	M	ADL	4.28E+00	1.00E+00	8.19	2.00E-01	1.00E-01
118	220 µg/L	D	1D	1DF1	F	3.99	1.31E+00	2.43E-01	8.19	2.00E-01	1.00E-01
119	220 µg/L	D	1D	1DF2	F	2916568	8.48E+00	1.59E+00	8.19	2.00E-01	1.00E-01
120	220 µg/L	D	1D	1DF3	F	6951133	8.57E+00	8.69E+00	8.19	2.00E-01	1.00E-01
121	220 µg/L	D	1D	1DF4	F	1403551	3.50E+00	8.01E-01	8.19	2.00E-01	1.00E-01
122	All values in red are values that were below detectable limits. The values are therefore 1/2 the LOQ.										
123	NR = not reportable										
124	NS = no sample										
125	ADL = above detection limit										
126	NA = not applicable										

Worksheet Title	DEST Term	Equivolent Term in Supporting Document
Pre-Exposure Observations	Tank ID Pre-test Day	Spawning Group ID Pre-exposure Day
Survival and Reproduction (Daily)	Name of Treatment Group	Nominal Concentration
Individual Observations (Day 21)	Name of Treatment Group Body Weight (g) Body Length (mm)	Nominal Concentration Wet Weight (g) Total Length (mm)
Nuptial Tubercle Scores (Day 21)	Name of Treatment Group	Nominal Concentration
Clinical Signs (Daily)	Name of treatment group Secondary sex characteristics and other Tubercles Present Fatpad Present Color Bars Present Territorial Agression	Nominal Conc. Observations TP Present FP Present CB Present TA Present
Male Gonadal (Histo)pathology	Name of treatment group	Nominal concentration
Female Gonadal (Histo)pathology	Name of treatment group	Nominal concentration
Clinical Chemistry (Day 21)	Vitellogenin (ng/mL plasma)	Conc. X Dil.

APPENDIX 10 – IODIDE ANALYSIS

Report Number: 94739 GALBRAITH LABORATORIES, INC Report Date: 2017-01-04

Sample: 6128-28Jul16
Lab ID: 2016-D-2519 **Received:** 2016-12-15

Analysis	Method	Result	Basis	Sample Amount Used	Date (Time)
I1D: Iodide by ICP-MS	GLI Procedure ME-71	5.28 µg/L	As Received	10 mL	2017-01-04

Sample: 6128-18Aug16
Lab ID: 2016-D-2520 **Received:** 2016-12-15

Analysis	Method	Result	Basis	Sample Amount Used	Date (Time)
I1D: Iodide by ICP-MS	GLI Procedure ME-71	4.29 µg/L	As Received	10 mL	2017-01-04

Sample: 6126-7Sep16
Lab ID: 2016-D-2521 **Received:** 2016-12-15

Analysis	Method	Result	Basis	Sample Amount Used	Date (Time)
I1D: Iodide by ICP-MS	GLI Procedure ME-71	3.20 µg/L	As Received	10 mL	2017-01-04

Sample: 6126-28Sep16
Lab ID: 2016-D-2522 **Received:** 2016-12-15

Analysis	Method	Result	Basis	Sample Amount Used	Date (Time)
I1D: Iodide by ICP-MS	GLI Procedure ME-71	4.77 µg/L	As Received	10 mL	2017-01-04

Sample: 6120-22Sep16
Lab ID: 2016-D-2523 **Received:** 2016-12-15

Analysis	Method	Result	Basis	Sample Amount Used	Date (Time)
I1D: Iodide by ICP-MS	GLI Procedure ME-71	9.48 µg/L	As Received	10 mL	2017-01-04

Sample: 6120-13Oct16
Lab ID: 2016-D-2524 **Received:** 2016-12-15

Analysis	Method	Result	Basis	Sample Amount Used	Date (Time)
I1D: Iodide by ICP-MS	GLI Procedure ME-71	4.90 µg/L	As Received	10 mL	2017-01-04

APPENDIX 11 – HISTOPATHOLOGY

Experimental Pathology Laboratories, Inc.

SMITHERS VISCIENT
STUDY NUMBER 13784.6120
EPL PROJECT NUMBER 237-080

PROTOCOL FOR CONDUCTING A SHORT-TERM REPRODUCTION ASSAY WITH FATHEAD MINNOW (*PIMEPHALES PROMELAS*) FOLLOWING OPPTS 890.1350 AND OECD 229 GUIDELINES

PATHOLOGY REPORT

Submitted by:

Experimental Pathology Laboratories, Inc.

Street Address:
45600 Terminal Drive
Sterling, VA 20166

Mailing Address:
P.O. Box 169
Sterling, VA 20167-0169

(703) 471-7060

Original submitted to:

Test Facility
Smither's Viscient
Wareham, MA 02571-1037

Copy submitted to:

Sponsor Representative
Battelle Memorial
Columbus, OH 43201

March 23, 2018

FINAL REPORT

EPL® Experimental Pathology Laboratories, Inc.

TABLE OF CONTENTS

	Page
PATHOLOGY NARRATIVE	1
COMPLIANCE STATEMENT	7
QUALITY ASSURANCE FINAL CERTIFICATION	8
SUMMARY INCIDENCE TABLES	
Males	I-1
Females	I-2
HISTOPATHOLOGY INCIDENCE TABLES	
Males	II-1
Females	II-4
APPENDIX A: GRADING CRITERIA	A-1
APPENDIX B: FIGURES AND LEGENDS	B-1

PATHOLOGY NARRATIVE

Experimental Pathology Laboratories, Inc.

SMITHERS VISCIENT
STUDY NUMBER 13784.6120
EPL PROJECT NUMBER 237-080

PROTOCOL FOR CONDUCTING A SHORT-TERM REPRODUCTION ASSAY WITH FATHEAD MINNOW (*PIMEPHALES PROMELAS*) FOLLOWING OPPTS 890.1350 AND OECD 229 GUIDELINES

PATHOLOGY NARRATIVE

INTRODUCTION

This study involved the performance of a short-term assay with the fathead minnow (*Pimephales promelas*) that was based on the protocol developed by Ankley et al. (2001), and meets the requirements of the OPPTS 890.1350 (US EPA, 2009) and OECD 229 (OECD, 2009) guidelines for the fish short-term reproduction assay (FSTRA). The purpose of this test was to assess the potential for the test substance, 2-ethylhexyl paraben (2-EHHB) to interact with the endocrine system in fathead minnows exposed under flow-through conditions.

The experimental design is presented in Table 1.

Table 1. Study Design

2-EHHB Treatment Group (µg/L)	Number of Males Examined per Treatment Group	Number of Females Examined per Treatment Group
0 (control)	8	16
14	8	14
35	8	15
88	8	15
220	8	16
TOTALS	40	76

METHODS

Gonads from 116 fathead minnows were submitted to Experimental Pathology Laboratories, Inc. (EPL®), Sterling, Virginia, for histologic processing and pathologic evaluation. Additionally, the carcasses of four male and four female fish randomly selected from controls and each treatment concentration (one male and one female from each of the four replicate tanks) were submitted for thyroid histopathology processing. At EPL, the gonad samples were processed for paraffin embedding on an automated processor using routine methods. Each set of gonads was embedded horizontal to the long axis to allow for longitudinal sectioning. For each block, excess paraffin was trimmed away until the first section was obtained at the point at which approximately half the gonad tissue had been removed and the size of the section was maximized. The second and third sections were acquired at 50 micron intervals, respectively, following the first section. All three of the 4-6 micron-thick sections were placed on one or two glass slides.

To obtain thyroid gland specimens, each head was excised from the carcass and decalcified using a commercial formic acid/EDTA decalcification solution. The heads were processed routinely for paraffin embedding using an automated tissue processor. Each head was embedded so that the caudal surface would be microtomed first, and five transverse serial sections (each ~5 microns thick) were obtained at the level of the gill arch ventral to the eye. All histologic sections were stained with hematoxylin and eosin, and mounted with a glass coverslip using an appropriate permanent mounting medium.

The pathologist evaluated all sections using brightfield microscopy, with awareness of the treatment group status of individual animals. Histopathologic findings were scored for severity according to the following grading system: P = present, Grade 1 = minimal, Grade 2 = mild, Grade 3 = moderate, and Grade 4 = severe. A modified scoring system based on OPPTS 890.1350 was used for gonadal stage scoring, and a modified grading scale based on OCSPP 890.2200 was used for the grading of increased oocyte atresia and post-ovulatory follicles (Appendix A). Gonad phenotype scoring was performed as follows: Phenotype 1 = entirely testicular tissue, Phenotype 2 = predominantly testicular tissue, Phenotype 3 = approximately equal testicular and ovarian components,

Phenotype 4 = predominantly ovarian tissue, and Phenotype 5 = entirely ovarian tissue. Results were recorded into a proprietary electronic data recording system, and were subsequently converted into spreadsheet format. Individual animal results were tabulated in the Histopathology Incidence Table (HIT) and summarized in the Summary Incidence Table (SIT).

RESULTS

Findings associated with 2-EHHB exposure occurred in the gonads of male and female fish and included: intravascular and interstitial proteinaceous fluid in the testes of 88 and 220 µg/L dose group males (minimal to mild), and in the ovaries of 220 µg/L dose group females (mild); increased mean testis stage scores, especially in males of the 88 and 220 µg/L dose groups; a dose-dependent increase in the prevalence and severity of oocyte atresia (minimal to moderate); decreased post-ovulatory follicles in females of the 88 and 220 µg/L dose groups; and shifts in ovarian stage scores in females, especially in females of the 88 and 220 µg/L dose groups. Representative examples of various findings are illustrated in Figures 1-7. Although mean ovarian stage score were comparable among control and 2-EHHB-exposed females, there was a shift away from females in the spawning or recently-spawned stages (i.e., Stages 4.0, 2.0, and 2.5) toward stages of oocyte maturation (i.e., Stages 3.0 and 3.5). That shift is consistent with the decreased post-ovulatory follicles observed in females of the 88 and 220 µg/L dose groups, because Stage 3.0 and 3.5 ovaries typically contain few, if any, of those residual structures.

An unusual finding that was not related to 2-EHHB exposure was the presence of multiple testicular oocytes in the collecting ducts of a control male, Animal No. 5AM2 (Fig. 8).

There were no thyroid gland findings in this study.

The relatively few additional diagnoses recorded in this study were background types of findings observed typically in fathead minnow gonads, and were present in comparable numbers of control and 2-EHHB-treated fish generally at low prevalence and severity; thus none were considered to be treatment-related.

Table 2. Prevalence and Severity of Selected Findings in the Testes of Male Fathead Minnows					
2-EHHB Dosage (µg/L)	0 (control)	14	35	88	220
n	8	8	8	8	8
Proteinaceous Fluid, Intravascular/Interstitial	0	0	0	2	8
minimal	-	-	-	2	2
mild	-	-	-	-	6
Testis Stage Score					
Stage 1.5	5	4	4	3	1
Stage 2.0	3	2	2	2	5
Stage 2.5	-	1	2	2	1
Stage 3.0	-	1	-	1	1
Mean Testis Stage Score	1.7	1.9	1.9	2.1	2.1

Table 3. Prevalence and Severity of Selected Findings in the Ovaries of Female Fathead Minnows					
2-EHHB Dosage (µg/L)	0 (control)	14	35	88	220
n	16	14	15	15	16
Proteinaceous Fluid, Intravascular/Interstitial	0	0	0	0	2
mild	-	-	-	-	2
Increased Oocyte Atresia	0	1	2	3	5
minimal	-	1	2	2	1
mild	-	-	-	1	2
moderate	-	-	-	-	2
Post-ovulatory Follicles	10	9	11	3	7
Grade 1[a]	1	1	1	1	3
Grade 2	2	1	1	-	2
Grade 3	7	7	9	2	2
Ovary Stage Score					
Stage 1.0	-	-	-	1	-
Stage 2.0	2	2	-	-	-
Stage 2.5	3	3	5	1	1
Stage 3.0	2	3	3	4	5
Stage 3.5	6	5	4	8	10
Stage 4.0	3	1	3	1	-
Mean Ovary Stage Score	3.2	3.0	3.2	3.2	3.3

[a] Grades are listed rather than severity scores because post-ovulatory follicles are normal structures.

DISCUSSION

The presence of proteinaceous intravascular and interstitial fluid in the gonads of male and female fathead minnows is consistent with increased hepatic vitellogenin production as might be induced by substances with estrogenic activity. A number of *in vitro* and *in vivo* studies have demonstrated estrogen-like effects in fish exposed to various parabens, including vitellogenin induction (Pedersen et al., 2000; Inui et al., 2003; Yamamoto et al., 2011). Although not specific for enhanced estrogenic activity, increased oocyte (follicular) atresia is another commonly reported effect of xenoestrogen exposure (Dietrich and Krieger, 2009).

CONCLUSION AND SUMMARY

Findings associated with 2-EHHB exposure included intravascular and interstitial proteinaceous fluid in the testes of 88 and 220 µg/L dose group males and in the ovaries of 220 µg/L dose group females; increased mean testis stage scores especially in males of the 88 and 220 µg/L dose groups; a dose-dependent increase in the prevalence and severity of oocyte atresia in 2-EHHB-exposed females; decreased post-ovulatory follicles in females of the 88 and 220 µg/L dose groups; and shifts in ovarian stage scores in females, especially in the 88 and 220 µg/L dose groups. Overall, clear treatment-related effects were observed primarily in fish of the 88 and 220 µg/L dose groups. There were no thyroid gland findings in this study.

23 March 2018

JEFFREY C. WOLF, DVM, Diplomate, ACVP Date
Senior Pathologist

JCW/cb

REFERENCES

Dietrich DR, Krieger HO (2009) Histological Analysis of Endocrine Disruptive Effects in Small Laboratory Fish. John Wiley & Sons, Inc., Hoboken, NJ, 121 pp.

Inui M, Adachi T, Takenaka S, Inui H, Nakazawa M, Ueda M, Watanabe H, Mori C, Iguchi T, Miyatake K (2003) Effect of UV screens and preservatives on vitellogenin and choriogenin production in male medaka (*Oryzias latipes*). Toxicology, 194(1-2):43-50.

OECD. (2009) Fish Short Term Reproduction Assay. OECD Guidelines for the Testing of Chemicals: Test No. 229. Adopted 7 September 2009, 40 pp.

Pedersen KL, Pedersen SN, Christiansen LB, Korsgaard B, Bjerregaard P (2000) The preservatives ethyl-, propyl- and butylparaben are oestrogenic in an in vivo fish assay. Pharmacol Toxicol. Mar;86(3):110-113.

US EPA. (2009) Endocrine Disruptor Screening Program Test Guidelines: OPPTS 890.1350 Fish Short-Term Reproduction Assay.

US EPA. (2015) Endocrine Disruptor Screening Program Test Guidelines: OCSPP 890.2200: Medaka Extended One Generation Reproductive Test (MEOGRT).

Yamamoto H, Tamura I, Hirata Y, Kato J, Kagota K, Katsuki S, Yamamoto A, Kagami Y, Tatarazako N (2011) Aquatic toxicity and ecological risk assessment of seven parabens: Individual and additive approach. Sci Total Environ, 410-411:102-111.

EPL® Experimental Pathology Laboratories, Inc.

COMPLIANCE STATEMENT

Test Facility	Smithers Viscient	EPL Principal Investigator	Dr. Jeffrey C. Wolf
Study No.	13784.6120	EPL Pathologist	Dr. Jeffrey C. Wolf
Species	*Pimephales promelas* (fathead minnow)	EPL Project Number	237-080
Study Title	Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines		
Test Article	2-ethylhexyl paraben		

The Histopathology portions of the above-referenced study were conducted in compliance with the Good Laboratory Practice regulations of the Environmental Protection Agency as stipulated by 40 CFR Part 160 (FIFRA); and all applicable amendments.

EPL Principal Investigator

23 March 2018
Date

EPL® Experimental Pathology Laboratories, Inc.

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: Protocol for Conducting a Short-Term Reproduction Assay with Fathead Minnow (*Pimephales promelas*) Following OPPTS 890.1350 and OECD 229 Guidelines

Client Study: 13784.6120 EPL Principal Investigator: Dr. Jeffrey C. Wolf

EPL Project Number: 237-080 EPL Pathologist: Dr. Jeffrey C. Wolf

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Principal Investigator and Management are indicated below.

Area Inspected	Dates	
	Inspection	Reporting
EPL Project Sheets	12/14,15/16; 2/7/17	12/15/16; 2/7/17
Project Setup	1/3/17; 1/9/17	1/3/17; 1/9/17
In-Process - Checkout	2/13/17	2/13/17
Data Review	2/14/17; 2/16/17	2/14/17; 2/16/17
Draft Pathology Report	4/19/17; 4/23/17	4/20/17; 4/24/17
Final Pathology Report	3/6,7,8,9,23/18	3/23/18

Date reported to Study Director/Management 3/23/18

Date of last annual facility inspection 12/17

Barbara Merrell
EPL Quality Assurance Unit

23 Mar 2018
Date

Form No. 6-2 (10/10/16)

SUMMARY INCIDENCE TABLES

TERMINAL SACRIFICE

SUMMARY INCIDENCE TABLE

13784.6120
Terminal Sacrifice
Male *Pimephales promelas*
(fathead minnow)

	GROUP Control	GROUP 14ug/L	GROUP 35ug/L	GROUP 88ug/L	GROUP 220ug/L	
TESTIS (NO. EXAMINED)	(8)	(8)	(8)	(8)	(8)	
Collecting Ducts, Mineralization	3	2	2		5	
Collecting Ducts, Oocyte	1					
Inflammation, Granulomatous					1	
Interstitial Cells, Decreased					1	
Interstitial Cells, Increased	4		1	1	1	
Mineralization	1	1			2	
Phenotype 1	7	8	8	8	8	
Phenotype 2	1					
Proteinaceous Fluid, Intravascular/Interstitial				2	8	
Stage 1.5	5	4	4	3	1	
Stage 2	3	2	2	2	5	
Stage 2.5		1	2	2	1	
Stage 3		1		1	1	
Testicular Degeneration	2	2	1	1	1	
THYROID (NO. EXAMINED)	(4)				(4)	

SUMMARY INCIDENCE TABLE

13784.6120
Terminal Sacrifice
Female *Pimephales promelas*
(fathead minnow)

	GROUP Control	GROUP 14ug/L	GROUP 35ug/L	GROUP 88ug/L	GROUP 220ug/L	
OVARY (NO. EXAMINED)	(16)	(14)	(15)	(15)	(16)	
Infection, Fungi	1					
Inflammation, Granulomatous	1					
Oocyte Atresia, Increased		1	2	3	5	
Ooplasm Dysgenesis	1	1	2			
Phenotype 5	16	14	15	15	16	
Post-ovulatory Follicles	10	9	11	3	7	
Proteinaceous Fluid,						
Intravascular/Interstitial					2	
Stage 1				1		
Stage 2	2	2				
Stage 2.5	3	3	5	1	1	
Stage 3	2	3	3	4	5	
Stage 3.5	6	5	4	8	10	
Stage 4	3	1	3	1		
THYROID (NO. EXAMINED)	(4)				(4)	

HISTOPATHOLOGY INCIDENCE TABLES

TERMINAL SACRIFICE

HISTOPATHOLOGY INCIDENCE TABLE

13784.6120 Terminal Sacrifice Male *Pimephales promelas* (fathead minnow)	GROUP Control									GROUP 14ug/L									
ANIMAL	5AM1	5AM2	5BM1	5BM2	5CM1	5CM2	5DM1	5DM2		4AM1	4AM2	4BM1	4BM2	4CM1	4CM2	4DM1	4DM2			
TESTIS																				
Collecting Ducts, Mineralization		2		2		2						1				2				
Collecting Ducts, Oocyte		2																		
Inflammation, Granulomatous																				
Interstitial Cells, Decreased																				
Interstitial Cells, Increased			2	1	1			2												
Mineralization				2								1								
Phenotype 1	P		P	P	P	P	P	P		P	P	P	P	P	P	P	P			
Phenotype 2		P																		
Proteinaceous Fluid, Intravascular/Interstitial																				
Stage 1.5	P	P		P	P			P		P			P		P		P			
Stage 2			P			P	P					P		P						
Stage 2.5																P				
Stage 3											P									
Testicular Degeneration	1					1						1			1					
THYROID	X		X		X		X													

Key: X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

13784.6120 Terminal Sacrifice Male *Pimephales promelas* (fathead minnow)		GROUP 35ug/L									GROUP 88ug/L										
	ANIMAL	3AM1	3AM2	3BM1	3BM2	3CM1	3CM2	3DM1	3DM2		2AM1	2AM2	2BM1	2BM2	2CM1	2CM2	2DM1	2DM2			
TESTIS																					
Collecting Ducts, Mineralization				1					1												
Collecting Ducts, Oocyte																					
Inflammation, Granulomatous																					
Interstitial Cells, Decreased																					
Interstitial Cells, Increased				2												2					
Mineralization																					
Phenotype 1		P	P	P	P	P	P	P	P		P	P	P	P	P	P	P	P			
Phenotype 2																					
Proteinaceous Fluid, Intravascular/Interstitial																1	1				
Stage 1.5		P	P					P	P				P			P	P				
Stage 2					P		P				P							P			
Stage 2.5				P		P						P		P							
Stage 3															P						
Testicular Degeneration			1													1					

Key: X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

13784.6120
Terminal Sacrifice
Male *Pimephales promelas*
(fathead minnow)

	GROUP 220ug/L																			
ANIMAL	1AM1	1AM2	1BM1	1BM2	1CM1	1CM2	1DM1	1DM2												
TESTIS																				
Collecting Ducts, Mineralization	1	1	1		1		2													
Collecting Ducts, Oocyte																				
Inflammation, Granulomatous						2														
Interstitial Cells, Decreased	1																			
Interstitial Cells, Increased						2														
Mineralization		1	1																	
Phenotype 1	P	P	P	P	P	P	P	P												
Phenotype 2																				
Proteinaceous Fluid, Intravascular/Interstitial	2	2	2	2	1	1	2	2												
Stage 1.5			P																	
Stage 2	P	P			P		P	P												
Stage 2.5				P																
Stage 3						P														
Testicular Degeneration						2														
THYROID	X			X	X			X												

Key: X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

GROUP
Control

13784.6120
Terminal Sacrifice
Female *Pimephales promelas*
(fathead minnow)

ANIMAL	5AF1	5AF2	5AF3	5AF4	5BF1	5BF2	5BF3	5BF4	5CF1	5CF2	5CF3	5CF4	5DF1	5DF2	5DF3	5DF4				
OVARY																				
Infection, Fungi											2									
Inflammation, Granulomatous											2									
Oocyte Atresia, Increased																				
Ooplasm Dysgenesis																2				
Phenotype 5	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P				
Post-ovulatory Follicles	2	2		3	3		3			3	3	1	3			3				
Proteinaceous Fluid, Intravascular/Interstitial																				
Stage 1																				
Stage 2	P			P																
Stage 2.5					P								P			P				
Stage 3		P										P								
Stage 3.5			P			P		P	P					P	P					
Stage 4							P			P	P									
THYROID	X				X						X		X							

Key: X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

GROUP
14ug/L

13784.6120
Terminal Sacrifice
Female *Pimephales promelas*
(fathead minnow)

ANIMAL	4AF1	4AF2	4AF3	4BF1	4BF2	4BF3	4CF1	4CF2	4CF3	4CF4	4DF1	4DF2	4DF3	4DF4						
OVARY																				
Infection, Fungi																				
Inflammation, Granulomatous																				
Oocyte Atresia, Increased						1														
Ooplasm Dysgenesis				2																
Phenotype 5	P	P	P	P	P	P	P	P	P	P	P	P	P	P						
Post-ovulatory Follicles	3		3	3		3	3	2		3	3	1								
Proteinaceous Fluid, Intravascular/Interstitial																				
Stage 1																				
Stage 2			P			P														
Stage 2.5	P			P							P									
Stage 3							P	P				P								
Stage 3.5		P			P				P				P	P						
Stage 4										P										

Key: X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

GROUP
35ug/L

13784.6120
Terminal Sacrifice
Female *Pimephales promelas*
(fathead minnow)

ANIMAL	3AF1	3AF2	3AF3	3BF1	3BF2	3BF3	3BF4	3CF1	3CF2	3CF3	3CF4	3DF1	3DF2	3DF3	3DF4					
OVARY																				
Infection, Fungi																				
Inflammation, Granulomatous																				
Oocyte Atresia, Increased		1									1									
Ooplasm Dysgenesis							1		2											
Phenotype 5	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P					
Post-ovulatory Follicles	3	3			2	3	3		3		3	3	3	3	1					
Proteinaceous Fluid, Intravascular/Interstitial																				
Stage 1																				
Stage 2																				
Stage 2.5						P	P		P		P			P						
Stage 3	P				P										P					
Stage 3.5			P	P				P		P										
Stage 4		P										P	P							

Key: X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

13784.6120
Terminal Sacrifice
Female *Pimephales promelas*
(fathead minnow)

	GROUP 88ug/L																			
ANIMAL	2AF1	2AF2	2AF3	2BF1	2BF2	2BF3	2BF4	2CF1	2CF2	2CF3	2CF4	2DF1	2DF2	2DF3	2DF4					
OVARY																				
Infection, Fungi																				
Inflammation, Granulomatous																				
Oocyte Atresia, Increased				2				1							1					
Ooplasm Dysgenesis																				
Phenotype 5	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P					
Post-ovulatory Follicles	3						1		3											
Proteinaceous Fluid, Intravascular/Interstitial																				
Stage 1								P												
Stage 2																				
Stage 2.5	P																			
Stage 3			P	P								P			P					
Stage 3.5		P			P	P	P			P	P		P	P						
Stage 4									P											

Key: X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

GROUP
220ug/L

13784.6120
Terminal Sacrifice
Female *Pimephales promelas*
(fathead minnow)

ANIMAL	1AF1	1AF2	1AF3	1AF4	1BF1	1BF2	1BF3	1BF4	1CF1	1CF2	1CF3	1CF4	1DF1	1DF2	1DF3	1DF4				
OVARY																				
Infection, Fungi																				
Inflammation, Granulomatous																				
Oocyte Atresia, Increased		1			3			2						3		2				
Ooplasm Dysgenesis																				
Phenotype 5	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P				
Post-ovulatory Follicles	1	1	3					2		1		2	3							
Proteinaceous Fluid, Intravascular/Interstitial					2									2						
Stage 1																				
Stage 2																				
Stage 2.5													P							
Stage 3	P	P	P							P		P								
Stage 3.5				P	P	P	P	P	P		P			P	P	P				
Stage 4																				
THYROID	X						X			X				X						

Key: X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=mild 3=moderate 4=severe
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

APPENDIX A
GRADING CRITERIA

Appendix A

Grade	Oocyte Atresia, Increased
1	Average of 3-5 atretic follicles per ovary section
2	Average of 6-9 atretic follicles per ovary section
3	Average > 9 atretic follicles per ovary section, but less than the vast majority
4	Vast majority of oocytes are atretic

Grade	Post-ovulatory Follicles (POF)
1	Average of 3-5 POF per ovary section
2	Average of 6-9 POF per ovary section
3	Average > 9 POF per ovary section, but less than vast majority
4	Vast majority of follicles are POF

Stage	Testes	Ovaries
Juvenile	Entirely spermatogonia	Entirely oogonia
0.0	Entirely immature phases (spermatogonia to spermatids) with no spermatozoa	Entirely perinucleolar oocytes
1.0	Immature phases predominate (areawise); many tubules still do not have an obvious lumen; small numbers of spermatozoa are evident; the germinal epithelium is thicker than in Stage 1.5	Vast majority (e.g., >90%, areawise) of follicles are pre-vitellogenic, predominantly perinucleolar through cortical alveolar phases
1.5	Immature phases predominate; more spermatozoa are evident as compared to Stage 1.0; tubule lumina are larger than in Stage 1; the germinal epithelium is thinner than Stage 1.0 and thicker than Stage 2.0.	Majority of follicles are pre-vitellogenic, predominantly perinucleolar through cortical alveolar; some mid-vitellogenic follicles may be present
2.0	Spermatocytes, spermatids, and spermatozoa are present in roughly equal proportions; the germinal epithelium is thinner than Stage 1.5 but thicker than Stage 2.5; most tubule lumina do not coalesce	At least half of observed follicles are early and mid-vitellogenic phases
2.5	All stages may be observed, however, mature sperm predominate; the germinal epithelium is thinner than Stage 2.0 but thicker than Stage 3.0; many tubule lumina are coalescing	Most follicles are early and mid vitellogenic, but some late vitellogenic follicles are present also
3.0	All stages may be observed, however, mature sperm predominate; the germinal epithelium is thinner than it is during Stage 2.5, appearing as small segments of spermatocysts and most tubule lumina are coalescing	Majority of developing follicles are late vitellogenic phase
3.5	All stages may be observed, however, mature sperm predominate; the germinal epithelium is barely apparent (often a single cell layer or less), and large coalescing tubule lumina are filled with spermatozoa	Majority of developing follicles are late vitellogenic phase; a few follicles are larger than in Stage 3 and have large yolk granules (mature / spawning follicles)
4.0	Not used	Many follicles are mature / spawning phase, with large yolk granules

APPENDIX B
FIGURES AND LEGENDS

Experimental Pathology Laboratories, Inc.

Battelle Memorial Institute Study Number 13784.6120

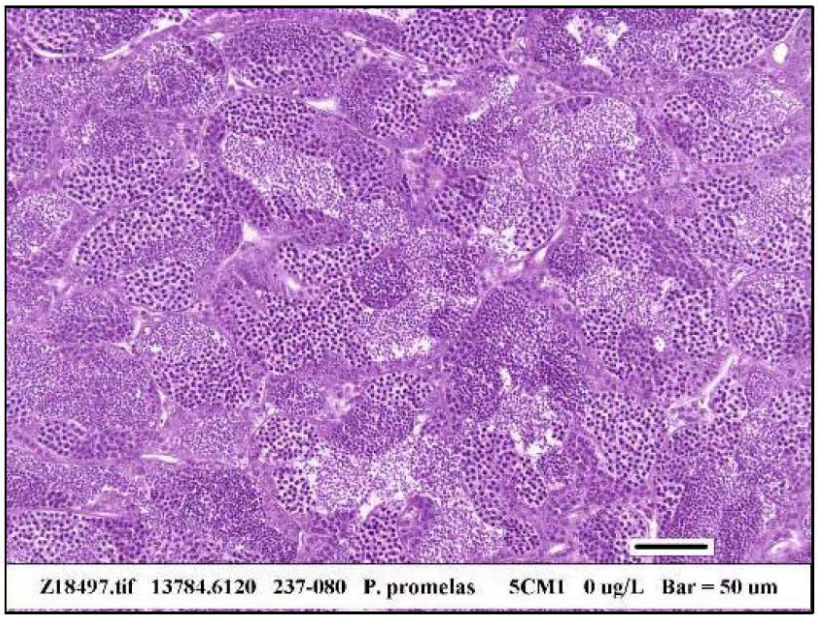


Figure 1 (Z18497). Testis from a control male, Animal No. 5CM1. Provided for comparison. H&E.

Experimental Pathology Laboratories, Inc.

Battelle Memorial Institute Study Number 13784.6120

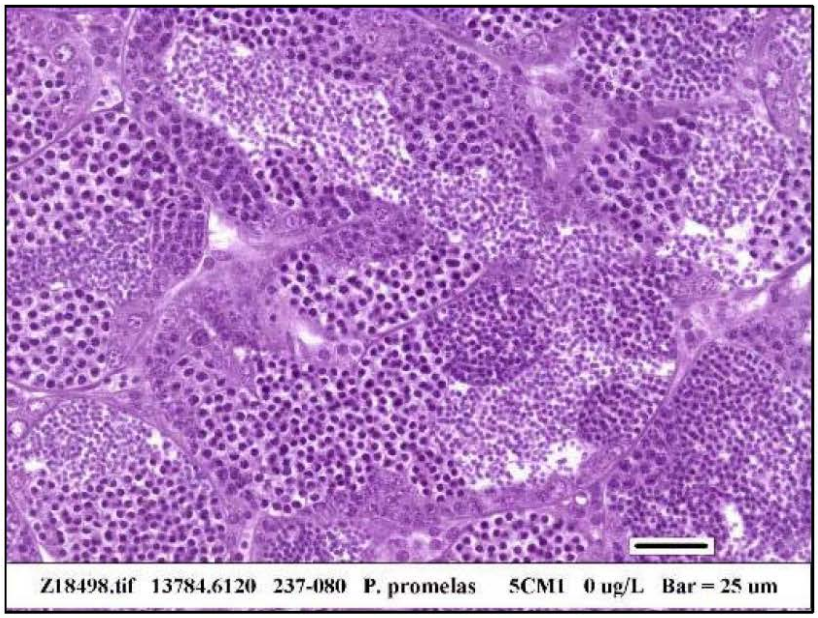


Figure 2 (Z18498). Higher magnification of testis from previous figure. H&E.

Experimental Pathology Laboratories, Inc.

Battelle Memorial Institute Study Number 13784.6120

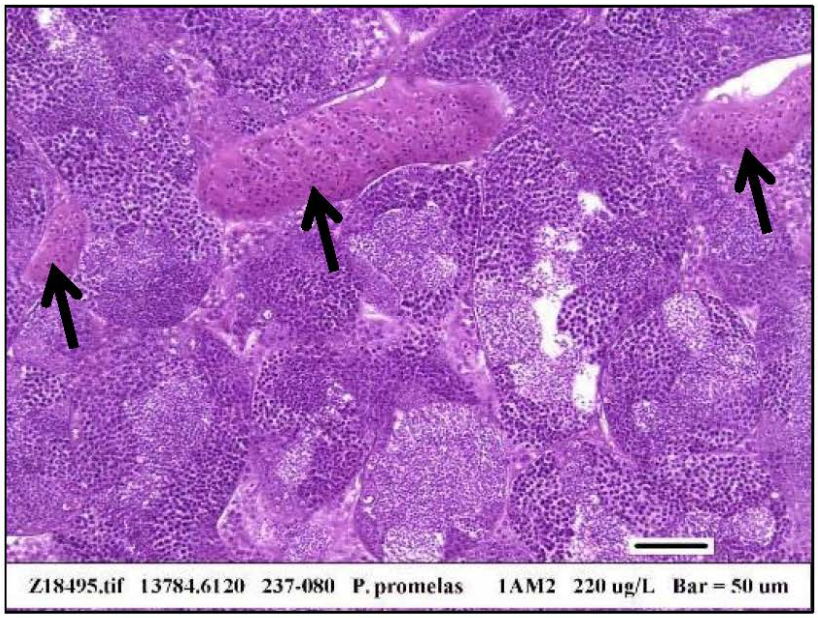


Figure 3 (Z18495). Testis from a male exposed to 220 µg/L 2-EHHB, Animal No. 1AM2. Proteinaceous fluid fills and expands testicular veins (arrows). H&E.

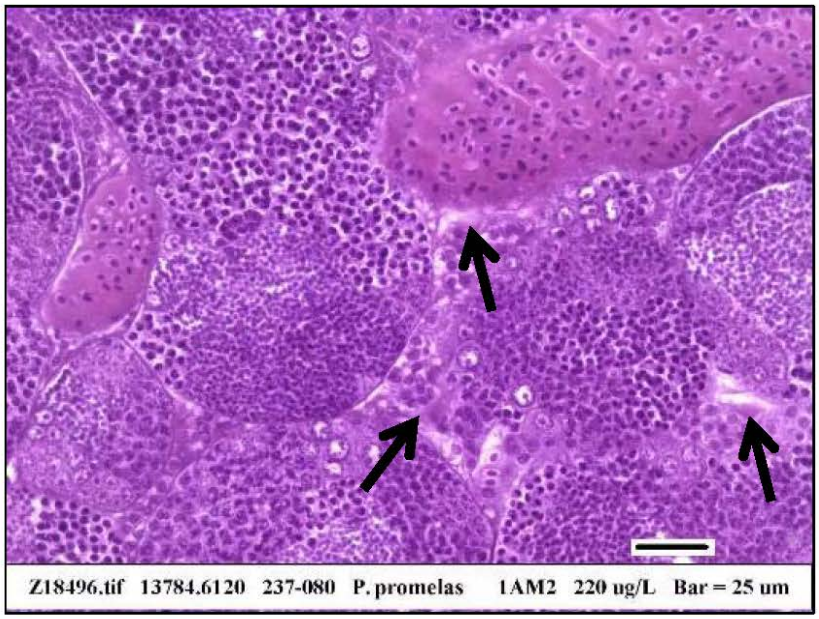


Figure 4 (Z18496). Higher magnification of testis from preceding figure. Although somewhat difficult to visualize in this image (as compared to the actual microscope slide), there are small amounts of proteinaceous fluid within the inter-tubular interstitium (arrows). H&E.

Experimental Pathology Laboratories, Inc.

Battelle Memorial Institute Study Number 13784.6120

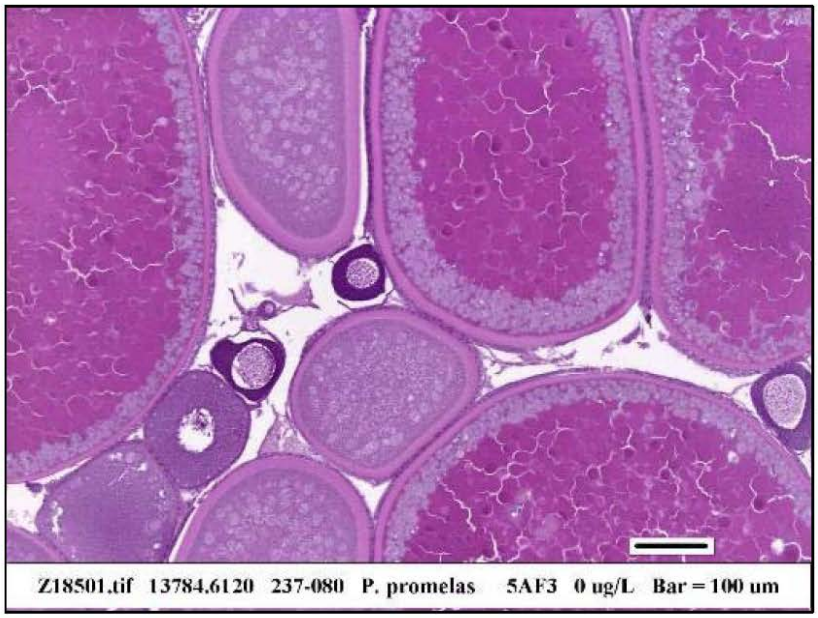


Figure 5 (Z18501). Ovary from a control female, Animal No. 5AF3. Provided for comparison. H&E.

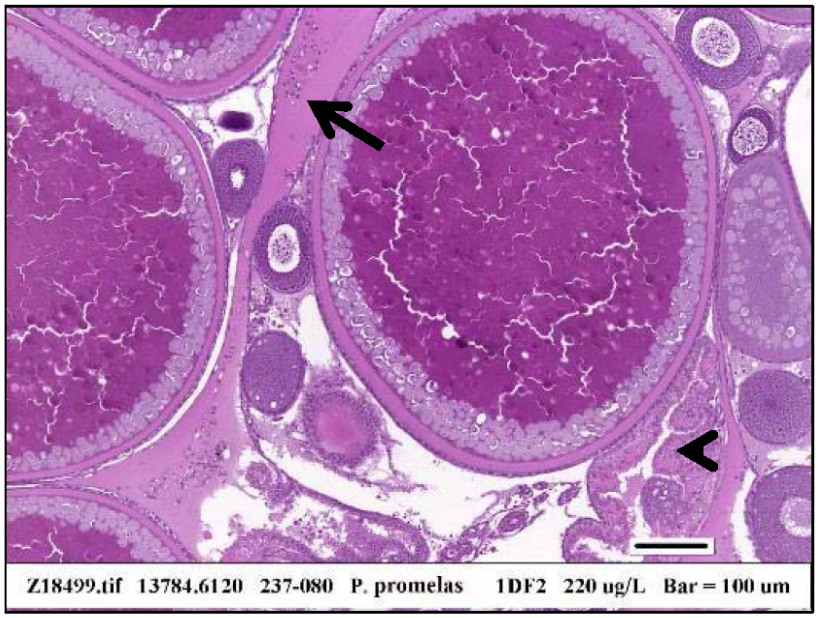


Figure 6 (Z18499). Ovary from a female exposed to 220 µg/L 2-EHHB, Animal No. 1DF2. Interstitial proteinaceous fluid is evident (arrow). However, unlike the situation in the males, in females it can be difficult to distinguish proteinaceous fluid caused by increased plasma vitellogenin from proteinaceous fluid (deteriorating yolk) that leaks from atretic oocytes. An atretic oocyte/follicle is also evident in this image (arrowhead). H&E.

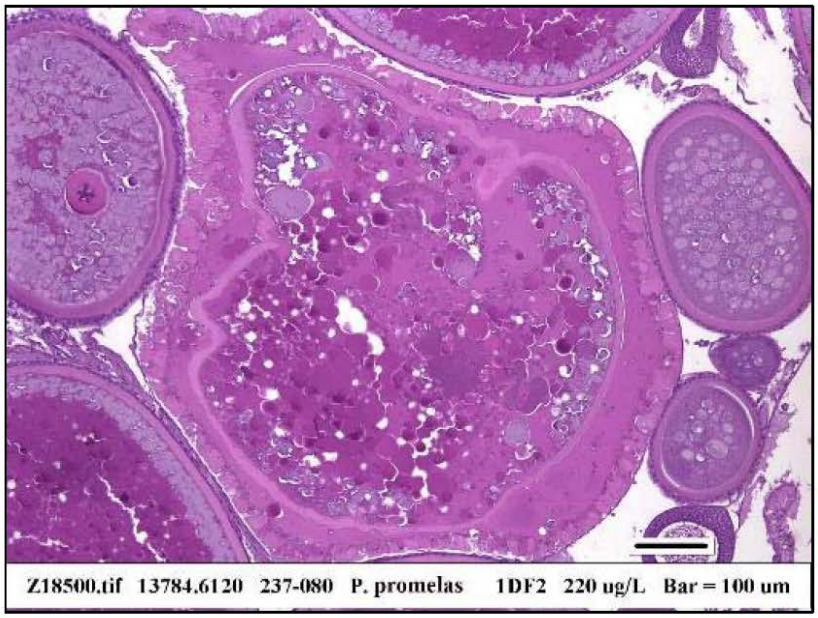


Figure 7 (Z18500). Another area from the same ovary depicted in the prior figure. The center of the image is occupied by a large atretic oocyte. H&E.

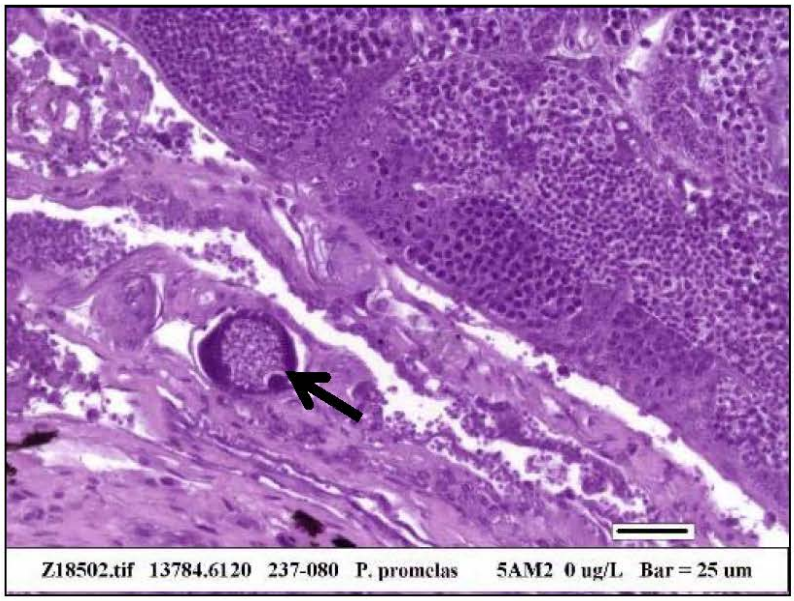


Figure 8 (Z18502). Testis from a control male, Animal No. 5AM2. A perinuclear phase oocyte (arrow) is evident within a collecting duct, which is a highly unusual location for testicular oocytes. This finding was not treatment-related. H&E.