
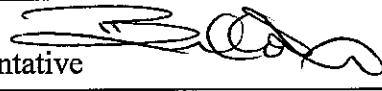


**AMENDMENT TO FINAL REPORT**

Study Number:	2901/001	
Amendment Issue Date:	May 2010	
Amendment Number:	2	
Study Title:	PFH Ammonium Salt: Fish, Early Life Stage Toxicity Test to <i>Oncorhynchus mykiss</i> (Rainbow Trout)	
Authorised By:		
	J Burke Study Director 	Date 28 May 2010
Quality Assurance Audit:		
	H Sarker QA Representative 	Date 28 May 2010
Distribution:	Sponsor	
This amendment to final report contains six pages including this one.		
Documentation		
Section(s) amended and explanation for the change		
<p>Following client request this report amendment is issued to supply additional information to support regulatory submission.</p> <p>Page 1: Title page amended to read 'Amended Final Report 2', issue date May 2010 and number of pages 1 of 67.</p> <p>Page 1: The Study monitor has been changed to Hiroyuki Iwai, D.V.M., M.S. This change has been made as the original study monitor, Dr. Tozuka, has retired from Daikin Industries, Ltd.</p> <p>Page 8: Additional line added to the table of contents detailing Appendix 5.</p> <p>Pages 65 to 67: Additional appendix added, Appendix 5, to document the life stages of the fish in the test.</p> <p>The headers have changed to read 'Amended Final Report 2'</p>		

# Amended Final Report 2

Study Title	PFH Ammonium Salt: Fish, Early Life Stage Toxicity Test to <i>Oncorhynchus mykiss</i> (Rainbow Trout)
Study Guideline	OPPTS Biological Effects Test Guidelines 850.1400 Fish Early-Life Stage Toxicity Test
Authors	J Burke A Scholey
Sponsor	Planning Dept. Chemical Division Daikin Industries, LTD. Umeda Center Bldg., 2-4-12, Nakazaki-Nishi Kita-Ku, Osaka, 530-8323 Japan
Study Monitor	Hiroyuki Iwai, D.V.M., M.S.
Test Facility	Covance Laboratories Ltd Otley Road, Harrogate North Yorkshire HG3 1PY UNITED KINGDOM
Study Number	2901/001
Covance Report Number	2901/001-D2149
Report issued	May 2010
Page Number	1 of 67

*All procedures to be carried out on live animals as part of this study will be subject to the provisions of United Kingdom National Law, in particular the Animals (Scientific Procedures) Act 1986, to ensure that minimum suffering and distress is caused to animals on study.*

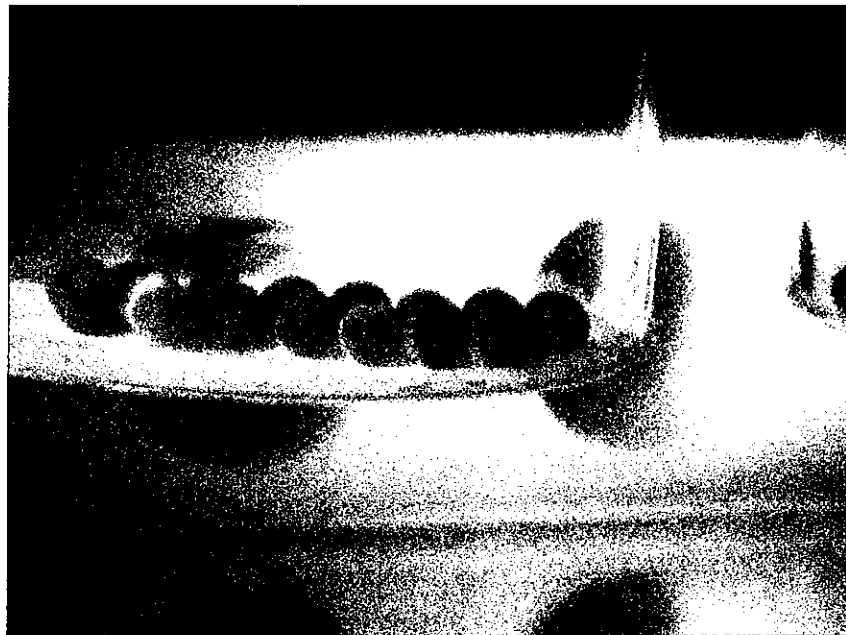
Table 6 Dry Weights of Fish (g) at the End of the Fish, Early-life Stage Test - 28 Days Post-Hatch .....	29
Table 7 Temperature (°C) Measurements during the Fish, Early Life Stage Test .....	33
Table 8 pH Measurements during the Fish, Early Life Stage Test.....	34
Table 9 Dissolved Oxygen (% ASV) Measurements during the Fish, Early Life Stage Test .....	36
Table 10 Dissolved Oxygen (mg/L) Measurements during the Fish, Early Life Stage Test.....	38
Table 11 Water Hardness and Residual Chlorine Measurements in Dilution Water during the Fish, Early Life Stage Test.....	40
<b>FIGURES .....</b>	<b>41</b>
Figure 1 Chromatogram of a 0.02 µg/mL Standard Solution of Perfluorohexanoic acid in hplc mobile phase (Internal Standard of Perfluoroheptanoic acid at 0.01 µg/mL).....	42
Figure 2 Chromatogram of a 0.0002 µg/mL Standard Solution of Perfluorohexanoic acid in HPLC mobile phase (Internal Standard of Perfluoroheptanoic acid at 0.01 µg/mL) .....	42
Figure 3 Chromatogram of a Control Test Water Sample at Day 0.) (Dilution factor = 10).....	43
Figure 4 Chromatogram of a 0.2 mg/L Test Water Sample at Day 0 (Dilution factor = 10) .....	43
Figure 5 Chromatogram of a 21 mg/L Test Water Sample at Day 0 (Dilution factor = 1000) .....	44
Figure 6 Chromatogram of a 0.2 mg/L Test Water Sample at Day 1 (Dilution factor = 100) .....	44
Figure 7 Chromatogram of a 21 mg/L Test Water Sample at Day 1 (Dilution factor =1000) .....	45
Figure 8 Chromatogram of a 0.2 mg/L Test Water Sample at Day -1 Post Hatch (Dilution factor = 100).....	45
Figure 9 Chromatogram of a 21 mg/L Test Water Sample at Day -1 Post Hatch (Dilution factor = 1000).....	46
Figure 10 Chromatogram of a 0.2 mg/L Test Water Sample at Day 0 Post Hatch (Dilution factor = 100).....	46
Figure 11 Chromatogram of a 21 mg/L Test Water Sample at Day 0 Post Hatch (Dilution factor = 1000).....	47
Figure 12 Chromatogram of a Control Test Water Sample at Day 28 Post Hatch (Dilution factor = 100).....	47
Figure 13 Chromatogram of a 0.2 mg/L Test Water Sample at Day 28 Post Hatch (Dilution factor = 100).....	48
Figure 14 Chromatogram of a 21 mg/L Test Water Sample at Day 28 Post Hatch (Dilution factor = 1000).....	48
Figure 15 Chromatogram of a New 3.2 mg/L Stock Solution at Day 0 (Dilution factor = 200)....	49
Figure 16 Chromatogram of a New 356 mg/L Stock Solution at Day 0 (Dilution factor = 20000) .....	49
Figure 17 Chromatogram of an Old 3.2 mg/L Stock Solution at Day 4 (Dilution factor = 200) ...	50
Figure 18 Chromatogram of an Old 356 mg/L Stock Solution at Day 4 (Dilution factor = 20000) .....	50
Figure 19 Typical Calibration Line for Perfluorohexanoic Acid over the range of 0.0002 to 0.02 µg/mL.....	51
<b>APPENDICES.....</b>	<b>52</b>
Appendix 1 Typical Water Characteristics.....	53
Appendix 2 Analytical Procedure .....	55
Appendix 3 Statistical Analysis .....	62
Appendix 4 Protocol Deviations .....	64
Appendix 5 Documentation of Life Stages of <i>Oncorhynchus mykiss</i> during the Early Life Stage Test.....	65

## Appendix 5 Documentation of Life Stages of *Oncorhynchus mykiss* during the Early Life Stage Test

This appendix provides documentation of the progressive life stages of *Oncorhynchus mykiss* and timing of these life stages during the course of the Definitive test conducted between 18 February and 15 April 2008.

Date	Day	Observations	Feed
18/02/2008	Day 0 pre-hatch	Eggs fertilised at 20:20 hrs, addition to test vessels complete by 22:21hrs	-
03/03/2008	Day 14 pre-hatch	Eye spots visible @ 09:35 hrs	-
15/03/2008	Day 26 pre-hatch	Hatching commenced and observed @ 15:15 hrs	-
18/03/2008	Day 0 post-hatch	>90% hatching observed designated Day 0 post hatch @ 16:05	-
28/03/2008	Day 10 post-hatch	Larvae observed at "swim up" and released from chambers into tank @ 14:25	Suspension of ground nutrafry feed in diluent water supplied
29/03/2008	Day 11 post-hatch	-	Suspension of ground nutrafry feed in diluent water supplied
30/03/2008	Day 12 post-hatch	-	Suspension of ground nutrafry feed in diluent water supplied
31/03/2008	Day 13 post-hatch	-	Suspension of ground nutrafry feed in diluent water supplied
01/04/2008	Day 14 post-hatch	Fish in all test vessels (V1-V12) observed feeding and majority swimming towards surface whilst feeding and taking food 17:25	Nutrafry 00 feed added ad-libitum in excess 3 times per day
02/04/2008	Day 15 post-hatch	Fish observed scavenging/searching for food @ 16:15	As above
15/04/2008	Day 28	Test terminated	As above

The following pictures were taken during the definitive test:



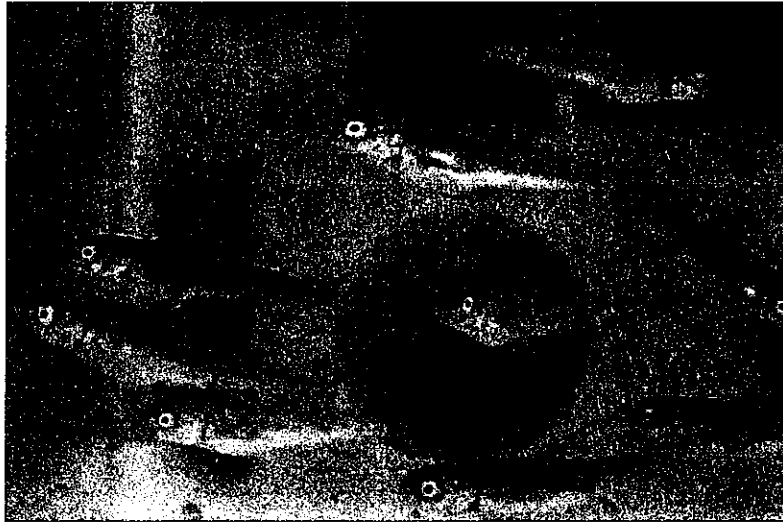
**14 March 2008 (ca Day 25 Pre-hatch)**



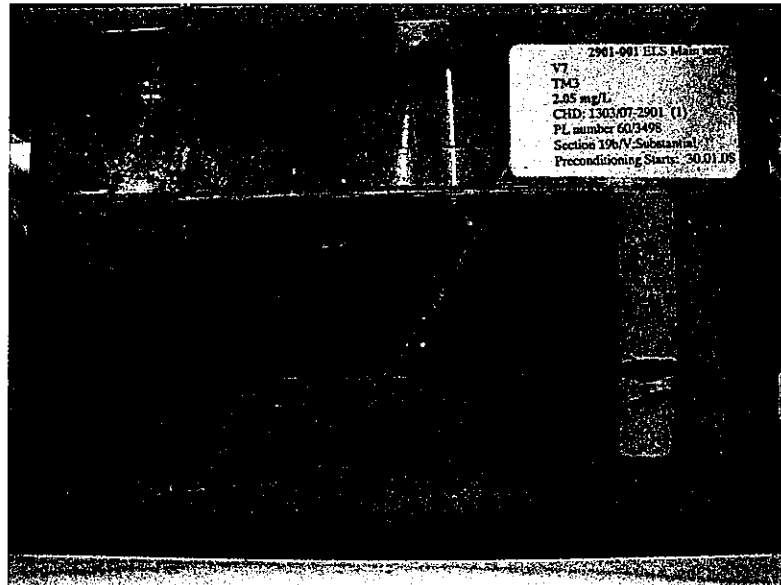
20 March 2008 (*ca* Day 2 post-hatch)



28 March 2008 *ca* Day 10 post-hatch, swim-up observed and released into outer tank



10 April 2008 Day 23 post-hatch



10 April 2008 Day 23 post-hatch