Ovaplant® (Salmon Gonadotropin-releasing Hormone Analogue) Clinical Field Trials - INAD 11-375

2011 Annual Summary Report on the Use of sGnRHa - Ovaplant® in Clinical Field Efficacy Trials

Prepared by:

Bonnie Johnson, Biologist
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, Montana

Summary

Spawning aids such as Ovaplant® (Salmon Gonadotropin-releasing Hormone Analogue, sGnRHa), luteinizing hormone-releasing hormone analogue ($LHRH_a$), human chorionic gonadotropin, and common carp pituitary are routinely used in aquaculture to induce gamete maturation in fish to enhance fish propagation programs. The U.S. Food and Drug Administration has authorized the use of Ovaplant® under the Compassionate Investigational New Animal Drug (INAD) Exemption #11-375 for the purpose of gathering efficacy data to support a new animal drug approval for Ovaplant®. In calendar year 2011 (CY11), 14 trials were conducted under this INAD to evaluate the efficacy of Ovaplant® to induce gamete maturation in a variety of fish species. Trials involved 3,781 treated fish and 112 control fish and were conducted at eight different hatcheries, including three U.S. Fish and Wildlife Service fish hatcheries, three state hatcheries, one private hatchery, and one tribal hatchery during this period. Efficacy was determined by whether or not treated fish produced or yielded more eggs or milt than untreated fish. Overall results of trials conducted during this period indicated that

64% of the trials appeared efficacious, 7% were ineffective, and 29% were characterized as inconclusive.

Introduction

The use of hormones to induce spawning in fish is critical to the success of many federal, state, private, and tribal fisheries programs. A wide variety of programs, including many that involve the restoration of threatened/endangered species, are dependent upon hormone treatment to complete final gamete maturation and ensure successful spawning.

The time of spawning is by its own nature a stressful period for all fish species. Both sexes are undergoing significant changes in physiology, morphology, and behavior (Hoar, 1969). The additional handling of fish required during the spawning process complicates an already delicate situation. This is particularly true for wildstock species that must endure the added stresses of capture, handling, and confinement in an un-natural environment. In fact, with respect to some wildstock species, the stress of capture alone is often sufficient to cause complete reproductive failure unless spawning is induced by hormone treatment. Hormone treatment in a variety of fish species is essential to ensure optimal spawning success.

Studies have shown that final gamete maturation (ovulation and spermiation) in fish can be induced by the administration of a variety of hormones (Donaldson and

Hunter 1983; Goetz 1983). Investigations have found that synthetic analogues of gonadotropin releasing hormones (GnRHa) to be one of the most effective means of inducing final gamete maturation. These compounds, which may be similar to native gonadotropins found in either fish or mammals, are attractive choices as they typically exhibit both high biological activity and low species specificity. Although a number of these analogues are available, the most commonly used analogue for fish culture to date has been luteinizing hormone releasing hormone (LHRHa; Alvarino et al. 1992; Donaldson et al. 1981; Erdahl and McClain 1987; Fitzpatrick et al. 1984; Taranger et al. 1992; and Van der Kraak et al. 1983). Effective treatment has been reported using both injection and pellet implant therapy.

The use of implants that contain GnRH analogues has been evaluated over the last 15 years (Crim et al., 1983a). In early attempts to use implants, peptide was imbedded in cholesterol pellets that contained cellulose to affect release rate (Sherwood et al., 1988). In this system, a 5% carboxymethyl cellulose / 95% cholesterol pellet containing mammalian GnRHa (mGnRHa) released an initial burst of mGnRHa followed by a sustained release of peptide over the next 28 days. Several researchers have demonstrated that these types of implants were capable of inducing maturation in a variety of species including: Atlantic salmon (Crim et al., 1983a; Crim and Glebe, 1984), herring (Carolsfeld et al., 1988), sea bass (Almendras et al., 1988), rainbow trout (Crim et al., 1983b; Crim et al., 1988) and milkfish (Lee et al., 1986; Marte et al., 1988). In all of these studies, mGnRHa was the imbedded peptide that induced maturation either in advance of, or synchronously within, a population.

The inclusion of salmon GnRHa (sGnRHa) instead of mGnRHa in Ovaplant® implants designed for inducing maturation in cultured fish is a logical one. In both in vitro (pituitary fragments or cell cultures) and in vivo studies sGnRHa has been found to be more potent in effect than mGnRHa for many species including: goldfish (Peter et al., 1985, 1987), Atlantic salmon (Crim et al., 1988), rainbow trout (Crim et al., 1988; Weil et al., 1992), winter flounder (Crim et al., 1988) and catfish (Namvongchong et al., 1992b; Schulz et al., 1994). This potency may be attributed to high pituitary binding affinity and gonadotropin hormone (GtH) releasing capacity, even though sGnRH itself may not be an indigenous form for some of the species tested (Schulz et al., 1993). Moreover, sGnRHa produces a sustained level of GtH from pituitary cells with a low therapeutic dose (Peter et al., 1987). Additionally, sGnRHa either as peptide alone or as Ovaprim® (sGnRH + a domperidone, Syndel International, Inc.) has proven to be effective in inducing final gamete maturation in a variety of cultured fish including, but not limited to, chinook salmon (Powell, 1995), coho salmon (Powell et al., 1998), catfish (Namvongchong et al., 1992b; Schulz et al., 1993), and ricefield eel (Tao and Lin, 1993). Furthermore, sGnRHa is an attractive therapy for aquaculture use as it has been shown to be ineffective in mammals (Millar et al., 1993), and has a short half life in fish (Goren et al., 1990; Zohar et al., 1990; Weil et al., 1992). Conversely, mGnRHa is superactive in humans and has a prolonged half-life in fish and water (Sherwood and Harvey, 1986) which potentially could constitute a human safety risk. Collectively, the above-described considerations indicate that sGnRHa (Ovaplant®) is an attractive choice for further evaluation and development as a candidate compound for a new

animal drug approval for use to induce final gamete maturation in a variety of fish species.

Purpose of Report

The purpose of this report is to summarize the results of Ovaplant® field efficacy studies conducted under INAD exemption #11-375 in CY11. We anticipate that data generated in these trials will be used to enhance data in the existing Ovaplant® database established from previous years, and will be considered in the "body of evidence" for the purpose of developing an appropriate label claim for the use of Ovaplant® in aquaculture

Facilities, Materials, and Treatment Procedures

1. Facilities

Field efficacy trials were conducted at eight different fish culture facilities during CY11, including three U.S. Fish and Wildlife Service fish hatcheries, three state hatcheries, one private hatchery, and one tribal hatchery. Water temperature during treatments at the various testing facilities ranged from 45.0 to 80.0°F. Overall mean treatment temperature from all trials was 58.6 °F.

2. Chemical material

Western Chemical Inc. of Ferndale, WA an Aquatic Life Sciences Company was the supplier for all Ovaplant® used in trials conducted during the reporting period.

3. Drug dosages

The Study Protocol authorized the use of up to 250 ug sGnRHa per pellet and administration as a single treatment event only. Drug dosages used by Investigators in CY11 ranged from 10 to 159.78 ug sGnRHa. Male hickory shad received a higher then allowed dosage due to smaller then expected fish size and the pellet size that was used. Fish treated by pellet implant either 1) have been or will be euthanized at the hatchery and properly disposed of; or 2) will remain on station and will not be released.

Fish Species and Sex Treated

1. Fish Species Treated

Field efficacy trials were conducted on six different fish species under INAD #11-375 during the reporting period, including the following two salmonids, three non-salmonids, and one marine non-salmonid species:

Salmonids

fall chinook salmon *Oncorhychus tshawytscha*

steelhead trout *O. mykiss*

Non-salmonids

American shad *Alosa sapidissima*

hickory shad *A. mediocris*

striped bass *Morone saxatilis*

Marine non-salmonid

cobia *Rachycentron canadum*

2. Gender of treated fish

Ovaplant® was used on 1,979 female and 1,802 male fish during the reporting period. Typically, females were treated with spawning hormone to shorten the gamete maturation period (i.e. advance maturation), while males were treated to ensure that sufficient milt would be available for egg fertilization.

Data Collected

1. Primary response variable (Maturation)

The primary response variable for evaluating the effect of Ovaplant® on fish was the percentage of ripe fish following treatment. These percentages reflected the number of female fish that ovulated and the number of male fish that reached active spermiation.

2. Egg development and milt evaluation

Secondary response variables for females included the relative number of eggs that reached the eyed stage and the number hatched. Secondary response

variables for males included the volume of milt (ml) available from individual fish and an evaluation of milt motility (percent motile spermatozoa).

Discussion of Study Results

1. General observations on the efficacy of Ovaplant® to induce gamete maturation in salmonid and non-salmonid fish (Note: Tables 1 & 2 provides summaries of all efficacy trials; Table 3 lists the number of treatment trials, number of fish and species treated, and treatment regimens used; and Table 4 describes all trials conducted during CY11 under INAD #11-375.)

A. Efficacy of Ovaplant® on male fish treated between 10 and 159.78 ug/kg body weight (1 - 2 implants)

Male fish were treated in six trials and implanted 1 or 2 times with Ovaplant® at a dosage between 10 and 159.78 ug/kg body weight (Table 1). During two trials, the Investigators did not evaluate whether treatment induced gamete maturation. In these cases, it's implied that the relative level of gamete maturation was undetermined. The investigators noted fish were tank spawned so individual ripeness could not be determined; however, viable fry were produced. Fish will not be available for human consumption. Below are the treatment regimens used to induce gamete maturation in four fish species treated with Ovaplant® at the dosages described above:

1. Salmonids:

   Ovaplant® was used at 10 ug/kg in one trial involving steelhead trout and were implanted with one pellet implant. No control fish were used. Results showed that there was a 97% spermiation in the treated fish. Treatment appeared efficacious in this trial.

2. Non-salmonids

   Ovaplant® was used at 41 - 159.78 ug/kg in five trials involving American shad, hickory shad, and striped bass and were implanted with 1 - 2 pellet implants. Control fish were used in two trials involving American shad. Results showed that there was an unknown level of spermiation in two trials of treated fish and 100% spermiation in the other three trials involving treated fish; as compared to unknown and no spermiation in the control trials. In the trials where the spermiation was unknown, individual fish were not checked to see if they were ripe after treatment; however, the investigator noted there were fry produced. In two trials males were ripe at the time of treatment. Fish are implanted to ensure spermiating males throughout the spawning process - these trials are characterized as inconclusive. Treatment appeared efficacious in two trials and was characterized as inconclusive in three trials.

Overall, treatment resulted in an unknown spermiation or 97 - 100% spermiation in the male treated fish; as compared to no spermiation or an unknown percent in the

control fish. Treatments appeared efficacious in three trials and was characterized as inconclusive in three trials.

B. Efficacy of Ovaplant® on female fish treated at a dosage between 12.5 and 150 ug/kg body weight (1 - 2 implants)

Female fish were implanted one to two times with Ovaplant® pellets at a dosage between 12.5 and 150 ug/kg body weight (Table 2) in eight different trials. During two trials, the Investigators did not evaluate whether treatment induced gamete maturation. In these cases, it's implied that the relative level of gamete maturation was undetermined. Fish will not be available for human consumption. Below are the treatment regimens used to induce gamete maturation in six fish species treated with Ovaplant® at the dosages described above:

1. Salmonids:

Ovaplant® was used at 12.5 - 40 ug/kg in two trials involving fall chinook salmon and steelhead trout and were implanted with one pellet implant. No control fish were used. Results showed that there was a 97 - 100% ovulation in treated fish. Treatment appeared efficacious in both trials.

2. Non-salmonids

Ovaplant® was used at 39 - 150 ug/kg in five trials involving American shad, hickory shad, and striped bass and were implanted with 1 - 2 pellet implants. Control fish were used in two trials involving American shad.

Results showed that there was an unknown level of ovulation in two trials of treated fish and 64 - 100% ovulation in the other three trials involving treated fish; as compared to no ovulation or an unknown percent of ovulation in the control trials. In the trials where the ovulation was unknown, individual fish were not checked to see if they were ripe after treatment; however, the investigator noted there were fry produced. Treatment appeared efficacious in four trials and was characterized as inconclusive in one trial.

3. Marine non-salmonid

Ovaplant® was used at 25 ug/kg in one trial involving cobia and were implanted with 1 pellet implant. Control fish were not used. Results showed that there was no ovulation in the treated fish. Treatment was not effective in this trial.

Overall, treatment resulted in either an unknown percent ovulation (due to fish not evaluated for ovulation by the Investigator) or a 0 - 100% ovulation in the female treated fish; as compared to no ovulation or an unknown ovulation in the control fish. Treatment appeared efficacious in six trials, ineffective in one trial, and was characterized as inconclusive in one trial.

2. Observed Toxicity

No toxicity or adverse effects relating to Ovaplant® treatments were reported in any of the trials conducted in CY11.

3. Observed Withdrawal Period

The investigators noted that treated fish will not be stocked, released, or harvested for human consumption. All treated fish will ultimately be destroyed.

Current Study Protocol for Ovaplant® INAD #11-375

No changes have occurred to the current study protocol for Ovaplant® INAD #11-375.

Facility Sign-up List

Please see "Table 5. Facilities and Names of Investigators" for facilities that signed-up to participate in the Ovaplant® INAD #11-375 during CY11.

The following facility had Ovaplant on hand during CY11; however, no treatments took place: Marine Finfish Reproduction Center

Correspondence sent to Ovaplant® Participants

Please see the attached correspondence that was sent to all Ovaplant® participants after the AADAP Office received their sign-up form for CY11.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY11 was 3,781. The total number of treated fish since December 15, 2005 is 14,866.

Summary of Study Results

Ovaplant® was used in 14 efficacy trials to induce gamete maturation in six different fish species (n = 3,781 treated fish; 112 untreated control fish) at dosages ranging from 10 - 159.78 ug/kg bw. Ovaplant® was administered as a pellet implant. Fish treated by pellet implant will be euthanized at the hatchery and properly disposed of or will not be released from the facility. Water temperature during treatments ranged from 45.0 - 80.0°F. Overall, results showed that Ovaplant® treatment appeared efficacious in 64% of the trials, ineffective in 7% of the trials, and was inconclusive in 4% of the trials. Data from the CY11 trials indicate that Ovaplant® treatment was efficacious in inducing gamete maturation in a variety of fish species. Although it is anticipated that the majority of future efficacy data collected under INAD #11-375 will also be ancillary data, efforts will be made to improve the quality of data whenever possible.

References

Alvarino, J.M.R., S. Zanuy, F. Prat, M. Carrillo, and E. Mananos. 1992. Stimulation of ovulation and steroid secretion by $LHRH_a$ injection in the sea bass (Dicentrarchus labrax): effect of time of day. Aquaculture. 102: 177-186.

Almendras, J.M., C. Duenas, J. Nicario, N.M. Sherwood, and L.W. Crim. 1988. Sustained hormone release III: Use of gonadotropin-releasing hormone analogues to induce multiple spawnings in the sea bass, *Lates calcarifer*. Aquaculture. 74: 97-111.

Carolsfeld, J., N.M. Sherwood, H. Krieberg, and S.A. Sower. 1988. Induced sexual maturation of herring using GnRH 'quick release' cholesterol pellets. Aquaculture. 70: 169-181.

Crim, L.W., A.M. Sutterlin, D.M. Evans, and C. Weil. 1983a. Accelerated ovulation by pelleted LHRH analogs treatemtn by spring-spawning rainbow trout (*Salmo gairdneri*) held at low temperature. Aquaculture. 35: 299-307.

Crim, L.W., D.M. Evans, and B.H. Vickery. 1983b. Manipulation of the seasonal reproductive cycle of the landlocked salmon (Salmo salar) by LHRH analogues administered at various stages of gonadal development. Can. J. Fish. Aquat. Sci. 40: 61-67.

Crim, L.W. and B.D. Glebe. 1984. Advancement and synchrony of ovulation in Atlantic salmon with pelleted LHRH analog. Aquaculture. 43: 47-56.

Crim, L.W., N.M. Sherwood, and C.E. Wilson. 1988. Sustained hormone release. II. Effectiveness of LHRH analog (GnRHa) administration by either single time injection or cholesterol pellet implanted on plasma gonadotropin levels in bioassay model fish, the juvenile rainbow trout. Aquaculture. 74: 87-95.

Donaldson, E.M., G.A. Hunter, and H.M. Dye. 1981. Induced ovulation in coho salmon (Oncorhynchus kisutch). II. Preliminary study of the use of LH-RH and two high potency LH-RH analogues. Aquaculture. 26: 129-141.

Donaldson, E.M., and G.A. Hunter. 1983. Induced final maturation, ovulation, and spermiation in cultured fish. Pages 351-403 in W.S. Hoar, D.J. Randall, and E.M. Donaldson, editors. Fish physiology, volume 9. Part B. Academic Press, New York.

Erdahl, D.A., and J McClain. 1987. Effect of LH-RH analogue treatment on egg maturation (ovulation) in lake trout broodstock. Progressive Fish-Culturist. 49: 276-279.

Fitzpatrick, M.S., B.K. Suzumoto, C.B. Schreck, and D. Oberbillig. 1984. Luteinizing hormone-releasing hormone analogue induces precocious ovulation in adult coho salmon (Oncorhynchus kisutch). Aquaculture. 43: 67-73.

Goetz, F.W. 1983. Hormonal control of oocyte maturation and ovulation in fishes. In: Fish Physiology Vol IX, Part B. Eds. W.S. Hoar, D.J. Randall and E.M. Donaldson. Academic Press, New York. pp. 117-169.

Goren, A., Y. Zohar, M. Fridkin, E. Elhanati, and Y. Koch. 1990. Degradation of gonadotropin releasing hormone in the gilthead seabream, *Sparus aurata.* I. Cleavage of native salmon GnRH and LHRH in the pituitary. Gen. Comp. Endocrinol. 79: 291-305.

Hoar, W.S. 1969. Reproduction. In: Fish Physiology Volume III. Eds. W.S. Hoar and D.J. Randall. Academic Press, New York and London. pp.1-72.

Lee, C.S., C.S. Tamaru, J.E. Banno, C.D. Kelley, A. Bocek, and J.A. Wyban. 1986. Induced maturation and spawning of milkfish, Chanos chanos Forsskal, by hormone implantation. Aquaculture. 52: 199-205.

Marte, L.M., N. Sherwood, L. Crim, and J. Tan. 1988. Induced spawning of the maturing milkfish (*Chanos chanos*) using human chorionic gonadotropin and mammalian and salmon gonadotropin releasing hormone analogues. Aquaculture. 73: 333-340.

Millar, R.P., J.S. Davidson, C. Flanagan, N. Illing, I. Becker, G. Jacobs, and I. Wakefield. 1993. Gonadotropin-releasing hormone receptor structure and function. Proceedings of the “Perspectives in Comparative Endocrinology”. XII International Congress on Comparative Endocrinology. Toronto, Ontario, Canada. 16-21 May. pp. 264-268.

Ngamvongchon, S., J.E. Rivier, and N.M. Sherwood. 1992b. Structure-function studies of five natural, including catfish and dogfish, gonadotropin-releasing hormones and eight analogs on reproduction in Thai catfish (*Clarias macrocephalus*). Regul. Pept. 42: 63-73.

Peter, R.E., C.S. Nahorniak, M. Sokolowska, J.P. Chang, J.E. Rivier, W.W. Vale, J.A. King, and R.P. Millar. 1985. Structure-activity relationships of mammalian, chicken, and salmon gonadotropin releasing hormones *in vivo* in goldfish. Gen. Comp. Endocrinol. 58: 231-242.

Peter, R.E., C.S. Nahorniak, M. Sokolowska, J.P. Chang, J.E. Rivier, W.W. Vale, J.A. King, and R.P. Miller. 1987. Activity and position-8-substituted analogs of mammalian gonadotropin-releasing hormone (mGnRH) and chicken and lamprey gonadotropin-releasing hormones in goldfish. J. Comp. Endocrinol. 65: 385:393.

Powell, J.F.F., P. Swanson, and N.M. Sherwood. 1995. Induced ovulation in Pacific salmonids: gonadotropin levels in chinook salmon spawned out of seawater and freshwater. Proc. Amer. Fish. Soc. Apr 26-30, 1995. Victoria, B.C.

Powell, J.F.F., J. Brackett, and J. Battaglia. 1998. Induced and synchronized spawning of captive broodstock using Ovaplant and Ovaprim. Proc. Aquaculture Assoc. of Canada. 31 Jan - 4 Feb 1998, St. John's Nflnd. Canada.

Schultz, R.W., Bosma, P.T., Zanderbergen, M.A. van der Sanden, M.C.A., van Dijk, W., Peute, J., Bogerd, J, and Goos, H.J.Th. 1993. Two gonadotropin-releasing hormones in the African catfish, Clarias gariepinus: Localizationl, pituitary receptor binding, and gonadotropin release activity. Endocrinology. 133: 1569-1577.

Schultz, R.W., M.C.A. van der Sanden, P.T. Bosma, and H.J.Th. Goos. 1994. Effects of gonadotropin-releasing hormone during the pubertal development of the male African catfish (*Clarias gariepinus*): gonadotrophin and androgen levels in plasma. J. Endocrinol. 140: 265-273.

Sherwood, N.M., L.W. Crim, J.L. Carolsfeld, and S.M. Walters. 1988. Sustained release I: Characteristics of in vitro release of gonadotropin-releasing hormone analogue (GnRH-a) from pellets. Aquaculture. 74: 75-86.

Tao, Y.X. and H.R. Lin. 1993. Effects of exogenous hormones on serum steroid in female ricefield eel (*Monopterus albus*). Acta Zool. Sinica. 39: 315-321.

Taranger, G.L., S.O. Stefansson, and T. Hansen. 1992. Advancement and synchronization of ovulation in Atlantic salmon (Salmo salar L.) following injections of LHRH analogue. Aquaculture. 102: 169-175.

Van der Kraak, G., H.R. Lin, E.M. Donaldson, H.M. Dye, and G.A. Hunter. 1983. Effects of LHRH and desGly^{10}(D-Ala$_6$)LHRH-ethylamide on plasma gonadotropin levels and oocyte maturation in adult female coho salmon (Oncorhynchus kisutch). General Comparative Endocrinology. 49: 470-476.

Weil, C., B. Breton, S. Sambroni, N. Zmora, and Y. Zohar. 1992. *In vitro* activities of various forms of GnRH in relation to their susceptibility to degradation at the pituitary level in the rainbow trout *Oncorhynchus mykiss*. Ben. Comp. Endocrinol. 87: 33-43.

Zohar, Y., A. Goren, M. Fridkin, E. Elhanati, and Y. Koch. 1990. Degradation of gonadotropin releasing hormone in the gildhead seabream, *Sparus aurata*. II. Cleavage of native salmon GnRH and LHRH, and their analogs in the pituitary, kidney, and liver. Gen. Comp. Endocrinol. 79: 306-319.

Table 1. Summary of Year 2011 Ovaplant® Male Efficacy Results - Implant

					Treated			Control	
Number of Trials	Efficacy	Species	Facility	Spawning Interval	Number Treated	Dose (ug/Kg b.w.)	% Spermiate	Number of Controls	% Spermaite
1	inconclusive	AMS	Bears Bluff NFH	24 - 48 hrs	26	100 - 150	?	24	?
1	effective	AMS	Dennis Wildlife Center	15 - 72 hrs	323	64.7	?	35	0
1	effective	AMS	Muddy River Ecological Lab	48 hrs	545	78.9	100	0	-
1	inconclusive	HKS	Manning SFH (Matapeake)	72 hrs - ripe at time of implant	803	159.78	100	0	-
1	effective	STT	Dworshak NFH	15 & 28 days	80	10	97	0	-
1	inconclusive	STB	Warm Springs FHC & TC	ripe at time of implant	25	41 - 61	100	0	-

Table 2. Summary of Year 2011 Ovaplant® Female Efficacy Results - Implant

					Treated			Control	
Number of Trials	Efficacy	Species	Facility	Spawning Interval	Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	inconclusive	AMS	Bears Bluff NFH	24 - 48 hrs	12	100 - 150	?	18	?
1	effective	AMS	Dennis Wildlife Center	15 - 72 hrs	341	64.7 or 129.3	?	35	0
1	effective	AMS	Muddy River Ecological Lab	48 hrs	391	52.1	100	0	-
1	ineffective	COB	Bears Bluff NFH	24 - 48 hrs	2	25	0	0	-
1	effective	FCS	Nez Perce Tribal Hatchery	2 wks	500	12.5	97	0	-
1	effective	HKS	Manning SFH (Matapeake)	48 hrs	684	130.17	100	0	-
1	effective	STT	Wells SFH	7 - 14 days	21	40	100	0	-
1	effective	STB	Warm Springs FHC &TC	2 - 9 days	28	39 - 61	64	0	-

Table 3. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY11 Ovaplant® Efficacy Studies

Total Number of Treatment Trials	14
Number of Trials that Appeared Efficacious:	9
Number of Trials that Appeared Inefficacious:	1
Number of Trials that were Inconclusive:	4
Total Number of Treated Fish:	3,781
Number of fish treated in efficacious trials	2,913
Number of fish treated in inefficacious trials	2
Number of fish treated in inconclusive trials	866
Treatment Regimes Used:	
10 - 150 ug/Kg body weight	13 trials
159.78 ug/Kg body weight	1 trial
Water Temperature (°F) Range:	45.0 - 80.0
Fish Species Treated:	
Salmonids	
fall chinook salmon *Oncorhychus tshawytscha*	
steelhead trout *O. mykiss*	
Non-salmonids	
American shad *Alosa sapidissima*	
hickory shad *A. mediocris*	
striped bass *Morone saxatilis*	
Marine non-salmonid	
cobia *Rachycentron canadum*	
Size Class of Treated Fish:	Adults