Date of Approval: August 6, 1999

FREEDOM OF INFORMATION SUMMARY

NADA 140-927

CHORULON®

(chorionic gonadotropin)

"...as an aid in improving spawning function in male and female brood finfish"

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

Sponsored by:

Intervet Inc.

I. GENERAL INFORMATION

NADA Number: 140-927

Sponsor: Intervet Inc.
P.O. Box 318
405 State Street
Millsboro, DE 19966

Established Name: chorionic gonadotropin

Proprietary Name: CHORULON®

Marketing Status: A prescription (Rx) product which carries the following caution statement: "Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian."

Supplemental Effect: Provides for the use of chorionic gonadotropin (CHORULON®) as an aid in improving spawning function in male and female brood finfish.

II. INDICATION FOR USE

CHORULON® (chorionic gonadotropin) is indicated as an aid in improving spawning function in male and female brood finfish.

III. DOSAGE FORM, ROUTE OF ADMINISTRATION AND RECOMMENDED DOSAGE

A. Dosage Form

CHORULON® is supplied in cartons containing five vials of freeze-dried powder and five 10 mL vials of sterile diluent. When reconstituted, each 10 mL vial contains 10,000 I.U. chorionic gonadotropin (equivalent to 10,000 USP units chorionic gonadotropin) in phosphate-buffered water for injection.

B. Route of Administration

CHORULON® should be injected intramuscularly just ventral to the dorsal fin. Depending upon body weight and dosage administered, it may be necessary to divide the dosage among two or more injection sites to avoid injecting a large volume at a single site.

C. Recommended Dosage

CHORULON® should be administered, depending on the fish species, at a dose of 50 to 510 I.U. per pound body weight (BW) for males and 67 to 1816 I.U. per pound BW for females, for one to three injections. Table 3.1 contains the recommended dosages for several representative fish species. The dose of CHORULON® to be used in other species of finfish may differ from those listed in the table, but should fall within the suggested range of 50 to 510 I.U. per pound BW for males and 67 to 1816 I.U. per pound BW for females.

Table 3.1. Recommended dose/dose range, number of injections, and injection interval for representative fish species

Nomenclature Common name, Genus, Species, Family	Dose/Dose Range (I.U./lb BW/injection)		Number of Injections	Injection Interval (hours)
	Males	Females		
yellow perch, *Perca flavescens*, Percidae	nt*	67-300	1	–
striped bass, *Morone saxitilis*, Percichthyidae	50-500	75-252	1	–
white bass, *Morone chrysops*, Percichthyidae	65-510	91-750	1	–
razorback sucker, *Xyrauchen texanus*, Catostomidae	nt	100	3	24
walleye, *Stizostedion vitreum*, Percidae	75-400	145-830	1-3	72
red snapper, *Lutjanus campechanus*, Lutjanidae	250	500	1	–
sauger, *Stizostedion canadense*, Percidae	500	500-1000	1	–
Chinese catfish, *Clarius fuscus*, Clariidae	nt	1816	1	–

*nt = not tested

The total dose administered (all injections combined) is not to exceed 25,000 I.U. (25 mL) in fish intended for human consumption.

IV. EFFECTIVENESS

A. Contributors: The effectiveness of CHORULON® (chorionic gonadotropin, human chorionic gonadotropin, or HCG) as an aid in spawning function in brood finfish has been demonstrated in clinical trials conducted by the sponsor and by several investigators operating under compassionate Investigational New Animal Drug (INAD) exemptions.

Pivotal trials (INAD 9115) were conducted on three commercial species (striped bass, white bass and walleye) at hatcheries located in the southern and southeastern United States. The fish received a target dose of 150 I.U. HCG/lb body weight via intramuscular injection. Negative control fish received a placebo.

Dr. John Grizzle of the Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, Alabama, served as the Study Director for this study. The following investigators participated in the study.

Tom Curtis
Bayless Striped Bass Hatchery
Bonneau, SC 29431

James M. Smith
Eagle Bend Fish Hatchery
Clinton, TN 37716

Harry J. Warren, BS
Possum Kingdom Fish Hatchery
Graford, TX 76449

Gene Wilson
Table Rock Fish Hatchery
Morganton, NC 28655

Charles C. Starling
Richloam Fish Hatchery
Webster, FL 33597

Harry J. Warren, BS
Dundee State Fish Hatchery
Electra, TX 76360

Nick Nichols
Marion State Fish Hatchery
Marion, AL 36756

Michael C. Hearn, MS
Minor Clark Hatchery
Morehead, KY 40351

Various aquaculturalists, working within the FDA/CVM's compassionate INAD exemption program, also contributed data demonstrating the effectiveness of chorionic gonadotropin as an aid in improving spawning function in male and female brood finfish. A list of these aquaculturalists (including their INAD number and species of fish studied) is provided below.

Dr. Dave Erdahl U.S. Fish and Wildlife Service Bozeman National INAD Office Bozeman, Montana	INAD 8392	Razorback sucker
Dr. Myron Kebus University of Wisconsin-Madison Aquaculture Program Madison, Wisconsin	INAD 9130	Yellow perch, Walleye

Dr. John Grizzle Auburn University Dept. of Fisheries & Allied Aquacultures Auburn University, Alabama	INAD 8860	Striped & White Bass, Walleye, Red Snapper, Sauger
Dr. Larry Willis Illinois Dept. of Natural Resources Topeka, Illinois	INAD 8106	Striped bass, Walleye, Sauger

B. Summary of studies: The results presented below summarize data from the clinical effectiveness studies. The summary is organized by species and is presented in the order shown in Table 3.1.

1. Yellow perch (Percidae)

Source: INAD 9130 (year 1996), Dr. Myron J. Kebus, University of Wisconsin-Madison, Aquaculture Program, Madison, Wisconsin

a. University of Wisconsin Aquaculture Program, Madison, Wisconsin

One hundred thirty-two female yellow perch were injected with either 150 (n = 40) or 300 (n = 92) I.U. HCG/kg body weight (68 or 136 I.U. HCG/lb body weight, respectively). Forty females served as negative controls. Of the 40 treated females receiving 150 I.U. HCG/kg body weight, 36 (90.0%) spawned within 8 days after treatment whereas 88 (95.7%) of 92 treated females receiving 300 I.U. HCG/kg body weight spawned. Only 16 (40.0%) of 40 negative control females spawned by 8 days after enrollment. All 132 treated females and 34 (85.0%) of 40 negative control females spawned by 17 days after enrollment.

b. Coolwater Farms, LLC, 2844 Clearview Road, Cambridge, Wisconsin

Three hundred female yellow perch were injected with 66.7 (n = 100) or 300 (n = 200) I.U. HCG/kg body weight (30 or 136 I.U. HCG/lb body weight, respectively). Sixty females served as negative controls. Ninety-seven (98.0%) of 99 females treated with 66.7 I.U. HCG/kg spawned by 8 days after injection (one fish died). One hundred ninety-five (99.0%) of 197 females treated with 300 I.U. HCG/kg spawned by 8 days after injection (three fish died). Only four (6.7%) of 60 negative control females spawned by 8 days after enrollment. Negative control females continued to spawn through Day 11 after enrollment at which time 14 (23.3%) of 60 had spawned.

Conclusion: When data from the two trials were pooled, 416 (97.2%) of 428 female yellow perch receiving CHORULON® spawned by 8 days after treatment compared to 20 (20.0%) of 100 negative control females. CHORULON® shortened the time necessary for these yellow perch to spawn, with most fish spawning within 8 days after treatment. These data demonstrate CHORULON®, when administered at doses ranging from 67 to

300 I.U. HCG/lb body weight, aids spawning function in female yellow perch.

2. Striped bass (Percichthyidae)

   Sources:

   Intervet Inc. INAD 9115, Dr. John M. Grizzle, Dept. of Fisheries & Allied Aquacultures, Auburn University, Alabama

   INAD 8860 (year 1994), Drs. John M. Grizzle and Dehai Xu, Department of Fisheries and Allied Aquacultures; Auburn University, Alabama

   INAD 8860 (year 1993), Drs. Wilmer A. Rogers, John M. Grizzle and Dehai Xu, Department of Fisheries and Allied Aquacultures; Auburn University, Alabama

   INAD 8106 (years 1994-1995), Dr. Larry Willis, Illinois Department of Natural Resources, Topeka, Illinois

   a. INAD 9115, Intervet Inc.

      Two hundred sixty-three female striped bass were injected with CHORULON® at doses ranging from 75 to 188 I.U. HCG/lb body weight. Twenty-seven females injected with placebo served as negative controls. Striped bass females receiving CHORULON® had significantly ($P < 0.001$) greater spawning rates compared to negative control females. Of the 263 females receiving CHORULON®, 246 (93.5%) spawned to produce 180.5 million eggs with an average hatch rate of 47.2%. None of the negative control females spawned.

      Male striped bass were injected with either CHORULON® at doses ranging from 50 to 500 I.U. HCG/lb body weight or placebo. All male striped bass spawned regardless of whether they received CHORULON® or placebo. Most males were flowing milt at the time of injection.

   b. INAD 8860 (year 1994)

      Five hundred eighty-five female striped bass received CHORULON® at doses ranging from 108 to 252 I.U. HCG/lb body weight. One hundred three females injected with placebo served as negative controls. Of the 585 females receiving CHORULON®, 543 (92.8%) spawned and produced 404.9 million eggs with an average hatch rate of 36.7%. One negative control female spawned to produce 1.9 million eggs with a hatch rate of 2.6%.

      Six hundred ninety-six male striped bass received CHORULON® at doses ranging from 50 to 286 I.U. HCG/lb body weight. Sixty-eight males injected with placebo served as negative controls. All males receiving CHORULON® and 33 (48.5%) of 68 negative control males produced milt.

c. INAD 8860 (year 1993)

Seven hundred female striped bass received HCG at doses ranging from 150 to 225 I.U./lb body weight. Fifty-five females injected with placebo served as negative controls. Of the 700 females receiving HCG, 572 (81.7%) spawned. Two (3.6%) of the 55 negative control females spawned.

One thousand one hundred forty-eight male striped bass were injected with HCG at doses ranging from 75 to 220 I.U./lb body weight. Four males injected with placebo served as negative controls. Of the 1148 males receiving HCG, 1135 (98.9%) produced milt whereas none of the negative control males produced milt.

d. INAD 8106 (years 1994-1995)

Jake Wolf Memorial Fish Hatchery, 25410 North Fish Hatchery Road, Topeka, Illinois

1994: Five female and 10 male striped bass were injected with HCG at doses of either 150 I.U./lb (females) or 75 I.U./lb (males) body weight, respectively. All fish spawned or produced milt within 24 hours after treatment.

1995: Eight female and 18 male striped bass were injected with HCG at doses of either 150 (females) or 75 (males) I.U./lb body weight, respectively. Only one female spawned within 48 hours after treatment. Due to poor egg quality, second injections were not administered.

Conclusion: Of the 1561 female striped bass receiving HCG, 1367 (87.6%) spawned. Only three (1.6%) of 185 negative control females spawned. These data demonstrate HCG, when administered at doses ranging from 75 to 252 I.U./lb body weight, aids in spawning function of female striped bass. The data also indicate that HCG in doses ranging from 50 to 500 I.U./lb body weight aids milt production by male striped bass.

3. White bass (Percichthyidae)

Sources:

INAD 9115, Intervet Inc., Dr. John M. Grizzle, Dept. of Fisheries & Allied Aquacultures, Auburn University, Alabama

INAD 8860 (year 1994), Drs. John. M. Grizzle and Dehai Xu, Department of Fisheries and Allied Aquacultures; Auburn University, Alabama

INAD 8860 (year 1993), Drs. Wilmer A. Rogers, John. M. Grizzle and Dehai Xu, Department of Fisheries and Allied Aquacultures; Auburn University, Alabama

a. INAD 9115, Intervet Inc.

Seventy-six female white bass were injected with CHORULON® at doses ranging from 91 to 308 I.U. HCG/lb body weight. Six females were injected with placebo and served as negative controls. Females receiving CHORULON® had significantly ($P < 0.001$) greater spawning rates than negative control females. Seventy-three (96.1%) of 76 females receiving CHORULON® spawned to produce approximately 8.11 million eggs with an average hatch rate of 28.5%. Only one (16.7%) of six negative control females spawned.

Forty-six male white bass were injected with CHORULON® at doses ranging from 65 to 510 I.U. HCG/lb body weight. Of the 46 males injected with CHORULON®, 43 (93.5%) produced milt.

b. INAD 8860 (year 1994)

Six hundred sixty female white bass received CHORULON® at doses ranging from 100 to 480 I.U. HCG/lb body weight and 73 served as negative controls. Of the 660 females receiving CHORULON®, 591 (89.5%) spawned to produce approximately 89.3 million eggs. None of the negative control females spawned.

Eight hundred sixty-two male white bass received CHORULON® at doses ranging from 75 to 380 I.U. HCG/lb body weight while 24 served as negative controls. Of the 862 receiving CHORULON®, 851 (98.7%) produced milt whereas nine (37.5%) of 24 negative control males produced milt.

c. INAD 8860 (year 1993)

Two hundred fifty female white bass received HCG at doses ranging from 100 to 750 I.U./lb body weight while 45 served as negative controls. Of the 250 fish receiving HCG, 216 (86.4%) spawned. None of the negative control fish spawned.

Four hundred fifty-nine male white bass received HCG at doses ranging from 100 to 500 I.U./lb body weight while six males served as negative controls. All treated male white bass produced milt whereas none of the negative control males produced milt.

Conclusion: Of the 986 female white bass receiving HCG treatment, 880 (89.2%) spawned. Only one (0.8%) of 124 negative control females spawned. These data demonstrate HCG, when administered at doses ranging from 91 to 750 I.U./lb body weight, aids spawning function in female white bass. Doses ranging from 65 to 510 I.U. HCG/lb body weight aid milt production by male white bass.

4. Razorback sucker (Catostomidae):

Source: INAD 8392 (year 1995), Dr. Dave Erdahl, US Fish and Wildlife Service, Bozeman, Montana

Twenty-five female razorback suckers were injected with CHORULON® at a dose of 100 I.U. HCG/lb body weight once daily for 3 days. Five females served as negative controls. Of 25 treated females, 24 (96.0%) spawned and produced 2.1 million eggs with an average hatch rate of 51.4%. None of the negative control females spawned.

Conclusion: These data demonstrate CHORULON®, when injected at a dose of 100 I.U. HCG/lb body weight once daily for 3 days, is effective in aiding spawning function in female razorback suckers.

5. Walleye (Percidae):

Sources: Intervet Inc. INAD 9115, Dr. John M. Grizzle, Dept. of Fisheries & Allied Aquacultures, Auburn University, Alabama

INAD 8860 (year 1994), Drs. John. M. Grizzle and Dehai Xu, Department of Fisheries and Allied Aquacultures; Auburn University, Alabama

INAD 8860 (year 1993), Drs. Wilmer A. Rogers, John M. Grizzle and Dehai Xu, Department of Fisheries and Allied Aquacultures; Auburn University, Alabama

INAD 9130 (year 1996), Dr. Myron J. Kebus, University of Wisconsin-Madison, Aquaculture Program, Madison, Wisconsin

INAD 8106 (years 1994-1995). Dr. Larry Willis, Illinois Department of Natural Resources, Topeka, Illinois

a. INAD 9115, Intervet Inc.

One hundred fourteen female walleye were injected with CHORULON® at doses ranging from 145 to 257 I.U. HCG/lb body weight. Ten females received placebo and served as negative controls. Females that did not spawn by 72 hours after initial injection received a second injection of either CHORULON® at a dose of approximately 170 I.U. HCG/lb body weight (treated) or placebo (negative control). Female walleye receiving two CHORULON® injections had significantly ($P < 0.005$) greater spawning rates compared to negative control females receiving two placebo injections. Twenty-five (21.9%) of 114 females spawned after the initial CHORULON® injection. Fifty-seven (66.3%) of 86 females receiving a second CHORULON® injection spawned. Female walleye receiving CHORULON® produced 10.29 million eggs with an average hatch rate of 49.5%. Two of ten negative control females spawned and produced eggs that did not hatch.

Sixteen male walleye received CHORULON® at a dose of approximately 75 I.U. HCG/lb body weight and two received placebo. All male walleye produced milt regardless of whether they received CHORULON® or placebo.

b. INAD 8860 (year 1994)

One hundred twenty-six female walleye were injected with CHORULON® at doses ranging from 200 to 833 I.U. HCG/lb body weight. Ten females received placebo and served as negative controls. Of the 126 treated fish, 121 (96.0%) spawned to produce 9.54 million eggs with an average hatch rate of 50%. None of the negative control females spawned.

Forty-two male walleye were injected with CHORULON® at doses ranging from 180 to 400 I.U. HCG/lb body weight. All of the injected fish produced milt.

c. INAD 8860 (year 1993)

Eighty-one female walleye were injected with HCG at doses ranging from 200 to 800 I.U./lb body weight. Of the 81 females receiving HCG, 68 (84.0%) spawned.

Forty-five male walleye received HCG at doses ranging from 250 to 270 I.U. HCG/lb body weight. All males produced milt.

d. INAD 9130 (year 1996)

Aurora-Aqua, Inc., 12830 82nd Avenue NE, Spicer, Minnesota

Ten female walleye were injected with HCG at a dose of 227 I.U./lb body weight and four females served as negative controls. None of the fish spawned. It was noted that the gonads were not mature in these fish which may have contributed to the lack of spawning.

Max McGraw Wildlife Foundation, P.O. Box 9, Dundee, Illinois

Ten female walleye were injected with CHORULON® at a dose of 227 I.U. HCG/lb body weight and four females served as negative controls. Four of ten females receiving CHORULON® spawned whereas all four of the negative control females spawned. There were two mortalities and three incidences of blood in the egg masses from fish receiving treatment. Mortalities and blood in the egg masses may have been associated with mechanical trauma to the peritoneal contents.

e. INAD 8106

LaSalle Fish Hatchery (year 1994), 2649 North 21st Road, Marseilles, Illinois

Eighteen female walleye were injected with 500 I.U. HCG/lb body weight. One untreated female served as a negative control. All 18 treated females spawned whereas the negative control female did not spawn.

LaSalle Fish Hatchery (year 1995), address above

Fifty-five female walleye were injected with 500 I.U. HCG/lb body weight. Six untreated females served as negative controls. Forty-eight (87.3%) of 55 treated females and two (33.3%) of six negative control females spawned.

Jake Wolf Memorial Fish Hatchery (year 1994), address above

Twenty-one female walleye were injected up to two times with 500 I.U. HCG/lb body weight. Three females served as negative controls. Fourteen (66.7%) of 21 treated females spawned after the initial injection. Six (85.7%) of the remaining seven treated females spawned after a second injection. In total, 20 (95.2%) of 21 treated females spawned after receiving either one or two injections. None of the negative control females spawned.

Conclusion: Of the 435 female walleye receiving HCG treatment, 361 (83.0%) spawned. Eight (21.1%) of 38 negative control females spawned. These data demonstrate HCG, when administered at doses ranging from 145 to 830 I.U./lb body weight, aids spawning function in female walleye. All 103 male walleye produced milt after receiving doses ranging from 75 to 400 I.U. HCG/lb body weight.

6. Red snapper (Lutjanidae):

Sources: INAD 8860 (year 1994), Drs. John. M. Grizzle and Dehai Xu, Department of Fisheries and Allied Aquacultures; Auburn University, Alabama

INAD 8860 (year 1993), Drs. Wilmer A. Rogers, John. M. Grizzle and Dehai Xu, Department of Fisheries and Allied Aquacultures; Auburn University, Alabama

a. INAD 8860 (year 1994)

Ten female red snapper were injected with CHORULON® at a dose of approximately 500 I.U. HCG/lb body weight and one female served as a negative control. Of the 10 females receiving CHORULON®, seven (70.0%) spawned to produce 753,000 eggs of which 541,000 (71.8%) hatched. The negative control female did not spawn.

Ten male red snapper were injected with CHORULON® at a dose of approximately 250 I.U. HCG/lb body weight and all produced milt.

b. INAD 8860 (year 1993)

Seventeen female red snapper were injected with 500 I.U. HCG/lb body weight. Of these 17 females, 11 (64.7%) spawned after treatment.

Twelve male red snapper were injected with 250 I.U. HCG/lb body weight and all produced milt.

Conclusion: Of the 27 female red snapper receiving HCG treatment, 18 (66.7%) spawned. The one negative control female did not spawn. These data demonstrate HCG, when administered at a dose of 500 I.U./lb body weight, aids spawning function in female red snapper. All 22 male red snapper produced milt after receiving a single dose of 250 I.U. HCG/lb body weight.

7. Sauger (Percidae):

Sources: INAD 8106 (years 1994-1995). Dr. Larry Willis, Illinois Department of Natural Resources, Topeka, Illinois

INAD 8860 (year 1993), Drs. Wilmer A. Rogers, John. M. Grizzle and Dehai Xu, Department of Fisheries and Allied Aquacultures; Auburn University, Alabama

a. INAD 8106

LaSalle Fish Hatchery (year 1994), address above

Two hundred thirty-one female sauger were injected with 500 I.U. HCG/lb body weight and 36 females served as untreated negative controls. By 96 hours after injection, 73 (31.7%) of the treated females spawned, whereas by 120 hours after injection, an additional 84 (36.5%) treated females spawned for a total of 157 (68.0%) out of 231 treated females spawned. Only two (5.6%) of 36 negative control females spawned.

LaSalle Fish Hatchery (year 1995), address above

One hundred twenty-two female sauger were injected with 500 I.U. HCG/lb body weight and 10 females served as untreated negative controls. Fifty-one treated females died during holding, presumably due to handling stress. Of the 71 remaining treated females, 51 (71.8%) spawned. None of the negative control females spawned.

b. INAD 8860 (year 1993)

Thirty-three female and 23 male sauger were injected with HCG at doses ranging from 800 to 1000 I.U./lb (females) or 500 I.U./lb (males) body weight. All 33 female sauger spawned and all 23 males produced milt.

Conclusion: Of the 386 female sauger receiving HCG treatment, 241 (62.4%) spawned (51 treated fish died during holding). Only two (4.3%) of 46 negative control females spawned. These data demonstrate HCG, when administered at doses ranging from 500 to 1000 I.U./lb body weight, aids spawning function in female sauger. All 23 male sauger produced milt after receiving a dose of 500 I.U. HCG/lb body weight.

8. Chinese catfish (Clariidae):

Source: INAD 8169 (years 1992-1995). Mr. Richard Bailey, University of Hawaii, Sea Grant Extension Program, Honolulu, Hawaii, and Dr. Paul G. Olin, Marine Advisor, University of California Sea Grant, Santa Rosa, California (Sections 7, 8 and 9).

a. INAD 8169 (year 1992)

One hundred sixty-six female Chinese catfish were injected with HCG at a dose of 4 I.U./gm (1816 I.U./lb) body weight. Of these, 158 (95.2%) spawned.

b. INAD 8169 (years 1993-94)

Five hundred forty-five female Chinese catfish were injected with HCG at a dose of 4 I.U./gm (1816 I.U./lb) body weight. All 545 fish spawned.

c. INAD 8169 (year 1995)

One thousand four hundred ninety-six female Chinese catfish were injected with HCG at a dose of 4 I.U./gm (1816 I.U./lb) body weight. Of these, 1466 (98.0%) spawned.

Conclusion: During a four year period (1992-1995), 2169 (98.3%) of 2207 Chinese catfish receiving HCG treatment spawned. These data demonstrate HCG, when administered at a dose of 4 I.U./gm (1816 I.U./lb) body weight, aids spawning function in female Chinese catfish.

V. ANIMAL SAFETY

A study was conducted to determine the safety of CHORULON® following intramuscular injection of 3 to 10 times the target dose in four representative species of brood fish: walleye (*Stizostedion vitreum*); grass carp (*Ctenopharyngodon idella*); channel catfish (*Ictalurus punctatus*); and white bass (*Morone chrysops*). Approximately six male and six female brood fish (approximately 3 fish/sex/treatment) of each species were administered a single intramuscular injection of either CHORULON® or placebo (Table 5.1).

Table 5.1. Target animal safety study treatment schedule

Nomenclature Common name, Genus, Species, Family	Treated Fish Dose = I.U. HCG/lb BW n = number of fish				Negative Control Fish Dose = mL vehicle/lb BW n = number of fish			
	Female		Male		Female		Male	
	Dose	n	Dose	n	Dose	n	Dose	n
Walleye, *Stizostedion vitreum,* Percidae	1500	3	750	3	0.6	3[a]	0.3	3
Grass carp*, *Ctenopharyngodon idella,* Cyprinidae	5000	3	2500	3	1.0	4	0.5	2
Channel catfish*, *Ictalurus punctatus,* Ictaluridae	5000	3	2500[b]	5	1.0	2[c]	0.5	7
White bass*, *Morone chrysops,* Percichthyidae	1500	3	750	3	1.5	5	0.75	1

*Several fish were initially mis-sexed. Sex was definitively determined at necropsy.

[a] Two negative control females died during the 14-day post-treatment observation period.

[b] Two treated males were initially mis-sexed as 'female' and received 5000 I.U. HCG/lb BW

[c] One negative control female jumped from the holding tank and died on Day 1 post-injection.

After injection, fish were observed twice daily for 14 days for any unusual clinical signs and then euthanized and necropsied on Day 15. At necropsy, gross observations were recorded and any abnormal tissues were collected and evaluated histopathologically. Weights of relevant tissues/organs were obtained and body weight change (pre- versus post-treatment weight), liver somatic index (LSI, a measure of nutritional state), and visceral somatic index (VSI, a measure of overall body condition), were calculated.

A. Results:

1. Walleye

Two negative control females died during the 14 day observation period; one at Day 1 due to handling stress, and one at Day 6 due to decomposition (rotting) of eggs within the body cavity. One negative control female exhibited loss of equilibrium and increased ventilation. No other unusual clinical signs were observed in treated or negative control fish. Histopathology revealed a necrotizing granulomatous steatitis (due to internal parasitism) in one treated male and mild fatty degeneration in one negative control male, neither of which was considered to be related to treatment. Table 5.2 provides values for variables calculated at necropsy.

Table 5.2. Variables (mean ± s.d.) calculated at necropsy for Walleye

Variable	Male		Female	
	Treated	**Negative Control**	**Treated**	**Negative Control**
Wt. Change (g)	28.2 ± 1.7*	16.0 ± 10.4	137.5 ± 88.4*	-28.6 ± 37.2
VSI	92.5 ± 1.3	90.7 ± 0.7	76.9 ± 11.3	79.2 ± 10.3
LSI	0.9 ± 0.1	0.8 ± 0.03	1.2 ± 0.7	1.3 ± 0.5
Visceral Wt. (g)	91.9 ± 24.2	122.7 ± 27.5	299.8 ± 177.7	357.4 ± 286.6
Heart Wt. (g)	1.8 ± 0.6	1.8 ± 0.9	1.6 ± 0.3	2.3 ± 0.8
GI Tract Wt. (g)	25.2 ± 8.0	30.9 ± 4.7	26.1 ± 2.0	37.1 ± 15.1
Liver Wt. (g)	11.0 ± 3.6	9.86 ± 1.6	13.3 ± 5.5	20.1 ± 9.8
Spleen Wt. (g)	1.68 ± 0.5	1.9 ± 0.6	1.2 ± 0.3*	1.7 ± 0.1
UG Tract Wt. (g)	12.5 ± 6.5	12.8 ± 3.0	226.0 ± 190.3	260.1 ± 280.9

*Significantly different ($P<0.05$) from negative control fish of same sex

Body weight changes differed between treated and negative control fish. Treated females gained 137.5 ± 88.4 g while negative control females lost 28.6 ± 37.2 g. Treated males gained 28.2 ± 1.7 g while negative control males gained only 16.0 ± 10.4 g. The weight gains observed were associated with increased gonadal development. The slight decrease (0.5 g) in spleen weight in treated females represents a physiologically insignificant finding.

2. Grass carp

Several fish (mostly females) in both the treated and negative control groups developed a fungal infection during the 14-day observation period. No other abnormalities were observed. Upon gross examination, all organ systems appeared normal. The sex of several negative control fish was not grossly discernible at necropsy and histopathology of the urogenital tract revealed that three negative control fish (two males and one female) were initially mis-sexed at injection. Table 5.3 provides values for variables calculated at necropsy

Table 5.3. Variables (mean ± s.d.) calculated at necropsy for Grass Carp

Variable	Male		Female	
	Treated	Negative Control	Treated	Negative Control
Wt. Change (g)	67.8 ± 4.63	86.1 ± 22.0	22.9 ± 102.5	27.0 ± 44.6
VSI	92.9 ± 1.5	93.2 ± 0.6	92.1 ± 0.6	97.2 ± 1.2
LSI	1.2 ± 0.2	1.4 ± 0.0	1.4 ± 0.0*	1.2 ± 0.1
Visceral Wt. (g)	250.3 ± 78.9	261.8 ± 46.7	374.7 ± 6.7*	285.0 ± 71.9
Heart Wt. (g)	4.7 ± 0.8	5.5 ± 1.0	6.4 ± 0.6	5.5 ± 0.6
GI Tract Wt. (g)	45.2 ± 11.2	36.6 ± 3.5	55.9 ± 3.7	47.3 ± 8.2
Liver Wt. (g)	41.6 ± 8.1*	55.2 ± 4.7	67.2 ± 3.8*	45.0 ± 10.1
Spleen Wt. (g)	4.9 ± 0.9*	7.4 ± 0.8	10.4 ± 1.4*	6.85 ± 1.4
UG Tract Wt. (g)	3.7 ± 2.3	7.5 ± 8.7	72.7 ± 35.9	41.2 ± 55.4

*Significantly different ($P<0.05$) from negative control fish of same sex

Visceral, liver and spleen weights in treated females were somewhat higher than in negative control females; however, gross examination revealed no abnormalities. Because the LSI was also higher in treated females compared to negative controls, the increased visceral, liver and spleen weights may have been due to better overall nutritional status among treated females. Liver and spleen weights were slightly lower in treated males compared to negative controls; however, gross examination revealed no abnormalities.

3. Channel catfish

No unusual clinical signs were observed during the 14-day observation period, although several fish exhibited healing bite marks (a result of fighting prior to injection and segregation). Upon gross examination, organ systems in most fish appeared normal, although several fish (two negative control females and one treated female) exhibited hepatic granulomas indicative of chronic parasitism. One negative control female exhibited a cardiac melanosis considered to be a physiologic change unrelated to treatment. Upon necropsy, it was determined that two treated males and three negative control males were initially mis-sexed as females. Therefore, two of five treated males received 5,000 I.U. HCG/lb BW. Table 5.4 provides values for variables calculated at necropsy.

Table 5.4. Variables (mean ± s.d.) calculated at necropsy for Channel Catfish

Variable	Male		Female	
	Treated	**Negative Control**	**Treated**	**Negative Control**
Wt. Change (g)	-34.8 ± 20.0	-13.2 ± 15.2	-15.7 ± 21.3	-4.4 ± 7.4
VSI	94.2 ± 1.5	94.0 ± 0.9	90.9 ± 3.0	91.4 ± 1.3
LSI	1.0 ± 0.2	1.1 ± 0.1	1.1 ± 0.3	1.4 ± 0.2
Visceral Wt. (g)	74.4 ± 56.7	55.8 ± 16.8	118.1 ± 76.5	166.6 ± 101.9
Heart Wt. (g)	0.9 ± 0.5	0.6 ± 0.2	0.7 ± 0.4	1.3 ± 0.4
GI Tract Wt. (g)	27.6 ± 20.8	22.6 ± 8.6	27.3 ± 15.0	48.1 ± 15.3
Liver Wt. (g)	12.3 ± 7.5	9.7 ± 2.4	14.6 ± 8.4	24.4 ± 7.7
Spleen Wt. (g)	1.5 ± 1.0	1.1 ± 0.4	1.5 ± 1.0	3.0 ± 1.0
UG Tract Wt. (g)	3.8 ± 1.6	2.0 ± 0.8	45.2 ± 67.8	9.7 ± 11.6

No significant differences in any of the variables evaluated were observed.

4. White bass

No unusual clinical signs were observed during the 14-day observation period. Gross examination at necropsy revealed an abnormal spleen (cystic dilation of splenic lymphatic vessels) in one treated female. This observation was considered to be an incidental finding unrelated to treatment. Gross examination also revealed that two negative control females were initially mis-sexed as males at injection. Table 5.5 provides values for variables calculated at necropsy.

Table 5.5 Variables (mean ± s.d.) calculated at necropsy for White Bass

Variable	Male		Female	
	Treated	Negative Control[a]	Treated	Negative Control
Wt. Change (g)	6.47 ± 7.1	18.9	29.0 ± 11.2	16.9 ± 7.2
VSI	92.9 ± 1.2	87.6	87.1 ± 3.9	88.2 ± 1.7
LSI	1.1 ± 0.5	1.9	2.1 ± 0.2	2.0 ± 0.3
Visceral Wt. (g)	30.7 ± 5.0	70.7	77.9 ± 25.3	47.2 ± 8.5
Heart Wt. (g)	0.6 ± 0.1	0.8	0.7 ± 0.1	0.44 ± 0.1
GI Tract Wt. (g)	11.5 ± 3.3	16.2	18.0 ± 2.5	16.8 ± 3.7
Liver Wt. (g)	4.8 ± 2.2	10.9	12.5 ± 2.3	8.2 ± 2.0
Spleen Wt. (g)	1.2 ± 0.3	0.8	1.1 ± 0.2	0.8 ± 0.4
UG Tract Wt. (g)	5.6 ± 1.8	21.6	33.4 ± 18.1	12.5 ± 3.5

[a] Only one male negative control fish was represented; therefore, s.d. could not be calculated

No significant differences in any of the variables evaluated were observed.

B. Conclusion: This target animal safety study, conducted in four representative species of fish at doses of 750, 1500, 2500 or 5000 I.U./lb BW per injection indicates CHORULON® can be administered to brood fish at the levels recommended in the product labeling without significant adverse effects. Tested species/dose combinations found to be safe are summarized in Table 5.6.

Table 5.6 Tested species/dose combinations found to be safe

Nomenclature Common name, Genus, Species, Family	Tested Dose(s) (I.U./lb BW/injection)		Number of Injections
	Males	Females	
white bass, *Morone chrysops*, Percichthyidae	750	1500	1
walleye, *Stizostedion vitreum*, Percidae	750	1500	1
grass carp, *Lutjanus campechanus*, Lutjanidae	2500	5000	1
channel catfish, *Ictalurus punctatus*, Ictaluridae	2500	5000	1

VI. HUMAN SAFETY

A. Toxicity Studies

The safety of chorionic gonadotropin was established in the sponsor's original approval for use in cattle under NADA 140-927.

The human food safety information was updated as part of the approval of this supplement for HCG as a spawning aid for use in male and female brood fish. A study entitled, "An Oral Drug Safety Study with PG 600 in Hypophysectomized Rats", Report No. 86, was performed by Small Animal Plant, Intervet International. The report was finalized on January 25, 1990, and submitted by the sponsor as a Drug Experience Report under NADA 140-856 on January 4, 1991. The purpose of the study was to demonstrate the safety of PG 600 (a mixture of HCG and pregnant mare serum gonadotropin (PMSG)) when given orally in hypophysectomized rats. Twenty-four hypophysectomized rats/sex/group plus 10 intact rats/sex/group were administered PG 600 via gavage once daily for 14 days. Dose administration of PG 600 reported as PMSG, HCG, and total gonadotropin was as follows.

Group	I	II	III
PMSG	0	400 I.U./rat	4000 I.U./rat
HCG	0	200 I.U./rat	2000 I.U./rat
Total Gonadotropin	0	600 I.U./rat	6000 I.U./rat

Increased absolute and relative uterine and testicular weights were observed in rats treated with 6,000 I.U. total gonadotropin compared to negative controls. A no-observed-effect-level (NOEL) of 600 I.U. total gonadotropin/rat/day was established.

B. Safe Concentration of Total Residues and Tolerances

1. No-Observed-Effect-Level (NOEL):

   A NOEL of 600 I.U. per rat of gonadotropin (low dose - 200 I.U. of HCG plus 400 I.U. of PMSG) was established based upon the increases in absolute uterine and testicular weights. Given an average body weight of 0.142 kg for the treated rats, the NOEL is 4,225 I.U./kg body weight/day.

2. Calculation of the Acceptable Daily Intake (ADI) and the Safe Concentration

   It may be assumed that PMSG and HCG contribute equally to the observed effects. Using a 100-fold safety factor (considering the increased sensitivity of the hypophysectomized rat model), an acceptable daily intake for total gonadotropin (HCG and PMSG) may be calculated as:

$$\text{ADI} = \frac{(4225\ I.U./kg/day)}{100} = 42.25\ \text{I.U./kg body weight/day}$$

Using a consumption factor of 0.3 kg/day for fillet (muscle with adhering skin), the safe concentration for total gonadotropin (HCG and PMSG) is calculated as:

$$\text{safe concentration}_{\text{muscle with adhering skin}} = \frac{(42.25\ I.U./kg/day)\ x\ 60\ kg}{0.3\ kg}$$

or 8,450 I.U. total gonadotropin/kg consumed meat/day

or 2,535 I.U. total gonadotropin/0.3 kg of muscle or muscle with adhering skin consumed/person/day

It is possible that the entire injection site may be consumed in a single meal. The safe concentration for the injection site is calculated by adjusting the ADI by 10-fold to reflect that consumption of the entire administered dose in a single meal is a relatively rare event.

$$\text{safe concentration}_{\text{injection site}} = \frac{(422.5\ I.U./kg\ bw/day)\ x\ 60}{0.3\ kg}$$

or 84,500 I.U. total gonadotropin/kg consumed meat

or 25,350 I.U. total gonadotropin/0.3 kg of muscle or muscle with adhering skin consumed/person

C. Residue Chemistry

It is possible to make a number of conservative estimates regarding the possible concentration of HCG in edible tissues. It may be conservatively assumed that:

1. Most fish receiving multiples doses are unlikely to receive a single dose greater than 500 I.U. HCG per lb. of fish based on efficacy data.
2. The treatment is repeated three times.
3. There is no metabolism or excretion of the administered dose.
4. The administered HCG distributes evenly throughout the body of the fish.

Given these conservative assumptions, a 100 lb. fish would receive a maximum accumulated dose of 150,000 I.U. per fish. Distributing the dose throughout the body of the fish, converting to a per kilogram basis, and assuming consumption of 0.3 kg fillet with adhering skin, results in a consumed dose of:

$$\frac{(150{,}000\ I.U./fish)\ x\ (2.204\ lbs/kg)}{100\ lbs}\ x\ 0.3\ kg$$

= 992 I.U. in 0.3 kg daily consumption.

The calculated consumption of HCG in a fillet with adhering skin for even a 100 pound fish is well below the 2,535 I.U. total gonadotropin per day provided by the safe concentration for a 60 kg human.

Since the HCG is administered to the fish by injection, the human food safety concern for the injection site also must be considered. It is possible that the entire administered dose of HCG may be consumed in a single meal of fillet with adhering skin from the injection site. Therefore, based on the safe concentration for HCG residues at the injection site, the total dose administered to the fish should not exceed 25,350 I.U. HCG.

Considering all of the available information, and the conservative nature of the assumptions, the following human food safety limitation is provided for incorporation on the NADA label:

> No withdrawal period is required for brood fish treated according to label directions. The total dose administered (all injections combined) should not exceed 25,000 I.U. HCG (25 mL) per fish in fish intended for human consumption.

D. Regulatory Method

Since residues are anticipated to be well below the safe concentration in fish intended for human consumption, a regulatory method is not required.

VII. AGENCY CONCLUSIONS

The data submitted in support of this NADA supplement satisfy the requirements of Section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that chorionic gonadotropin, when administered as an intramuscular injection in accordance with the label directions, is safe and effective as an aid in improving spawning function in male and female brood finfish.

The human food safety information was updated as part of the approval of this supplement. The results of the 14-day oral bioavailability study in hypophysectomized rats permitted the determination of an acceptable daily intake (ADI) of 42.25 I.U. total gonadotropin/kg body weight/day. Conservative calculations of possible residues of human chorionic gonadotropin in fish treated with the maximum anticipated dose indicate that residues of total gonadotropin will be well below the safe concentration in the edible tissue of fish muscle, or muscle with adhering skin. Therefore, neither a withdrawal period nor regulatory method is required for brood fish treated according to label directions. Restriction of the total administered dose available to a brood fish intended for human consumption is based upon the potential for unsafe residues in the injection site. The total dose administered (all injections combined) should not exceed 25,000 I.U. human chorionic gonadotropin (25 mL) per fish in fish intended for human consumption.

Labeling restricts this drug to use by or on order of a licensed veterinarian. The Center for Veterinary Medicine (CVM) has concluded that this product shall continue to have prescription marketing status.

The agency has determined under 21 CFR 25.33(c) that this action is of the type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

Under the Center's supplemental approval policy (21 CFR 514.106(b)(2)(vii)), this is a Category II change. The approval of this change is not expected to have any adverse effect on the safety or effectiveness of this new animal drug and, therefore, did not require a reevaluation of the human food or target animal safety data in the parent application.

Under Section 512(c)(2)(F)(iii) of the FFDCA, this approval for food-producing animals qualifies for THREE (3) years of marketing exclusivity beginning on the date of approval because the supplemental application contains substantial evidence of the effectiveness of the drug involved, any studies of animal safety, or, in the case of food producing animals, human food safety studies (other than bioequivalence or residue studies) required for the approval of the application and conducted or sponsored by the applicant. The three years of marketing exclusivity applies only to the addition of the new species and indication, for which the supplemental application is approved.

VIII. APPROVED LABELING

A copy of the draft facsimile labeling is attached to this document.

A. CHORULON® Box Label

B. CHORULON® 10 mL Vial Diluent Label

C. CHORULON® 10,000 I.U. Vial Label

D. CHORULON® Package Insert

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