FREEDOM OF INFORMATION SUMMARY

Romet® -30 (sulfadimethoxine + ormetoprim) Premix for Fish

I.	Application:	Supplemental Submission NADA 125-933
	Sponsor:	Hoffmann-La Roche Inc. Animal Science Research Nutley, New Jersey 07110
	Generic Name:	sulfadimethoxine + ormetoprim (5:1)
	Pharmaceutical Classification:	Antibacterial
	Trade Name:	Romet ® -30
	Marketing Status:	over-the-counter (OTC)
	Effect of Supplement:	21 CFR Parts 556 and 558 provide for the use of a premix containing sulfadimethoxine 113.5 grams per pound and ormetoprim 22.7 grams per pound. The premix is intended for the manufacture of medicated feed for use in salmonids to control furunculosis. This supplement extends the claim to include control of enteric septicemia in catfish.

II. Indications For Use

The present application provides for the use of Romet® -30 at a dose level of 50 mg/kg of fish/day for 5 days in the feed for the control of bacterial infections in catfish caused by *Edwardsiella ictaluri* (enteric septicemia of catfish).

III. Dosage Form

Finished feeds manufactured from: Romet® -30 Premix.
Each pound of premix contains 113.5 g (25%) of sulfadimethoxine and 22.7 g (5%) of ormetoprim. Finished feeds will be medicated to provide 50 mg of total drug ingredients per kg of fish body weight per day.

Route of Administration

Oral administration via the feed.

Recommended Dosage

Romet 50 mg/kg of fish body weight/day for 5 consecutive days administered to catfish via the feed.

IV. Animal Efficacy

In the present NADA it has been established that Romet administered at a dose level of 50 mg/kg of fish/day for 5 consecutive days in the feed is effective in the control of natural outbreaks of enteric septicemia of catfish.

A. Pivotal Studies

Four well controlled studies were conducted to evaluate the therapeutic efficacy of Romet in the control of enteric septicemia of catfish (ESC). Two studies were conducted in the state of Mississippi and the other in the state of Alabama, the major producers of catfish in the country.

Romet at a dose level of 50 mg/kg of fish body weight was administered for 5 consecutive days in an effort to control two natural outbreaks of ESC.

In one study involving an 18 acre pond containing approximately 130,000 fish 349 g weight, three groups of 35 fish and one group of 41 were confined by means of holding nets within the 18 acres pond where the outbreak developed. The fish in the pond and in two of the holding nets received Romet medicated feed, while the fish in the other two holding nets received nonmedicated feed. In the second study involving a one acre pond containing approximately 55,000 fingerling catfish of 227 g weight, 1184 fish were confined in a holding net within the pond where the outbreak developed. The fish in the pond received Romet medicated feed, while those in the holding net received nonmedicated feed.

There was a rapid decrease in the daily mortality rate in the medicated, non-confined fish, from 0.05 to 0.01% in the first study and from 0.28 to 0.11% in the second study during the 5 day time of treatment. This very low incidence of mortality persisted during the 2 week post-treatment observation.

There was a statistically significant difference ($P<0.01$) between Romet medicated (1.88% mortality) and nonmedicated fish (3.53% mortality) in the second study while there was no statistically significant difference between Romet medicated (6.06% mortality) and nonmedicated fish (14.40% mortality) in the first study (Dr. M. Beleau, College of Veterinary Medicine, Mississippi State University, Delta Branch Experiment Station, Stoneville, MI).

Two field studies were conducted at the Auburn University Experiment Station to evaluate the therapeutic efficacy of Romet used at 50 mg/kg of fish body weight in the control of ESC. Six groups of 200 fingerling catfish were infected in each trial by immersion into water containing an appropriate concentration of *Edwardsiella ictaluri*, the agent of ESC. Each group of fish was then placed into a 3,125 gallon concrete raceway where the fish were kept until the end of the trial. In the first study medicated feed was administered to 3 randomly selected groups (infected, medicated fish) starting at 1 day after infection. In the second study the medicated feed was administered starting on the same day as the infection. The other 3 infected groups were maintained on nonmedicated feed (infected nonmedicated fish). In both studies the medicated feed was administered for 5 consecutive days and was followed by a 14 day post medication observation period. Three groups of noninfected, nonmedicated control fish were also included in each study. Mortality was the main parameter for evaluating the therapeutic efficacy of the compound.

In the first sudy the incidence of mortality in the Romet medicated fish (0.83%) was significantly ($P<0.01$) lower than that of nonmedicated fish (4.17%). In the second trial there was also a statistically significant ($P<0.05$) difference between the mortality of Romet medicated (2.83%) and nonmedicated (15.00%) fish (Dr. J. Plumb, Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL).

B. Supportive Studies

1. Aquarium Studies

Studies to establish the optimal dose level of Romet to be used in the control of ESC were conducted in two separate locations: one at the College of Veterinary Medicine, Mississippi State University (MSU), and the other at the Department of Fisheries, Auburn University.

Pond raised healthy channel catfish fingerlings were used as the experimental animals. After one week of acclimation, the fish were infected using *E. ictaluri* strains recently isolated from natural outbreaks of ESC. In every study, noninfected control fish were exposed to the same manipulations as those being infected, except for the absence of the infecting agent.

In the four aquarium studies conducted at the College of Veterinary Medicine, MSU, fingerling catfish were infected by intraperitoneal injection with *E. ictaluri*. In all studies the fish were kept in flow-through aquaria, 20 fish per aquarium, and each treatment regimen was administered to 5 randomly selected aquaria for 5 consecutive days followed by 14 days observation. Also 5 aquaria, each containing 20 placebo inoculated fish, were used in each study as noninfected, nonmedicated controls. Romet dose levels of 0, 50 and 100 mg/kg were administered in the first two studies. In the first study the mortality rate of the 50 mg/kg (84%) and 100 mg/kg (72%) treated fish was significantly lower ($P<0.01$) than that of the non-treated fish (99%). In the second study the mortality rate of the 50 and 100 mg/kg treated fish was respectively 55% and 44% and was also significantly lower ($P<0.01$) than that of the nontreated fish (88%).

In the third study Romet dose levels of 0, 25, 50, 100 and 150 mg/kg were administered and resulted in mortality rates of respectively 14.73, 4.21, 3.16, 5.26 and 1.05%. The mortality rate of all Romet medicated fish was significantly lower ($P<0.01$) than that of the infected nonmedicated fish.

In the fourth study Romet dose levels of 0, 12.5, 25, 50 and 75 mg/kg were administered. Mortality rates were respectively 18, 11, 11, 13 and 2%. Only the 75 mg/kg dose level mortality rate was significantly lower ($P<0.05$) than that of the infected nonmedicated fish.

Within each of the four studies there was no statistically significant difference in mortality rates between the different dose levels of Romet tested (Dr. P. Bowser, College of Veterinary Medicine, Mississippi State University, Mississippi State, MI).

Two dose level titration studies were conducted at Auburn University. Fingerling catfish were infected in bulk by immersion into water

contaminated with *E. ictaluri*. The fish were then subdivided into 25 fifty liter aquaria, 5 fish per aquarium and medicated using feed containing 0, 25, 50 and 75 mg of Romet per kg of fish body weight, starting at 3 days post infection. Each treatment regimen was administered to 5 randomly selected aquaria for 5 consecutive days and was followed by a 14-day post treatment observation period. Five aquaria of noninfected (inoculum placebo) fish were used as controls in both studies. The main parameter for evaluating the therapeutic efficacy of the treatments was mortality.

Mortality rates in the first study were respectively 88, 48, 40 and 32%. There was a statistically significant difference ($P<0.05$) between the 50 and 75 mg/kg treatment and the 0 mg/kg treatment regimens. Mortality rates in the second study were respectively 32, 28, 24 and 16%. Even though there was a numerical difference, there was no statistically significant difference between the 25, 50 and 75 mg/kg and the 0 mg/kg treatment regimen.

When the data of these two studies were pooled, there was a significantly lower mortality, as compared to the 0 mg/kg dose level, of fish treated with Romet dose levels of 25 mg/kg ($P<.10$), 50 mg/kg ($P<0.05$) and 75 mg/kg ($P<0.01$) (Dr. J. Plumb, Alabama Agricultural Experiment Station, Auburn University, Auburn, AL).

Despite the fact that the six dose level titration studies were conducted using different environmental conditions (water, feed, flow thru versus static aquaria), different size, age and genetic background fish, different *E. ictaluri* inoculum strains, different routes of infection (parenteral versus bath) and different treatment regimens (medication started at different times with respect to the infection), no optimal dose level of Romet could be selected for use in field studies.

On August 20, 1984, a conference to review the available aquarium dose level titration data and decide on the Romet dose level to be used in field studies was attended by the U.S. Department of Interior, Fish and Wild Life Service (FWS), University of Auburn and of Mississippi, Food and Drug Administration (FDA), IR-4 program and Hoffmann-La Roche representatives.

FWS representatives recommended the use of 50 mg of Romet per kg of body weight per day for five consecutive days for field studies. This recommendation was accepted by FDA representatives based upon the following considerations:

i. The difficulty encountered in interpretation of the data obtained from the dose titration studies conducted under varying environmental conditions (water, feed, flow through versus static aquaria), different size, age and genetic background fish, different *E. ictaluri* inoculum strains, different routes of infection (parenteral versus bath) and different treatment regimens (medication started at different times with respect to the infection), no optimal dose level of Romet could be selected for use in field studies.

ii. Information provided by Dr. F. Meyer provided historical information which demonstrates that drug use levels are very comparable between cold water species and warm water species. The dosage regimen of 50 mg/kg body weight for salmonids (cold water species) was the subject of a previously approved supplement to this NADA. Personal communications with Drs. G. Bullock, F. Meyer, and R. Herman (FWS) confirm the high level of efficacy under condition of use in the U.S.

2. In vitro Studies

Laboratory data showed that 1) potentiation in antibacterial activity was evident using the combination of sulfadimethoxine + ormetoprim at the 5:1 ratio and 2) in vitro resistance to Romet developed at a much slower rate than resistance to its two components: sulfadimethoxine and ormetoprim (Dr. G. Bullock, National Fisheries Center, Leetown, WV, Dr. G. Maestrone, Hoffmann-La Roche, Inc., Nutley, N.J.).

The in vitro activity of Romet against pathogenic fish origin bacteria was evaluated against 128 strains, isolated from natural outbreaks of systemic bacterial diseases of cold and warm water fish in various geographical areas of the United States.

Eighty percent of the strains were susceptible in vitro to Romet while only fifty-two percent were susceptible to sulfadimethoxine (Dr. G. Bullock, National Fisheries Center, Leetown, WV, Mr. A. Mitchell, Fish Farming Experiment Station, Stuttgart, AR, Dr. G. Maestrone, Hoffmann-La Roche Inc., Nutley, N.J.).

Five *Aeromonas hydrophila* catfish origin field isolates were evaluated for susceptibility to Romet using the agar diffusion procedure at the Fish Experimental Station in Stuttgart, Arkansas. Four out of the 5 strains were susceptible (Mr. A. Mitchell, Fish Farming Experiment Station, Stuttgart, AR).

Further information on the in vitro activity of Romet against catfish bacterial isolates was obtained in 1981, 1982 and 1983 in cooperation with the Diagnostic Laboratories of the Mississippi Cooperative Extension Service. One-thousand seventeen strains obtained from disease outbreaks of bacterial origin were tested for susceptibility to Romet using the agar diffusion procedure and 942 (92.7%) were susceptible. Of the 383 *E. ictaluri* strains tested, 368 (96%) were susceptible to Romet (Dr. T. Wellborn, Mississippi Cooperative Extension Service, Starkville, MI, Dr. T. Schwedler, Mississippi Cooperative Extension Service, Stoneville, MI, Dr. G. Maestrone, Hoffmann-La Roche Inc., Nutley, N.J.).

Seven strains of *E. ictaluri* isolated from ESC outbreaks in Mississippi were evaluated for susceptibility to Romet and to 11 other antibacterial agents using the agar diffusion procedure. All strains were susceptible to streptomycin, gentamicin, neomycin, chloramphenicol, tetracycline, nitrofurantoin, sulfadimethoxine, myxin, cephalothin and to potentiated sulfadimethoxine (Romet), 5 were moderately susceptible and 2 resistant to

penicillin, 5 were susceptible and 2 resistant to ampicillin (Dr. G. Maestrone, Hoffmann-La Roche Inc., Nutley, N.J.).

Data on the minimum inhibitory concentration (MIC) of Romet for 10 *E. Ictaluri* strains were obtained using the tube dilution procedure. Eight out of 10 strains were inhibited by concentrations of ≤0.8 mcg/ml while 1 was inhibited by a concentration of 4 mcg/ml and 1 was not inhibited by a concentration of 100 mcg/ml (Dr. P. Bowser, Mississippi State University, College of Veterinary Medicine, Mississippi State, MI).

3. Field Studies

The therapeutic efficacy of Romet medicated feed was evaluated in the treatment of 182 natural outbreaks of enteric septicemia of catfish in commercial production ponds (5 to 27 acres each) under the supervision of Dr. M. Beleau of Mississippi State University, Stoneville, Mississippi.

Out of the 182 trials, 163 (89.6%) showed various degrees of improvement while the remaining 19 (10.4%) showed no improvement. Within five days, a decrease in mortality of 80% or higher was present in 137 (75.3%) and a decrease in mortality of at least 50% was present in 160 (87.9%) trials. In many of the 10.4% of the trials which showed no improvement, a disease complex involving viral and/or parasitic infections were evident.

V. ANIMAL SAFETY

C. Toxicity:

The safety of Romet and its components, sulfadimethoxine and ormetoprim, for catfish was evaluated by exposing the fish to increasing concentrations of the solubilized compounds in water for 96 hours. This toxicity test is standardized in accordance with the Committee of Methods for Toxicity Tests for Aquatic Organism, EPA-60/3-75-009. The test parameters are annotated during exposure and for 14 days after exposure to the test compound.

The highest concentrations of the compounds evaluated: namely, 600 parts per million (ppm) of Romet, 400 ppm of sulfadimethoxine and 200 ppm of ormetoprim, were not toxic for catfish (Mr. L. Marking, FWS, National Fish Research Laboratory, La Crosse, WI).

The LC_{50} (concentration lethal to 50% of test species) was determined in the blue gill which belong to the same warm water family of fish as catfish. The LC_{50} was greater than 20,000 ppm.

D. Palatability-Toxicity:

The data are included in the Freedom of Information summary for Master File #5056 and is on display in the Dockets Management Branch (HFA-305), Food and Drug Administration, Room 4-62, 5600 Fishers Lane, Rockville, Maryland, 20857, from 9 a.m. to 4 p.m., Monday through Friday.

VI. HUMAN SAFETY

The toxicity of these compounds has been previously submitted, accepted, and used, as provided by 21 CFR 556.490 and 556.640, to establish tolerances of 0.1 part per

million (ppm) for ormetoprim and sulfadimethoxine in the edible tissues of chickens, turkeys, ducks, salmonids, and catfish.

Practicable regulatory analytical methods for determination of tissue residues of ormetoprim and sulfadimethoxine have been published and are on file in the Food Additives Analytical Manual on display in the Public Records and Document Center, Food and Drug Administration, Rockville, Maryland.

The regulatory procedure for sulfadimethoxine has been compared using both gas and liquid chromatography to validate the colorimetric readout using five tissues (liver, muscle, skin, kidney and intestinal fat) from five species (duck, chicken, beef, swine, and turkey) with all results found to be equivalent. This study was reported to NADA 40—209V on February 9, 1977.

The regulatory methods described were used for both sulfadimethoxine and ormetoprim assays in catfish fillets as the only edible portion with the method validation recovery values determined as part of the study. Sensitivity of the methods was 0.05 ppm.

A tissue depletion study of Romet in catfish was conducted, as part of an environmental study, by Dr. M. H. Beleau at the Mississippi State University Delta Experiment Station, Stoneville, Mississippi, from August 1, 1985 to September 14, 1985.

The trial site was a one acre production pond (DBES pond #35) estimated to contain fish weighing a total of 8,549 lbs. at the start of the experiment.

The catfish received 58.4 mg Romet (5:1, SDM:OMP) per kg body weight/day for five days in their sinking medicated feed. Ten fish were taken four hours after the last feeding of medicated diet with the muscle filleted and quick frozen. This process was repeated for post last dose withdrawal days 1 through 7, 9, 13 and 17 while the fish were being fed unmedicated diet.

The sulfadimethoxine and ormetoprim assays for the 10 catfish fillets on each withdrawal day are listed in the following table.

Withdrawal Day	Concentration (ppm + S.D.)	
	Sulfadimethoxine	Ormetoprim
0	0.77 ± 0.57	0.68*
1	0.07 ± 0.04	ND**
2	ND	ND
3	ND	ND
4	ND	-

*Value represents 1 sample out of 10
**ND = less than 0.05 ppm

Statistical analysis of the data using the 99% tolerance limit with 95% confidence from the residue depletion study supports the assignment of a 3-day withdrawal period for catfish treated with Romet at 50 mg/kg for 5 days.

Based on our knowledge of the metabolism of these compounds, it is the Agency's scientific judgment that no unique metabolites of toxicological concern would be

formed in catfish. Therefore, a metabolism study was not required for the approval of this minor species use of the drug product.

VII. AGENCY CONCLUSIONS

The use of sulfadimethoxine and ormetoprim combination in catfish is approvable under the Agency's minor species regulation (48 FR 1922; January 14, 1983).

The data submitted in support of this supplemental NADA comply with the requirements of Section 512 of the Act and demonstrate that sulfadimethoxine and ormetoprim combination when used under its proposed conditions of use is safe and effective for the control of enteric septicemia of catfish caused by *Edwardsiella ictaluri* strains susceptible to this combination product.

Generally, sulfonamide products for use in food producing animals are over-the-counter products. Furthermore, the diagnosis and treatment of enteric septicemia of catfish are conducted by laity. Moreover, this product has been approved for use in salmonids (21 CFR 558.575) and a similar combination product is approved for non-prescription use in food producing chickens, ducks, and turkeys. Therefore, this product is approved as an over-the-counter product.