

**STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE
INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION
FOR POTASSIUM PERMANGANATE (INAD #9246)**

Sponsor:

U.S. Fish and Wildlife Service, Division of Fish Hatcheries

Sponsor Signature

Date Approved

Manufacturer:

Carus Chemical Company
315 5th Street
Peru, IL 61354-0599

Facility for Coordination of Potassium Permanganate INAD:

Bozeman National INAD Office
4050 Bridger Canyon Road
Bozeman, MT 59715

Proposed Starting Date

June 1, 1995

Proposed Ending Date

May 31, 1996

Study Director

Mr. Jim Bowker

Study Director Signature

Date

Facility Location of Clinical Field Trial Including Trial Number:

Type or Print Facility Name

Trial Number

Investigator _____

Type or Print Name

Investigator Signature

Date

STUDY PROTOCOL FOR A COMPASSIONATE AQUACULTURE INVESTIGATIONAL NEW ANIMAL DRUG (INAD) EXEMPTION FOR POTASSIUM PERMANGANATE UNDER INAD #9246

I. STUDY ID AND TITLE

Clinical field trials to determine the effectiveness of potassium permanganate in controlling protozoan and metazoan ectoparasitic, bacterial, and fungal diseases on a variety of cultured warmwater fish species.

II. SPONSOR

Dr. David Erdahl, U.S. Fish and Wildlife Service, Branch Chief, Aquatic Animal Drug Approval Partnership Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-587-9265 x 125; Fax: 406-582-0242; Email: dave_erdahl@fws.gov

Manufacturer: Carus Chemical Company, 315 5th Street, Peru, IL 61354-0599.

Study Director: Mr. Jim Bowker, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program, 4050 Bridger Canyon Road, Bozeman, MT 59715; Phone: 406-587-9265 x 126; Fax: 406-582-0242; Email: jim_bowker@fws.gov.

Principal Regional INAD Coordinators: See Appendix I for names and addresses.

Study Monitors for Potassium Permanganate INAD: See Appendix II for names and addresses.

III. INVESTIGATORS/FACILITIES

See Appendix III for names and addresses. Each facility has been assigned a trial number that reflects the INAD number (9246) and a unique number for that facility (e.g., Lamar NFH 9246-01).

IV. PROPOSED STARTING AND COMPLETION DATES

Proposed Starting Date: June 1, 1995

Proposed Completion Date: May 31, 1996

V. BACKGROUND/PURPOSE

A. Protozoan and Metazoan parasitic disease:

Protozoan and metazoan ectoparasites are responsible for over 40% of diseases of cultured warmwater fishes. Among these are obligate parasites that require a fish host and other, normally facultative, organisms that appear to become pathogens when poor water quality, poor health, or poor nutrition cause unacceptably high stress on fish. The genera represented in this group of protozoan parasites include all Trichodinids, *Ichtyobodo*, *Ambiphyra*, *Trichophyra*, *Ichthyophthirius*, *Apiosoma*, and *Chilodonella*. A few other genera combine to represent about 1% of reported ectoparasitic diseases. Genera of metazoan ectoparasites known to cause fish diseases include *Cleidodiscus*, *Gyrodactylus*, and *Dactylogyrus*. Diagnosis is relatively simple and the different ectoparasites are easily differentiated, morphologically. When an ectoparasite infection is established, serious gill and surface damage can occur in a very short time. The outcome may be the death of the fish as a direct result of damage done by the parasites, or secondary infections may be established due to exposure of sensitive unprotected areas to water borne bacteria and fungi.

B. Flavobacteriosis:

Flavobacteriosis is an acute to chronic bacterial infection that has been reported as a mortality factor in several species of cultured catfish, bait minnows, goldfish, basses, and sunfish (Post 1987). The definition of Flavobacteriosis is found in Public Master File #5456 with FDA. This disease is reported to occur in 33% of warmwater diseases. Optimum temperatures for its occurrence are near 28 to 30°C, but epizootics will occur in cultured fishes at 10 to 17°C. Flavobacteriosis seldom occurs in waters below 10°C; thus outbreaks are the highest in the summer months. Stressors such as crowding and handling are predisposing factors, although highly virulent strains of flavobacteria can cause outbreaks even in cooler waters.

The transmission of Flavobacteriosis from fish to fish occurs directly through the water. Fish infected with the organism can harbor it over winter and then can become sources of infection during the summer months (Nelson et al. 1988).

Flavobacteriosis first invades the skin of the head region, including the mouth, lips, cheeks and gills or injuries to the body of the fish. The type of lesions varies with the species of fish. In scaleless fish such as channel catfish, the lesions are small and circular with gray-blue necrotic centers and red margins surrounded by a ring of inflamed tissue. In scaled fish, necrotic lesions begin at the outer margin of the fins and spread toward the body. The gills may be involved and demonstrate light-colored areas at the tips of the gill filaments. Infected areas on surface or gills may be found to contain many characteristic microscopically visible masses of bacterial growth. As the disease progresses, gill filaments are lost to advancing necrosis and sloughing of gill tissue (Bullock et al. 1986). The bacterium may invade the blood stream through a gill or skin lesion and become systemic. Flavobacteriosis disease is usually fatal within a relatively short time following development of a bacteremia (Post 1987).

C. Fungal disease:

The incidence of fungal diseases varies between 3 and 8% from year to year in warmwater fishes, and the severity in any year may depend largely on the severity of the winter season. Fungal species of the genera *Saprolegnia*, *Achlya*, *Aphanomyces*, *Leptomitus*, and *Pythium* have been reported as causing fish disease, with *Saprolegnia* as the most commonly involved. When the integrity of the skin or scale

barrier is broken or the mucus layer is removed, fungal growth may begin from invading aquatic borne fungal zoospores that are always present in fish culture water. Although these pathogens are considered to be secondary invaders, once a fungal colony is established it can continue to grow and spread and can, without treatment, lead to the death of large numbers of fish.

While precise identification of fungus specimens from lesions on fish requires a considerable level of familiarity with taxonomy of the aquatic Phycomycetes, the detection of a significant level of fungal involvement does not require rigorous classification. Fungal infection is recognized as white to brown or darker patches of cottony growth on lesions on the body surface, on the fins and barbels, or in the mouth. Microscopically, individual hyphae are evident that are non-septate and about 20 microns in diameter. Older segments of hyphae often terminate in zoosporangia containing zoospores. Once recognized, the growth of an aquatic phycomycete on lesions is difficult to confuse with any other aquatic form.

D. Control of parasites, flavobacteriosis, and fungal diseases:

In many situations potassium permanganate is the preferred treatment of fish with surface infections involving protozoan and metazoan parasites, flavobacteriosis, and aquatic fungi. The treatment is especially effective against Trichodinids, *Ambiphyra* and external bacterial columnaris, but generally less effective against *Trichophyra*. Potassium permanganate is the preferred chemical for treating these infections in circumstances that preclude the use of less expensive chemicals such as copper sulfate. Copper sulfate is not recommended for use in water with alkalinity less than 50 mg/L (as CaCO₃) because of its high toxicity to fish in low alkalinity water. This is especially applicable for salmonids and some other species that are very sensitive to copper toxicity.

Potassium permanganate is a strong oxidizing agent and its oxidizing potential is the key to its effectiveness for controlling external parasitic, bacterial, and fungal diseases. The amount of potassium permanganate needed to provide an effective treatment is determined by applying the chemical in increments of 2 to 4 ppm until a red color (indicative of residual permanganate) persists for 4 to 8 hours.

There is obviously room for a lot of uncertainty in this method of treatment and a procedure has been developed to reduce the error and uncertainty in treating fish culture water with potassium permanganate. The more precise estimation of the amount of potassium permanganate needed for disease control may be obtained by first determining the 15-minute potassium permanganate demand (15-min PPD) of the water to which the chemical is to be applied. The 15-min PPD is a measure of the amount of potassium permanganate that is destroyed by oxidizable organic material in the treated water in a 15-minute period. The amount of potassium permanganate required to provide an effective disease treatment is greater in water containing higher concentrations of oxidizable organic material. The oxidizing potential of potassium permanganate is available to kill parasites, bacteria, or fungi only after the organic material contained in the water has been oxidized.

VI. SPECIFIC OBJECTIVES

- A. The purpose of this compassionate INAD on potassium permanganate is to develop clinical field trial data that will extend the knowledge of the most appropriate treatment regimens and treatment times for controlling flavobacteriosis disease, and fungal diseases of selected cultured fishes. These data will be used to support a new animal drug application (NADA) for potassium permanganate.

The Service anticipates asking that the U.S. Food and Drug Administration (FDA) grant an extension of the potassium permanganate INAD for an additional 2 years at the end of the first treatment season. The Service feels that data from at least 3 treatment seasons will be required in order to adequately assess the effectiveness of potassium permanganate for treating ectoparasitic, bacterial, and fungal diseases of cultured fishes.

- B. This INAD permit will allow the Service to collect scientific data necessary to establish the effectiveness of potassium permanganate for treatment and control of ectoparasitic, bacterial, and fungal diseases of cultured fishes.
- C. Service fish culturists will have the opportunity to legally use potassium permanganate to control ectoparasites, parasites, bacteria, and fungal diseases of cultured fishes, and maintain healthy stocks of fish during the period of time necessary for collection of the data on its effectiveness for an NADA for potassium permanganate in fish.

VII. MATERIALS

Common Name:	Potassium Permanganate
Chemical Name:	Permanganic acid potassium salt
Trade Name:	Cairox
Molecular Formula:	KMnO_4
Appearance:	Dark purple crystals

(See Appendix XI for further information on nomenclature, chemical and physical properties of the pure chemical).

Manufacturer, Source of Supply:	Carus Chemical Company 315 5 th Street Peru, IL 61354-0599
------------------------------------	---

<u>Contact:</u>	Brenda Veronda Ph: 815-224-6557 Fax: 815-224-6697
-----------------	---

Strength and dosage form:

Potassium permanganate is considered 100% active ingredient for purposes of calculating the amount of chemical to be used to satisfy potassium permanganate demand and to use in treatment of fish diseases. The effectiveness of potassium permanganate in disease treatment is due to the oxidizing potential contained in the manganate (MnO_4^-) radical, however, the chemical has traditionally been dealt with as the total weight of the whole product.

VIII. VERIFICATION OF DRUG INTEGRITY AND STRENGTH

The manufacturer, Carus Chemical Company, will provide the analytical data necessary to establish purity of each lot of potassium permanganate supplied. The lot number and date of manufacture for each batch of potassium permanganate will be placed on the label of each container. The form "Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermal Food Animals" (Form 1) will clearly identify the lot number and date of manufacture of potassium permanganate that it accompanies. If the integrity of the potassium permanganate is compromised (i.e., by spilling or contamination of the stock container) the event will be carefully recorded, dated, and signed in the Chemical Use Log (Form 2). The Study Monitor assigned to the Investigator involved will be immediately notified and the remaining material will be returned to the Study Monitor along with the properly recorded Form 1.

IX. STORAGE CONDITIONS

Potassium permanganate will be stored in the original container supplied by the manufacturer with the appropriate investigational label attached. The container will be stored in a cool, dry location away from direct sunlight. The container will NOT be stored in a refrigerator because opening a cold container can cause condensation of moisture on potassium permanganate. Potassium permanganate should be stored in a secure location such as in a locked cabinet.

X. HANDLING PROCEDURES

Each Study Monitor and Investigator will be required to have a current copy of the Material Safety Data Sheet (MSDS) for Potassium permanganate (Appendix IV). Each person involved with the study and each person who may be present during the use of potassium permanganate shall be required to read the MSDS. Safety precautions as outlined in the MSDS will be followed at all times when working with potassium permanganate. Standard laboratory equipment such as gloves, lab coats or aprons, eye protection, etc., will be worn at all times. **As a special precautionary note, potassium permanganate is a powerful oxidizing material and may be explosive in contact with some other chemicals such as formaldehyde.**

XI. ENVIRONMENTAL CONDITIONS

XII. INVESTIGATIONAL LABELING

Copies of the labels to be attached to each container of potassium permanganate are provided in Appendix V. There is a specific label for drugs to be used on research animals, and another label for drugs to be used in clinical field trials. It will be the responsibility of each investigator to insure that a proper investigational label is attached to each container of potassium permanganate immediately upon receipt.

XIII. EXPERIMENTAL UNIT

The experimental unit in this clinical field trial will consist of a contained or isolated group of fish. This could be a group of fish contained in a tank, raceway, pond, etc. The experimental unit will **not** be individual animals.

XIV. TREATMENT GROUPS:

Separately confined, untreated, controls will not be required in the tests conducted to determine the effectiveness of potassium permanganate. Each group of treated fish will serve as its own control. Fish from a group will first be examined to determine if a treatment with potassium permanganate is required. When the treatment is underway or has been completed, fish from the same group (perhaps the same fish) will be examined to determine the effect of the treatment on the parameters used to sanction the treatment initially. This may consist (for example) of determining the percent reduction in the number of parasites per gill filament or per field of view and data will be recorded in these terms.

Whenever possible, investigators are encouraged to segregate subsamples of infected fish for use as untreated (negative) control groups to document the virulence of outbreaks of disease. Not only do control animals establish that disease is present or more severe when a drug is not used, they are useful to validate adverse drug reactions in the treated animals. Assignments to the control and treatment groups should be random and designed to avoid bias. Control animals should be kept under conditions as similar as possible to treated animals for valid comparison.

XV. ENTRANCE CRITERIA

A. The characteristics of the study animals:

Species, size, number, and all other pertinent information is presented in Appendix VI.

B. Diagnosis of disease:

1. Protozoan parasites

- a. Initial indications that protozoan parasites may be present on fish will involve changes in behavior and gross appearance. Most of the diagnosis of the disease will be provided by the Investigator. If there is any question concerning the diagnosis, the Study Monitor will confirm the diagnosis. Descriptions of fish behavior and appearance suggesting the presence of protozoan parasites may include the following:
 - 1) Flashing, scratching, or rubbing against objects in the pond or raceway. Twitching, darting, or convulsing.
 - 2) "Topping" or "piping" at the water surface; Appear to be gasping for air.
 - 3) Failure to feed.
 - 4) Gills appear swollen, discolored, mottled bright red, may bleed freely when touched.
 - 5) Areas of excess mucus. Nodules, white spots, or pustules appear on surface, fins, or gills.
 - 6) Frayed fins or tail, eroded tail.
- b. Fish selected for use as diagnostic samples should represent the diseased portion of the population. If such a collection is not possible, the second choice will be a random sampling of the fish. Selection techniques that produce the most healthy and vigorous fish, such as hook and line, should not be used.

The sampling techniques used for selecting diagnostic subjects should be used for selecting subjects for evaluating the results of the treatment.

- c. Definitive diagnosis depends on microscopic examination of excised gill filaments, fin clippings, or mucus scrapings for the presence of identifiable protozoans. A minimum of 5 fish should be selected and examined. Fish can be anesthetized with MS-222 to reduce trauma and to make the fish easier to handle. From each fish at least 5 gill filaments, clipped close to the gill arch should be wet-mounted on a clean slide, spread apart, and cover slipped. Terminal areas of fin rays should be clipped from each fish and wet-mounted. Mucus samples from the head and from the skin or scale surface (near the mid region along the lateral line) should be taken, placed on a slide and cover slipped.

The slides should be examined within 5 minutes of preparation. Mounted specimens should be scanned at dissecting scope range (25-40X), low power (100X), and high power (400-450X). Protozoans can be identified to genus level by reference to numerous guides for identification of fish parasites.

- d. The decision to apply potassium permanganate should be based on the presence and density of identifiable protozoan parasites. Prophylactic treatments unsupported by microscopically observed and recorded protozoan parasites are not to be conducted under this research protocol. The decision to treat with potassium permanganate may be reached in a number of ways, but any decision must be documented. Circumstances vary and parasite loads and distributions will differ. However the decision to apply a treatment is made, it is necessary to have a basis for treatment and to have a record of the kinds and numbers of parasites present before treatment, to make it possible to later evaluate the success or failure of the treatment. The following are examples of observations that would warrant treatment (modified from Schaperclaus 1986). These examples are not intended to restrict the freedom of managers in deciding when treatment is needed, and there are many other sets of observations that would support a decision to treat.
- 1) An average of 25 or more trichodinids per gill filament in a wet mount of excised gill tissue in 3 of 5 fish examined. For fry and small fingerlings (less than 3"), 4 or more trichodinids per filament.
 - 2) An average of 50 or more trichodinids per field (25-40X) in a wet mount of a clipped terminal fin ray in 3 of 5 fish examined. In fry or small fingerlings, 10 or more per field.
 - 3) An average of 25 or more *Chilodonella* sp. per filament in a wet mount of excised gill tissue in 1 of 5 fish examined. Treat if any are found on small fingerlings or fry.
 - 4) An average of 20 or more mixed protozoans per microscopic field (100X) in mucus samples from 5 of 5 fish examined.
 - 5) An average of 1 *Ichthyophthirius* per fish for 3 of 5 fish examined. In cooler temperatures, treat whenever seen in fry and young fingerlings.
 - 6) An average of 50 or more *Ambiphyra* per gill filament in 3 of 5 fish examined. In fry or small fingerlings, 10 or more per filament.
 - 7) An average of 25 or more *Costia* sp. per filament in 1 of 5 fish examined.

A lower level if seen in 4 of 5 fish examined and any level seen in fry and young fingerlings.

- 8) An average of 40 or more *Apiosoma sp.* per gill filament or per field of view (40X).

2. Fungal diseases

Fish most often have fungal infections during periods of cool to cold water temperatures (45 to 70°F); however, malnourishment and infections caused by other microorganisms may result in secondary fungal infections at any time of the year. The infection initially appears as white to dirty brown cottony patches that may be confined to the site of some other disease lesion or injury or may be spread over a larger portion of the animal.

Microscopic examination of excised gill filaments, scrapings from skin or scales, and fin clippings that reveal non septate hyphae that terminate in sporangia containing zoospores is sufficient evidence to warrant consideration of treatment. The presence of 5% or more of the total population displaying visible signs of fungal infections that prove positive microscopically, or in 2 of 5 selected fish, is sufficient to warrant treatment of the pond or raceway with potassium permanganate.

3. Flavobacteriosis

Lesions characteristic of flavobacteriosis initially occur on the body, head, fins, or gills as white to off-white spots that may be slightly eroded. Lesions on the skin usually become shallow ulcers that can progress deeply into the tissue and even expose underlying bone. On fins there is a loss of tissue from fin rays and the fins and tail take on a tattered appearance. Lesions often start on the back near the dorsal fin and progress around the sides and under the abdomen giving a saddle-back appearance. Surface lesions involving skin or fin surfaces may have a yellowish coloration. Lesions on gills consist of areas of necrosis that start at the distal end of filaments and progress toward the gill arch. Several filaments may be involved and the lesion may spread laterally as well as becoming more penetrating.

- a. The diagnosis of flavobacteriosis is based on the presence of long, thin, bacterial rods in necrotic lesions on the surface of the skin or gills. Microscopic examination of gills or other necrotic tissues will reveal piles of the rod-shaped bacterium in a "haystack" appearance in wet mount preparations. For this observation, the wet mount should be prepared and a period of 15 minutes allowed to lapse before examination. This delay gives time for the "haystack" characteristic to develop. Treatment can commence with the confirmation of this diagnosis. At least one of the following definitive diagnosis could follow to further identify the disease.
- b. Definitive diagnosis of flavobacteriosis requires isolation of the bacterium on cytophaga medium or slide agglutination test using flavobacteriosis antiserum. The growth of *Flavobacteria* on solid media is yellow-green, with flat, rough, spreading colonies which adhere to the media (Post 1987).
- c. Another definitive method (Griffin 1992) involves the biochemical and cultural characteristics that are unique to flavobacteriosis. Four characteristics can be observed directly on the original isolation plate: (1) growth in the presence of neomycin sulfate and polymyxin B; (2) color and colonial morphology

consistent with this typical bacterium; (3) production of a diffusible, gelatin-degrading enzyme; and (4) binding of aqueous Congo red dye in the surface secretions of the suspect colony. The last characteristic would be observed by using a brief test for the presence of chondroitin AC lyase activity in an agar block excised from the isolation plate. If the bacterium is present, a diffusible enzyme that degrades chondroitin sulfate will be produced. Specific identification of flavobacteriosis can be made in minutes after visible growth appears on primary isolation plates.

- C. Environmental conditions--See Appendix VII for details on environmental considerations at each facility.
- D. Ability of investigator to fulfill all the requirements of the Study Protocol--See Appendix III for example of knowledge required of hatchery managers (i.e., Investigators)

XVI. TREATMENT SCHEDULES:

- A. Routes of administration, volume and dosage calculations, and treatment times:

The route of administration of potassium permanganate will be waterborne. The volume of the rearing unit should be first determined. It is most convenient to express this volume in liters. Each time a treatment is required, it is necessary to determine the potassium permanganate demand (PPD). The amount of potassium permanganate needed to perform a disease treatment can then be calculated according to the formula:

$$\text{Total potassium permanganate (mg/L)} = 2.5 \times \text{PPD (mg/L)}$$

The procedure to obtain the 15-min PPD can be done in the following way:

1. Prepare a stock solution of 1,000 ppm of potassium permanganate by dissolving 1.00 gram of potassium permanganate in 1 liter of distilled water. This solution should be used the day of preparation.
2. Measure 8-1 liter portions of culture water into a set of 8 glass beakers.
3. Prepare from 1 through 8 ppm concentrations of potassium permanganate in the culture water samples by adding 1 to 8 mL of the 1,000 ppm stock solution to the beakers of culture water. Stir immediately and observe for 15 minutes.
4. After 15 minutes evaluate the differences in the colors in the beakers and determine the lowest concentration of potassium permanganate that still has a faint pink color. This concentration represents the 15-min PPD.

The treatment rate for the culture water, either tank or pond, can then be calculated by multiplying the 15-min PPD by 2.5. This multiple has been shown to very closely estimate the amount of potassium permanganate needed for an effective disease treatment. The chemical should still be applied in increments of 2 to 4 ppm to avoid excessively high short-time concentration. The maximum treatment rate will not exceed 10 ppm potassium permanganate.

The proper amount of dry chemical for a pond treatment can be broadcast evenly over the surface of the water, dissolved from a bag containing the chemical as the bag is pulled through the water, or applied as dry chemical into the wake of a paddle wheel or aerator that circulates the treated water throughout the container. No provision is made for replacement of the water when treatment is applied to a static pond.

The amount of potassium permanganate needed for treating a tank or raceway will be accurately weighed and dissolved in water. The stock solution will then be applied to the container holding the fish to be treated, and uniformly mixed to achieve the prescribed treatment concentration (bath) or metered for 1 hour at a flow rate adequate to achieve the desired treatment concentration (flowing system).

B. Dosing interval and repetition:

A single treatment with potassium permanganate is usually adequate to control an outbreak of protozoan parasites, fungal diseases, or bacterial columnaris disease, so a second or subsequent treatment is usually not called for. Potassium permanganate may be used again on the same population of fish, but in most cases the disease incidents will be unrelated.

Special variations of treatment are recommended for certain fish diseases and the special considerations are used in determining the proper treatment. The most notable exception to the "one disease:one treatment" rule is in the treatment of Ichthyophthiriosis. The protozoan, *Ichthyophthirius multifiliis*, cause of "Ich" or "white spot", has a life cycle which proceeds in stages that at times do, and at times do not, involve infection of the fish host. During the stage of host association, treatment is ineffective; the parasite is protected by the integument of the fish. When the protozoan is in a free swimming stage, away from the fish, it can be readily killed with potassium permanganate. The transformation from stage to stage takes 4 to 70 days, depending on water temperature and at temperatures above about 27°C the protozoan enters a non infective phase in its life cycle. While temperatures remain in the range of 7°C to 26°C the protozoan repeatedly cycles from the host associated stage to the free-swimming stage. To effectively remove the infection from a population of fish, the free-swimming stage must be treated each time it appears, so repeated treatments must be applied, at intervals related to water temperature, until the disease is under control. The treatment should be repeated at the following frequency:

Water Temperature	Treatment Schedule
80°F	Every day
70°F	Every other day
60°F	Every third day
50°F	Every fourth day
40°F	Once a week

C. Detailed procedures for drug administration

Standard laboratory equipment such as gloves, lab coats or aprons, eye protection, etc. will be worn at all times when working with potassium permanganate. The chemical will be accurately weighed for each treatment, just prior to the treatment.

D. Permissible concomitant therapy

Because efficacy data are being collected during this INAD process, there should be no other concomitant therapy. Preferably, there will be no other therapy during a period of 1 week before and 1 week after potassium permanganate treatment. If concomitant therapy is required to protect stocks of fish, the treatment should be fully documented and appropriately identified so that data obtained during concomitant treatment will not be included in efficacy data for potassium permanganate.

XVII. TREATMENT RESPONSE PARAMETERS

A. Primary Response Parameter:

The primary parameter for evaluating the effectiveness of potassium permanganate in controlling external protozoan parasites, fungal infections, and flavobacteriosis disease, will be the change in the number or density of the microorganisms that were observed before the treatment was conducted and which provided the basis for deciding to perform the treatment.

Fish will be examined after the treatment is completed; this will be after 1 hour, after 4 hours, and after 18 hours, or at times near those intervals, as scheduling permits. Examinations will be performed in the same way as for fish before the treatment.

Data will be recorded in the same quantitative terms that were employed when fish were examined before treatment. A thorough description of before and after treatment conditions of fish should be the objective of the examinations.

B. Secondary Response Parameters:

Secondary parameters that should be documented include mortality, non-quantitative observations of behavior or gross appearance.

C. Adverse Reactions

Any adverse reactions to the treatments should be reported immediately to the study monitor who will in turn notify the INAD Coordinator. This might include changes in water quality, adverse responses of the fish, or hazards to the applicator.

XVIII. ACCOUNTABILITY

Each USFWS Investigator will notify FDA prior to any shipment of potassium permanganate for use under this INAD. Immediately upon placing an order with the approved supplier for potassium permanganate, the investigator will complete Form 1, "Guide for Reporting Investigational New Animal Drug Shipments for Poikilothermic Food Animals" and send it to the Study Monitor. The Study Monitor will then send the original plus two copies to the FDA. The Study Monitor will also send a single copy to the Study Director at the Bozeman National INAD Office. The Investigator will keep one copy of the completed Form 1 for the facility's INAD file. Arrangements should be made between Investigators and Study Monitors to insure completed Form 1s are received by the FDA within 7 days of the date an order was placed.

Potassium permanganate will be shipped in pre-weighed containers of 50 kg from Carus Chemical Company to each of the Principal Regional INAD Coordinators, who will in turn ship the amount of chemical estimated to be required by each Investigator in prepackaged amounts along with a signed Form 1 to the Investigators to which they have been assigned. Form 1 will be signed and dated by each person accepting responsibility for potassium permanganate at each custody transfer. Upon receipt of potassium permanganate, the Investigator will send the original signed Form 1 plus two copies to the INAD Coordinator. Then fax one copy to the Regional INAD Coordinator and one copy to the Study Monitor.

A Chemical Use Log (Form 2) will be supplied to each investigator. Each time any potassium permanganate is used, the weight of the chemical and container will be recorded and compared with the weight of chemical and container recorded after the previous use. The person weighing potassium permanganate will also record the weight of chemical removed, the name and number of the study involved, his/her signature, and the date. After weighing out the

amount of potassium permanganate needed, the container and remaining chemical will be weighed and recorded. At the conclusion of the study, all remaining potassium permanganate will be shipped to the Study Monitor along with the properly recorded Form 1 and the Chemical Use Log (Form 2). The Study Monitor will then verify the Chemical Use Log against the quantity of potassium permanganate remaining. All remaining potassium permanganate will then be returned to the Manufacturer.

A number of items will be necessary for sample collection, observations, etc. Sampling and diagnostic equipment should include scissors, clean microscope slides, cover slips, a compound microscope, and a dissecting microscope.

When the Study Protocol has been approved and treatments are scheduled, the Investigator at each facility covered by the potassium permanganate INAD will need to complete several forms:

Form 1. Guide for reporting investigational new animal drug shipments for poikilothermic food animals.

Form 2. Chemical use log for clinical field trials on potassium permanganate under INAD #9246.

Form 3. Treatment and dispensing record for clinical field trials on potassium permanganate under INAD #9246.

Form 4. Disposal record for animals from clinical field trials on potassium permanganate under INAD #9246.

Copies of these forms are attached to this Study Protocol. A separate record of names, signatures, initials, and dates employed must be maintained until the completion of the study for all persons who sign or initial Forms 1-4.

XIX. RECORD KEEPING PROCEDURES

The data should be recorded in permanent ink (preferably black). The data should be recorded on the official data record forms at the time the observations are made. The raw data should be original, i.e., they should be the first recording of the observations, rather than a transcription of original observations to another data sheet. Each original data sheet should be signed and dated by the person making the observation and recording the entry. If more than one person makes and records the observations, entries should be properly attributed to each person. The data should be accurate and legible. If a mistake is made, it should be crossed out using a single strike-through and the correct data should be recorded next to it; each change to the raw data should be initialed and dated by the person making the change, and a statement should be provided explaining why the change was made. If the data sheet needs to be copied, all data should be transferred, including the properly noted changes; the original record should be retained and submitted with the revised copy, along with a memo explaining the reason for the copying.

XX. DISPOSITION OF INVESTIGATIONAL ANIMALS

Animals that die during a treatment will be disposed of in an approved manner.

We request approval to release treated fish no less than 7 days after treatment. Data do not exist about bioaccumulation of manganese following treatment of fish with therapeutic levels of potassium permanganate. Studies of possible bioaccumulation of manganese suggest that levels of manganese in edible fish tissues (muscle) is controlled homeostatically, in keeping with current knowledge of control mechanisms for other essential metals. Data in Appendix APP-1 show that manganese levels are quite similar in muscle from a variety of fish collected over many years from different locales. Homeostatic control is most evident in a controlled study of bluegill in which whole body and muscle concentrations of manganese were maintained at constant levels against a concentration gradient, for the 500 day duration of the study (See Appendix APP-1, Weiner and Geisy 1979). This is thought to suggest that exposure to manganese through therapeutic use of potassium permanganate for fish diseases will not lead to excessive bioaccumulation of manganese in the edible flesh. Empirical evidence is not available, however, and until such information is available, we request that arbitrary restriction not be applied to management of fish and their release into public waters. The Investigator records the disposition of all treated fish on Form 4.

XXI. DISPOSITION OF INVESTIGATIONAL DRUG

Potassium permanganate will be used only in the manner and by the individuals specified in the Study Protocol. Any potassium permanganate remaining at the end of the study must be returned to the Study Monitors who will verify the use records and the quantity of material remaining and then return unused potassium permanganate to the manufacturer. The investigational drug may not be redistributed to others not specified by the protocol and may not be retained by the Investigator after completion of the study.

XXII. DATA HANDLING, QUALITY CONTROL, MONITORING, ADMINISTRATIVE RESPONSIBILITIES

- A. Drug distribution--See Section VII.A.6. Accountability (page 9) for information and details.
- B. Study Monitors--The Study Monitors are generally fish health professionals with experience in diagnosing and treating fish diseases. There is one Study Monitor assigned for each facility within the USFWS that is covered by the potassium permanganate INAD. The list of these Study Monitors, along with their addresses and phone numbers is presented in Appendix II. The Study Monitors are responsible for supervision of the trials, adherence of the Investigator to the Study Protocol, and inspection of the site.
- C. Special equipment and materials--Most of the equipment and materials required for this study (with the exception of potassium permanganate itself) are already available at each fish hatchery. Diagnosis and treatment of diseases of fish is a common occurrence at most fish hatcheries. Fish hatchery managers (i.e., Investigators) are well trained and well equipped to handle these situations (Appendix IIIb). If any additional equipment or materials are required, they will be provided by the Study Monitors (See Section VII.B. Items needed for sample collection, observations, etc.) (page 10).
- D. Administrator of the drug--Potassium permanganate will be administered directly by the assigned Investigator (fish hatchery manager) or under the Investigator's direct supervision (see Appendix IIIa for names). Potassium permanganate will be

maintained in a secure location and only the Investigator or a person under his/her direct supervision will have access.

- E. Drug accountability records--See Section VII.A.6. Accountability (page 9) for details and Forms 1-4 for actual forms to be used in the study.
- F. Recording observations--The Investigator or a person under his/her direct supervision will be responsible for implementing the Study Protocol, making observations, collecting samples, and recording data during the clinical field trials. After the data have been collected and recorded on the forms, the Investigator sends the data quarterly to the Study Monitors who ensure that all the information is provided. The Study Monitors send the data to the INAD Coordinator through the Principal Regional INAD Coordinators. The INAD Coordinator process the data yearly to be analyzed and summarized, and prepare an annual report that will be submitted to the FDA.
- G. Data storage--The Investigators are responsible for complete and accurate data collection. The originals of Forms 1 and 2 are retained by the Investigator until the completion of the study when they are forwarded to the INAD Coordinator through the Principal Regional INAD Coordinator. The Investigators will make copies of Forms 3 and 4 and retain the copies in secure files. The original raw data on Form 3 will be sent to the Study Monitors on a quarterly basis and Form 4 will be sent on an annual basis. The Study Monitors will carefully check each set of raw data for accuracy and completeness. If there are any discrepancies in the raw data, the Study Monitor will contact the Investigator immediately to rectify the problem. After their review, the Study Monitors will submit the data collected during that quarter to the INAD Coordinator through the Principal Regional INAD Coordinator. The complete set of raw data will be archived by the INAD Coordinator.

XXIII. DATA ANALYSIS

Data analysis will be completed at the Lead Research Center for potassium permanganate, Fish Farming Experimental Laboratory, Stuttgart, Arkansas 71857. Data from the treatment year will be summarized through tabulation and appropriate statistical analysis. An annual report will be prepared for submission to the Sponsor (USFWS, Fisheries) who will in turn submit the report to the FDA. This submission will probably include a request for an extension of the INAD based on the data collected during that year. When sufficient data are collected, the entire INAD data set will be summarized in a final report for submission to support a full NADA.

XXIV. PROTOCOL AMENDMENTS/DEVIATIONS

A signed copy of the Study Protocol must be retained by each Investigator. At any time before the study begins, desired changes in the Study Protocol should be brought to the attention of the Study Director. The desired changes will be fully described in the form of an amendment along with the reason for the change. The amendment will be signed by the Sponsor (or its representative), INAD Coordinator and Investigator. Amendments must be reviewed and approved by the FDA before the study begins. Copies of the signed amendment will be attached to each copy of the Study Protocol. Investigators will be liable for non-compliance violation if drugs are used without a study protocol or differently than specified in the Study Protocol, if forms are not filed on time, or if the study data are not properly collected, maintained, and reported. The Study Monitor is responsible for determining if all the INAD procedures are being followed as defined by the Study Protocol.

Deviations from the established Study Protocol occasionally cannot be avoided. If deviations occur, the Study Monitor should be contacted immediately for advice. Protocol deviations should be fully documented and should be accompanied by a written explanation of what happened, why, and what steps were taken to mitigate the deviation. Deviation statements should be signed and dated. These should be forwarded to the Study Monitor along with the quarterly data summaries and will ultimately be submitted to the INAD Coordinator.

LITERATURE CITED

- Abedin, M.Z., B. Pahlavanpour, and T. Hassan. 1986. Atomic absorption spectrophotometric (AAS) analyses of zinc and manganese in Libyan fresh and canned tuna fish. *Bull. Mar. Biol. Res. Cent., Tajura* 7:46-59.
- Bullock, G.L., Hsu, T.C., and Shott, E.B., Jr., 1986. Columnaris disease of fishes. U.S. Fish and Wildlife Service, Fish Disease Leaflet 72. 92pp.
- Goodyear, P. and C.E. Boyd. 1972. Elemental composition of largemouth bass (Micropterus salmoides). *TAFS* 101:545-547.
- Griffin, B.R. 1992. A simple procedure for identification of Cytophaga columnaris. *J. Aquat. An. Health* 4:63-66.
- Hoffman, G.L. 1967. Parasites of North American freshwater fishes. University of California Press, Berkeley, CA. p. 1-42.
- Hoffman, G.L. and Meyer, F.P. 1974. Parasites of freshwater fishes. T.F.H. Publications, Inc. Neptune City NJ. 224 pp.
- Hughes, J.S., Wellborn, T.L. and Mitchell, A.J. 1990. Parasites and diseases of striped bass and its hybrids. [in *Culture and propagation of striped bass and its hybrids*. ed. Harrell, R.M., Kerbey, J.H., and Minton, R.V.] American Fisheries Society. Bethesda, MD. p.217-238.
- Macmillan, J.R. 1985. Infectious diseases. [in *Developments in aquaculture and fisheries science, Volume 15; Channel catfish culture*. ed. C.S. Tucker] Elsevier, New York. p. 405-496.
- Marshall, K.C. 1979. Biogeochemistry of manganese minerals. [in *Biogeochemical cycling of mineral-forming elements*. eds. P.A. Trudinger and D.J. Swaine] Elsevier, New York. pp 253-292.
- Moore, B.R., Mitchell, A. J., Griffin, B.R. and Hoffman, G.L. 1984. Parasites and diseases of pond fishes. [in *Third Report to the Fish Farmer*, ed. H.K. Dupree.] U. S. fish and Wildlife Service p. 177-205.
- National Research Council. Committee on medical and biologic effects of environmental pollutants. 1973. Manganese. National Academy of Sciences. Washington, D.C. 191 p.
- Nettleton, J.A., W.H. Allen, Jr., L.V. Klatt, W.M.N. Ratnayake, and R.G. Ackman. 1990. Nutrients and chemical residues in one- and two-pound Mississippi farm-raised channel catfish (Ictalurus punctatus). *J. Food Sci.* 55:354-358.
- Post, G.W. 1987. Textbook of fish health. Revised and expanded edition. T.F.H. Publications, Inc., Ltd., Neptune City, NJ. pp.238.
- du Preez, H.H. and G.J. Steyn. 1992. A preliminary investigation of the concentration of selected metals in the tissues and organs of the tigerfish (Hydrocynus vittatus) from the Olifants River, Kruger National Park, South Africa. *Water SA* 18:131-136.
- Schaperclaus, W. 1986. Fish Diseases. [ed. W. Schaperclaus, H. Kulow, and K. Schreckenbach] Akademie-Verlag, Berlin. Translated by M.S.R. Chari. 1991 [Ed. V.S. Kothekar] Amerind Publishing Co. Pvt. Ltd., New Delhi. p. 645-728.

Sorensen, E.M.B. 1991. Metal poisoning in fish. CRC Press. Boca Raton. p. 235-284.

Tucker, C.S. 1989. Method for estimating potassium permanganate disease treatment rates for channel catfish in ponds. *Prog. Fish-Cult*, 51:24-26.

Tucker, C.S. and Robinson, E.H. 1990. Channel catfish farming handbook. Van Nostrand Reinhold, New York, p. 317-380.

Weiner, J.G. and J.P. Geisy, Jr. 1979. Concentrations of Cd, Cu, Mn, Pb, and Zn in fishes in a highly organic softwater pond. *J. Fish. Res. Board Can.* 36:270-279.

FORMS

- Form 1: Guide for reporting Investigational New Animal Drug shipments for poikilothermic food animals
- Form 2: Chemical use log for field trials of potassium permanganate. INAD #9246
- Form 3: Diagnosis and treatment record for field trials of potassium permanganate. INAD #9246
- Form 4: Disposal record for animals from field trials of potassium permanganate. INAD #9246

APPENDICES

- Appendix I: Principal Regional INAD Coordinators for Potassium Permanganate INAD #9246
- Appendix II: Study Monitors for Potassium Permanganate INAD #9246
- Appendix IIIa: USFWS Facilities, and Names of Investigators for Potassium Permanganate INAD #9246
- Appendix IIIb: Sample of Knowledge Required for Position of USFWS Hatchery Managers (i.e., Investigators)
- Appendix IVa: Material Safety Data Sheet for Potassium Permanganate
- Appendix IVb: Toxicity Profile for Potassium Permanganate
- Appendix IVc: Carus Chemical Company Label for Potassium Permanganate Application to Drinking Water.
- Appendix V: Investigational Labels for Potassium Permanganate INAD
- Appendix VI: Treatment Data for Potassium Permanganate INAD
- Appendix VII: National Pollution Discharge Elimination System (NPDES) Information for Potassium Permanganate INAD
- Appendix VIII: Environmental Conditions for Facilities under Potassium Permanganate INAD
- Appendix IX: Common and Scientific Names of Species under Potassium Permanganate INAD
- Appendix X: Investigational New Animal Drug Quality Control Program
- Appendix XI: Levels of Manganese in Edible Tissues of Fish

Appendix IIIb
Sample of Knowledge Required for Position
of USFWS Hatchery Manager
(i.e. Investigators)

Professional knowledge of all facets of fishery biology as well as the ability to apply new scientific findings, developments, and advances toward the resolution of critical propagation problems involving the rearing a variety of fish species under a variety of water quality conditions, water temperatures, water chemistry, etc.

Knowledge of general bacteriology, parasitology, and water chemistry sufficient to treat fish for various diseases.

Skill in interpreting biological observations and ability to draw sound conclusions from available data.

Skill in developing and coordinating available resources to ensure effective management and utilization of manpower, equipment, and funds relative to established priorities and needs.

Skill in coordination of sometimes divergent resource issues to obtain common objectives, including interaction with other Federal and State agencies.

Knowledge of USFWS policy, programs, and organizational structure in order to be able to modify and adapt standard techniques/processes and to devise new strategies and plans necessary to overcome resource problems.

Knowledge of and skill in the use of effective management and supervisory techniques to provide support, guidance, and motivation to hatchery staff.