



FHS NEWSLETTER

FISH HEALTH SECTION-AMERICAN FISHERIES SOCIETY

Volume 21, Number 1

Page 1

Winter 1993

Strain Differentiation of IHNV: Fish Health Management Applications

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An important control measure for infectious hematopoietic necrosis (IHN) involves containment of the disease within areas or watersheds where it is already known to exist and the avoidance of introduction of infected fish and eggs into areas where the virus is not enzootic. This type of control policy is valuable but allows for movements of infected fish within areas where IHN has occurred. This may be detrimental if different strains of the virus exist, especially if these differences are in virulence. Information on strain differences is also essential if effective vaccines are to be developed.

Management schemes for infectious hematopoietic necrosis virus (IHNV) are beginning to implement strain differentiation methodologies. However, there has been no standardization of procedures or management decisions based on results. To date only one serotype of

IHNV has been identified but there are many antigenic subtypes, electropherotypes, and strains which exhibit virulence differences in different species of fish. There also exist a variety of different strain typing schemes. From a fish health management perspective certain parameters that must be defined include criteria to differentiate IHNV

strains, potential methods available, capabilities of diagnostic laboratories, and commercial availability of reagents.

Five biochemical types of IHNV were distinguished by an electropherotyping procedure with sodium dodecyl sulfate, polyacrylamide gel electrophoresis (Hsu et al. 1986). In that study, molecular weight differences were detected primarily in the nucleocapsid (N) protein of the most commonly isolated strains of IHNV. A classification scheme was designed based on these molecular weight differences that allowed placement of most isolates into type 1 - 3 IHNV strain categories. Type 4 and 5 categories were also designated but these included isolates that are no longer detected or that exhibited no distinguishing characteristics. The information to date suggests that type 1 strains are present primarily in British Columbia and Alaska, type 2's in Washington, Oregon and Idaho, and type 3 strains are present in southern Oregon and California. Other studies have examined the susceptibility of different salmonid species to the type 1-3 strains of IHNV. LaPatra et al. (1990) compared a type 1 and type 2 strain of IHNV in different sizes of rainbow trout (*Oncorhynchus mykiss*) and kokanee salmon (*Oncorhynchus nerka*). Kokanee were consistently more susceptible to the type 1 strain and rainbow trout were consistently more susceptible to the type 2 strain. Another study compared the virulence of type 1, 2, and 3 strains (6 total) obtained from fish at hatcheries in Oregon, Washington, California, and Idaho in two different sizes of steelhead (*Oncorhynchus mykiss*) and chinook salmon (*Oncorhynchus tshawytscha*). The results showed that Columbia River basin type 2 IHNV strains were most virulent for steelhead and type 3 IHNV from southern Oregon and California were most virulent for chinook salmon. A type 1 electropherotype was less

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virulent for both species (LaPatra et al. in press). These studies and others suggest that there are biochemical types of IHNV that exhibit virulence differences in different species of salmonids.

Ristow et al. (1989) produced two monoclonal antibodies against the viral nucleoprotein of IHNV. Antibody 1NDW14D recognizes an epitope on all IHNV isolates examined. Another antibody, 2NH105B, recognizes a unique epitope on biochemical type 2 strains of IHNV only. These two antibodies have been used to serologically confirm a replicating agent as IHNV and to determine if that isolate is a type 2 strain (unpublished observations; Mark Engelking, Oregon Department of Fish and Wildlife, personal communication).

These two antibodies can be used in a dot blot immunoassay (Ristow et al. 1991) or fluorescent antibody test (LaPatra et al. 1989; Ristow et al. 1989) for strain typing IHNV isolates. The antibodies available would indicate if the replicating agent was IHNV and if it was a type 2 electropherotype. Definitive strain identification for those isolates that did not react with 105B would not be possible without additional testing. However, by using these two antibodies management agencies and other fish health professionals can begin collecting information for a data base of past and present isolates from fish in a particular watershed or geographic area. Because 105B can identify type 2 strains of IHNV and information available suggests that different electropherotypes exhibit virulence differences in different salmonid species this may be a prudent management tool. Recently, the Pacific Northwest Fish Health Protection Committee unanimously endorsed the typing of IHNV isolates by this scheme. Recommended virus isolate testing frequencies, specific research that could improve this technique, and a commercial source for the monoclonal antibodies were also identified.

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"GREAT SCOT! How Do We Deal With INADS?"

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No, not the misspelled first name of a virologist who works for Clear Springs Food Company. "SCOT" is an acronym for Sub-Committee On Therapeutants - an ad hoc group of the Pacific Northwest Fish Health Protection Committee (PNFHPC) organized to deal with the challenging drug and chemical issues. We recognized last year that the livelihood of public, private and tribal aquaculture in our region had the potential to be severely impacted as a result of the change in the regulatory discretion at the U.S. Food and Drug Administration (FDA). To address these FDA issues and develop regional strategies, SCOT came into being.

SCOT serves many functions within the PNFHPC. First, and perhaps most important, it educates the membership on what is happening in the FDA and other federal agencies on all aspects of drug research, development and regulation. Second, the sub-committee serves as the focal point for initiatives on therapeutants within the region to include: setting the priorities on needed compounds; coordinating the development and implementation of regional compassionate investigational new animal drug application (INADs); and, investigating funding sources to operate INADs. Finally, SCOT prepares communications from the PNFHPC to outside

organizations such as the FDA, International Association of Fish and Wildlife Agencies, Congress, state legislators, and the Joint Sub-Committee on Aquaculture.

There are many examples of SCOT's accomplishments. Working closely with Dr. Christine Moffitt, SCOT has organized two regional INAD's for erythromycin - one for injection, the other for oral application. Erythromycin is used to control bacterial kidney disease caused by *Renibacterium salmoninarum*. The sub-committee spearheaded a letter-writing campaign alerting Congress to the crisis in aquaculture and the critical need for therapeutants. SCOT's current efforts are directed at developing and implementing new INADs in the Pacific Northwest region. Given the current state of affairs, I expect that SCOT will be keeping busy for years to come!

Another Fish Virus or What's TSV?

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A recent published report in the Journal of Aquatic Animal Health describes a new virus that was isolated from ovarian fluid samples taken from Tokul Creek winter steelhead broodstock collected in January, 1990. The virus called Tokul Steelhead Virus (TSV) is described as a small RNA virus like picornavirus or enterovirus. A similar virus has been isolated from Ford Hatchery brown trout broodstock and trout species in California and Oregon. The isolated and purified TSV was reinjected into rainbow trout resulting in no death and the virus could no be reisolated from these fish.

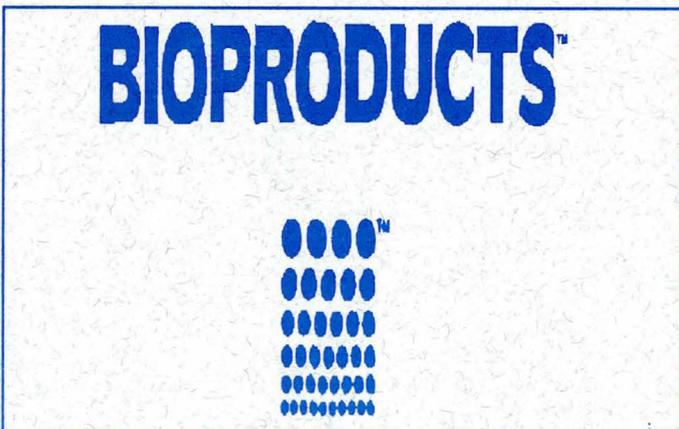
The Effectiveness of Ozone Water Treatment at Cowlitz Trout Hatchery

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Approximately \$3,000,000 was spent by the Tacoma Public Utilities to construct an ozone water treatment system at the Cowlitz Trout Hatchery for control of *Ceratomyxa shasta*. In the past, outbreaks of ceratomyxosis caused steelhead mortality to reach as high as 98% before fingerlings reached smolt. Initial results of the effectiveness of the ozone water treatment are impressive. Mortality has significantly decreased from 1990 and 1991 to 1992 (Table 1) and the number of surviving fish to reach smolt size (180 mm) has increased.

Table 1. Effectiveness of ozone water treatment on survival and growth of summer run and winter run steelhead.

	Pre-ozone 1989-90	1990-91	With ozone 1991-92
Winter steelhead			
Mortality	77%	69%	6%
to smolt	40%	42%	96%
Summer steelhead			
Mortality	86%	41%	14%
to smolt	52%	77%	98%



Detection of An Iridovirus Infection in Kootenai River White Sturgeon

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An estimated 880 sturgeon inhabit the Kootenai River in northern Idaho. A small sturgeon hatchery was built in 1991 at Bonners Ferry, Idaho, on the Kootenai River and is operated by the Kootenai Tribe in a cooperative effort with Idaho Department of Fish and Game, Bonneville Power Administration, Montana Department of Fish, Wildlife and Parks, and BC Ministry of Fisheries. The purpose of the cooperative is to help reestablish natural production in the Kootenai River.

During November 1992, mortality in juvenile white sturgeon (*Acipenser transmontanus*) at the Kootenai Hatchery was reported after a temporary loss of water. Most of these fish were being cultured in dense rearing conditions. About 800 of the 5,000 affected sturgeon were subsequently transferred to the Sandpoint State Hatchery, Sandpoint, Idaho, to decrease fish densities and improve rearing conditions at Kootenai Hatchery. A total of 2,600 (52%) fish died as a result of the epizootic.

Tissues were collected from moribund fish at both sites, fixed in Bouin's solution for 24 h, rinsed and stored in 70% ethanol prior to paraffin embedding, sectioning and staining. Microscopic examination of epithelial tissues from fish at both sites revealed lesions typical of a white sturgeon iridovirus (WSIV) infection. Subsamples from fish at the Kootenai Hatchery used for the original histological diagnosis were deparaffinized and processed for transmission electron microscopy. Examination of these specimens revealed the presence of iridovirus-like particles with a morphology similar to WSIV (Figure 1).

Characteristics of this epizootic were similar to a recent report of WSIV in cultured juvenile sturgeon in southern Idaho (LaPatra et al. 1992. AFS Fish Health Newsletter 20(2):1-3). Mortality occurred in 6 month old sturgeon, 10-15 cm length, after periods of low water flows when fish densities were high. When densities were reduced and water flows increased, the mortality subsided. These observations suggest that juvenile sturgeon may have been infected with

WSIV but clinical signs, disease and mortality did not occur until the sturgeon were subjected to stressful conditions. This is supported by observations of other juvenile white sturgeon groups, from the same source, at Kootenai Hatchery but their densities were reduced prior to low flows and these sturgeon suffered no mortality. Sibling juvenile sturgeon that were reared at Sandpoint Hatchery from the fertilized egg stage and were not subjected to low water flows and high densities exhibited negligible mortality although WSIV lesions were observed in gill epithelium from moribund animal. It is not known if WSIV was present in this group originally or if the virus was introduced. Additionally, of the affected sturgeon transferred from Kootenai to Sandpoint, 75% died compared to 48% loss at Kootenai and the stress of transporting may have exacerbated the mortality. Since this disease appears size(age)-specific and stress-mediated, fish culture management strategies could be identified and used to avoid or minimize epizootics.

This represents the first report of WSIV in Kootenai River white sturgeon. We suspect the source of this virus was wild sturgeon caught from the Kootenai River that were used as broodstock, but this has not been confirmed. Spread of the pathogen from Kootenai River water to Kootenai Hatchery fish is not likely since all egg incubation and

rearing of larval and most juvenile sturgeon at the Kootenai Hatchery prior to the epizootic had been on treated city water. One group of 400 juvenile fish were experimentally reared in Kootenai River water and were not subjected to low flows and suffered no mortality. WSIV infections also occurred in progeny of wild adult Snake River white sturgeon in southern Idaho reared on spring water (LaPatra et al. AFS Fish Health Newsletter 20(2):1-3). This virus has also been consistently detected in young white sturgeon spawned from adults inhabiting the lower Columbia River basin and cultured in river water and it was speculated that if WSIV does occur in wild white sturgeon it may be present in most Northwest populations due to the species long life span and migratory patterns (Holt et al. 1992. AFS Fish Health Newsletter 20(3):4).

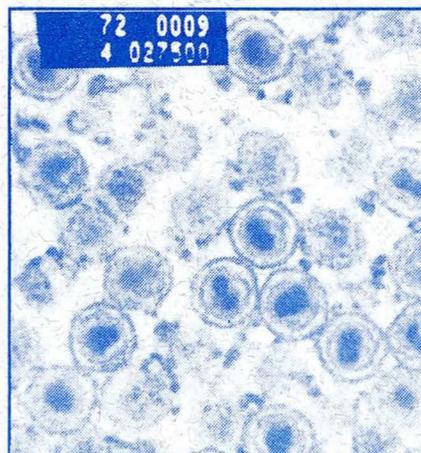


Figure 1. Iridovirus-like particles in nasal epithelium (uranyl acetate, x 44,500)

Thermal Management of *Edwardsiella ictaluri* Infections in Channel Catfish (*Ictalurus punctatus*)

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Costs to maintain fish in the laboratory for research purposes can be exorbitant. The purchase price of fish and continuous expenditures for feed, water treatment, and labor can make these research organisms extremely valuable. Loss of fish which have been acclimated to laboratory conditions can delay experimentation indefinitely and result in unanticipated expenditures to research budgets.

Epizootics of bacterial etiology among acclimated fish can leave the experimenter with the sole option of antibiotic chemotherapy unless the animals are destined for use in toxicological or pharmacological research. The extent to which antibiotics alter xenobiotic metabolism and gut flora in fish is not thoroughly understood. Alteration of either xenobiotic metabolism or gut flora could result in a fish which physiologically does not respond in a manner identical to the untreated animal.

Edwardsiella ictaluri is a gram negative bacteria that is the causative agent of enteric septicemia of catfish (ESC). The pathogen is responsible for wide-spread losses in the channel catfish industry. *E. ictaluri* demonstrates a distinct temperature-related pathogenicity to channel catfish which has been well documented through field observations and laboratory exposures. This report describes the mitigation of a natural ESC epizootic in a laboratory population of channel catfish through water temperature management.

Channel catfish (100-150 g) were maintained in flow-through conditions (water temperature 24.0 degrees C, dissolved oxygen 8.0 mg/L, pH 7.9, total hardness and alkalinity 26.5 and 176.0 mg/L as CaCO₃, respectively). Photoperiod was 12 h dark/ 12 h light and catfish were fed Sterling Silver Cup trout chow *ad libitum* daily. Catfish originated from the Ben Hur Aquaculture Research Station, Louisiana Agricultural Experiment Station, Baton Rouge, LA, and had no previous history of ESC.

Moribund catfish were submitted for examination to the Fish Disease Diagnostic Laboratory, Louisiana State University School of Veterinary Medicine. Fish clinical signs and the results of confirmatory biochemical tests of bacterial isolates were consistent with those of *E. ictaluri*.

Following diagnostic confirmation, temperature of water flowing to holding tank was lowered at a rate of 2.0 °C/day to 17.0 °C. Water temperature was held constant for ten days and then elevated to the initial temperature at the same acclimation rate.

Catfish mortality decreased correspondingly to lowered water temperature (figure 1) and ceased completely once water temperature was stabilized at 17.0 °C. Cumulative percentage mortality reached 14% during the first disease episode. On day 28, six days following reestablishment of the original thermal regime, ESC-related mortalities were again observed in the holding tank. The second epizootic occurred from days 28 to 32 and resulted in mortality to an additional 4% of the catfish population. No further mortalities were recorded among catfish following the second epizootic. Concurrent with the disease outbreak in the observed catfish population, ESC epizootics resulting in over 50% mortality were observed in other holding tanks within the laboratory.

In the absence of antibiotic therapy we were able to save many catfish which may have otherwise died (18% vs 50%). Thermal management of ESC requires further research as it appears to be an effective means of reducing mortality. Utilization of this practice may be economically feasible for small fish holding facilities, aquatic laboratories or intensive recirculating operations with some capacity to produce chilled water. Use of this method by larger facilities would be restricted unless large volumes of good quality water at the appropriate temperature (well water, a diverted cold water stream or the hypolimnion of a lake) were available at a low cost.

Methodologies to treat active ESC outbreaks in food fish are currently restricted to two antibiotics; Romet-30 (sulfadimethoxine/ormetoprim) and terramycin (oxetetracycline).

Resistant bacterial strains, extended withdrawal times, and sensitive bacterial populations in biofilters necessitate that other alternatives for ESC mitigation be developed. Thermal management may provide an attractive option for the moderation of ESC epizootics in some fish holding or culture systems.

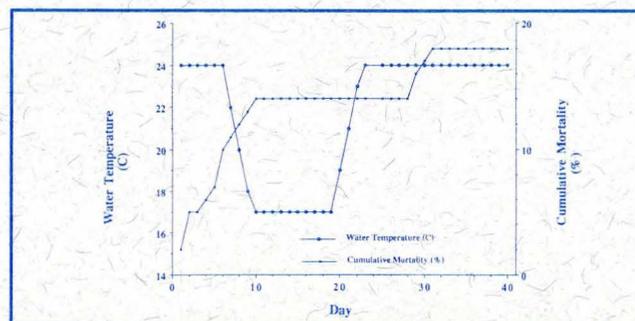


Figure 1. Water temp. and cumulative percentage mortality of channel catfish.

Specificity of Commercial Monoclonal Antibody Based Capture ELISAs for *Renibacterium salmoninarum*.

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Introduction

Renibacterium salmoninarum, the causative agent of Bacterial Kidney Disease (BKD), is a fairly ubiquitous intracellular gram positive pathogen of salmonid fish. It has been isolated from fish in North America, Japan, Chile, and Europe (1). Infection can, but does not always, lead to chronic systemic disease. The organism is thought to be able to evade the immune system by residing within the macrophage (2), and by directly interfering with normal immune function by the release of a soluble 57 kD protein (3). This soluble protein has been implicated in suppressing antibody responses in vitro (4), causing hemagglutination (5) and disease pathogenesis (6).

Transmission of *R. salmoninarum* has been shown to occur both horizontally (7) and vertically (8). The progression of infection to a disease state, like with so many other infectious agents, is thought to be through stressor mechanisms, both environmental and physiologic (9). Fryer and Sanders (10) have noted that the highest mortality rates are generally observed during the transfer of smolts from fresh to salt water and during spawning, a time that correlates with stress factors. It would appear that these would be two critical control points during which to test for the pathogen so that the proper management decisions can be made.

This research note presents information on two USDA licensed commercial capture ELISA diagnostic kits for *R. salmoninarum*. Both tests are based on monoclonal antibodies directed against different epitopes on the soluble 57 kD protein (11). One is a quantitative assay run in a microtiter plate format and is marketed under the tradename K-Dtect. The second diagnostic kit provides semi-quantitative results, is run in a tube format that lends itself to use in the field, and is marketed under the tradename KwiK-Dtect.

Materials and Methods:

Monoclonal Antibodies: The generation of the monoclonal antibodies (MAb) has been previously described (11). The capture MAb designated 4D3 was epitope mapped and identified as binding to the Group I

region (N terminus) of p57. The second MAb 3H1, which is biotinylated and serves as the reporter, was mapped and shown to bind in the Group II (middle) region.

Quantitative K-Dtect: The assay, which is provided as a complete ready-to-use kit, was performed according to the manufacturer's instructions. Briefly, the supernatant from a macerated kidney sample that was diluted 1:2 with buffer was added to duplicate wells of a microtiter plate coated with the capture MAb. The plate with test samples and positive controls of p57 that are used to generate a standard curve, was incubated for 1 hour at room temperature. The plate was washed and the biotinylated reporter MAb was added to all wells, before the plate was again incubated for 1 hour at room temperature. The plate was washed to remove unbound MAb, a streptavidin-horseradish peroxidase conjugate was added to all wells, and the plate incubated for 30 minutes. After the final wash step, the substrate (hydrogen peroxide)/chromogen (ABTS) solution was added to all wells, the plate incubated for 10 minutes and the reaction quenched with a solution of SDS. The optical density (O.D.) of the wells of the plate were read at 410 nm and a standard curve was drawn using the values for the positive controls. The quantity of p57 antigen in the test samples was extrapolated from the standard curve that was generated for each plate.

Semi-quantitative KwiK-Dtect: The general test procedure for the rapid field test was the same with a few exceptions. First, the sample, which was derived by absorbing kidney fluid with a cotton swab, was placed in a MAb coated tube containing sample dilution buffer. The sequence of steps for the assay was the same as for the K-Dtect, but the incubation times for the sample and reporter MAb were reduced to 30 minutes each. In addition, rather than reading the O.D. of the samples and extrapolating against a standard curve, the color change for each sample was compared to tubes of positive control antigen.

Test Pathogens: The following agents were used to test for cross reactivity in the two diagnostic systems: *Aeromonas salmonicida* (D. Rockey, Rocky Mountain Laboratory, Hamilton, MT), *Yersinia ruckeri*, *Vibrio*

anguillarum (J. Rohovec, Oregon State University), sporulating *Bacillus* (P. Barbash and J. Thoesen, USFWS, Fish Health Unit, Lamar, PA), *Lactobacillus piscicola*, *Streptococcus facium*, *Streptococcus fecalis* (by S. Kaattari, Oregon State University,), Viral Hemorrhagic Septicemia Virus (P. McAllister, National Fish Health Research Laboratory, Kearneysville, WV), Infectious Hematopoietic Necrosis Virus (S. Ristow, Washington State University, Pullman, WA), Infectious Pancreatic Necrosis Virus (B. Nicholson, University of Maine, Orono, ME), and Erythrocyte Inclusion Body Syndrome (R. Holt, Oregon Dept. Fish and Wildlife, Corvallis, OR).

When testing the viral agents a concentration of 1×10^8 to 1×10^{10} pfu/ml was used. For testing the individual bacterial pathogens, five colonies each measuring approximately 2 to 3 mm in diameter were used. When testing the sporulating *Bacillus*, fresh TSI slants were inoculated 24 hours prior to sample acquisition in accordance with the instructions of P. Barbash. Each of the pathogens were spiked into 1 ml of kidney tissue supernatant that had previously been identified as negative by the ELISA. The spiked supernatants were vortexed for 30 seconds before samples were taken for analysis.

Results: The data presented in Table 1 (page 8) summarizes the results from a minimum of five replicates with each assay format. None of the viral or bacterial agents tested with either the quantitative (K-Dtect) or semi-quantitative (KwiK-Dtect) capture ELISAs were identified as positive. For the K-Dtect the cutoff between a positive and negative sample is 6 ng/ml of p57, while the KwiK-Dtect value is 10 ng/ml. The level of sensitivity for the K-Dtect is 1 ng/ml and approximately 3 ng/ml for the KwiK-Dtect (data not shown).

Discussion : The results from this study and others indicates that the commercial monoclonal antibody based capture ELISA kits do not cross react with the pathogens tested. This is in contrast with many of the other serologic assay systems that are now currently available. There have been several reports regarding the variable specificity of these other serologic reagents (12, 13, 14). Other serologic reagents have been identified as cross reacting with some components of fish feeds (12, 13) and other gram positive bacteria (14).

Because some management decisions are made based on the prevalence of certain pathogens, it is imperative to have the most accurate information available as is possible. The misinformation that may result from the

identification of a higher pathogen prevalence than is actually present can lead to costly, and even irreversible management decisions. Although the detection assays utilized in the present study were not tested against every pathogen of salmonids, and there is always the potential for cross reactivity with any serologic reagent, the MAb based capture ELISAs appear to be superior to some other systems that are being used by the industry in terms of specificity.

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Table 1. Data is presented in terms of ng/ml of p57 antigen. Values <6 ng/ml on the K-Dtect and <10 ng/ml on the KwiK-Dtect are considered negative for *Renibacterium salmoninarum*.

PATHOGEN	K-Dtect Result	KwiK-Dtect Result
<i>Aeromonas salmonicida</i>	1.6 ng/ml	<10 ng/ml
<i>Vibrio anguillarum</i>	1.3	<10
<i>Yersinia ruckeri</i>	<1.0	<10
Sporulating Bacillus	<1.0	<10
<i>Lactobacillus piscicola</i>	(-)*	(-)*
<i>Streptococcus facium</i>	(-)*	(-)*
<i>Streptococcus fecalis</i>	(-)*	(-)*
IHN Virus	2.1	<10
IPN Virus	<1.0	<10
VHS Virus	<1.0	<10
EIBS**	<1.0	<10

* Pathogens tested in the laboratory of Dr. S. Kaattari

** Erythrocyte Inclusion Body Syndrome

Detection of a Naturally Occurring Coinfection of IHN and IPNV in Rainbow Trout

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Infectious pancreatic necrosis virus (IPNV) was first detected in the commercial rainbow trout (*Oncorhynchus mykiss*) industry in southern Idaho in the early 1960's. This virus caused mortalities in 1-5 g trout ranging from 10-80% (mean 25%). Infectious pancreatic necrosis virus was the only enzootic virus of this area until the introduction of infectious hematopoietic necrosis virus (IHN) in 1977. When IHN first appeared, infectious hematopoietic necrosis (IHN) was a chronic disease of large (100-500 g) fish. Infected production lots of rainbow trout were chronically debilitated and secondary bacterial infections were common. By 1979, IHN epizootics were occurring in smaller fish (1-5 g) that had also been infected with IPNV and losses were typically 30-50%. By 1982, IPNV was only occasionally detected and IHN induced mortality as high as 70% was being reported (Busch 1983). Over the past two years detection of IPNV has been infrequent (D. Ramsey, Rangen Inc., personal communication).

Recently, two rainbow trout production lots (mean weight, 1.5 g) were diagnosed as coinfecting with IHN and IPNV. Dual infection of rainbow trout fry with IHN and IPNV has been reported previously and we used similar methods for detection and confirmation (Mulcahy and Fryer 1976). Typical cytopathology (CPE) of both viruses was observed on CHSE-214 cells inoculated with visceral homogenates obtained from morbid animals after 48 h of incubation at 18°C. Characteristic plaque morphology indicative of IHN was also observed on EPC cells. The presence of these viruses was confirmed by serum neutralization and indirect fluorescent antibody tests. Serum neutralization tests used supernatant that was removed from primary cultures exhibiting one or both types of CPE and cross neutralized with polyclonal anti-IHN or anti-IPNV. The presence or absence of specific CPE after incubation with either antisera was used to determine if IPNV and/or IHN was present. Fluorescent antibody tests used coverglasses that were monolayered with CHSE-214 cells and subcultured with supernatant from the primary assays. Replicate coverglass cell cultures were fixed with methanol 16 h post-inoculation and reacted with rabbit anti-IHN or anti-IPNV using an indirect fluorescein isothiocyanate staining methodology. The observance of specific fluorescence after incubation with either antisera was used to determine if IPNV and/or IHN was present.

The results indicated that one production group had high IHN prevalence and concentration while IPNV was detected only at low concentrations in a few of the specimens examined. Daily mortality detected in this group was very high. The other group exhibited low concentrations of IHN and IPNV but both viruses were detected in almost all samples tested. Daily mortality in this group was very low but dead fish were consistently observed. Both groups, about 350,000 fish, were subsequently destroyed and their raceways disinfected. Examination of other production lots of fish in close proximity to these has showed no evidence of either virus.

These types of mortality patterns in small, susceptible fish are interesting. The group that was primarily infected with IHN exhibited high mortality which would be expected. However, the group that was equally infected with IHN and IPNV would also be expected to exhibit high mortality but did not. A possible explanation was recently reported by de Kinkelin and his colleagues (1992). They showed that rainbow trout alevins waterborne exposed to IPNV and subsequently waterborne challenged with IHN 17 d later exhibited only 11% cumulative mortality (CM) compared to fish exposed to IHN (61% CM) or IPNV and mock infected groups (both 3% CM). They termed this type of resistance as interference-mediated and speculated that induction of interferon by IPNV was responsible for the reduced mortality. However, no interferon was detected and protection decreased with time post-IPNV exposure.

It is tempting to relate these experimental results to the epizootiology of IPNV and IHN observed in southern Idaho. Infectious pancreatic necrosis (IPN) was the first and only enzootic viral disease within the commercial trout industry until 1977. However, when IHN was introduced, initial fish losses were low and both IPNV and IHN were routinely detected. Over time detections of IPNV decreased and fish mortalities attributed to IHN increased. Until recently IPNV has only rarely been detected in this area. The source of IPNV responsible for the recent isolations is unknown.

It is possible that IPNV has always been present but not detected or that this is a new introduction. During the course of other studies we have detected IPNV in skin scrapings of large (300-400 g) rainbow trout exhibiting

skin anomalies but no mortality. This type of manifestation of IPNV in trout epidermis is unique but has been reported experimentally (Yamamoto and Ke 1991). We speculated that this may be a different strain of IPNV but analysis of this isolate and five others obtained from 1980-1985 using a panel of monoclonal antibodies indicated that the viruses were serologically identical to the Buhl strain of IPNV originally detected in this area (P. Reno, Oregon State University, personal communication).

In de Kinkelin et al. (1992) studies, protection against viral hemorrhagic septicemia virus was also demonstrated in fish previously infected with IPNV. In collaboration with R. Hedrick's research team (University California, Davis) we have been examining interference-mediated resistance of fish to IHNV after exposure to an avirulent picornavirus (cutthroat trout virus or CTV; Hedrick et al. 1991). Waterborne exposure of fish to CTV followed by challenge with high concentrations of IHNV also resulted in significant protection compared to fish that were only exposed to IHNV. Fish exposed only to CTV exhibited no mortality. Studies are continuing in an effort to define the mechanism of protection exhibited by these interference-mediated resistance models for potential application in development of viral disease control strategies.

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ANNOUNCEMENTS

1993 Western Fish Disease Workshop:

meeting announcement and first call for papers. The workshop will be held in Port Ludlow, Washington at the Port Ludlow Golf and Meeting Retreat on June 15-16, 1993. Please call

the resort at 1-800-732-1239 to make reservations before May 1st, 1993, and refer to the Western Fish Disease Workshop to obtain these room rates. Contact Bob Rogers, Washington Department of Fisheries, at 206-902-2669 for more information.



10th Anniversary Meeting of the Aquaculture Association of Canada

meeting announcement and first call for papers. The meeting will be held in the Charlottestown Hotel, Charlottestown, P.E.I., Canada. Aquaculture Canada '93 will host the 15th Regional DFO Fish Health Workshop August 26th -27th. Contact: Chairman, Aquaculture Canada '93, Host Committee., P.O. Box 2000, Charlottestown, P.E.I., Canada, C1A 7N8, 902-368-5525, FAX 902-368-5542.

"Disease Diagnosis and Control in Marine Shrimp Culture" Shortcourse.

May 31 to June 11, 1993. The University of Arizona, Tucson, AZ. For information contact: Donald V. Lightner, Department of Veterinary Science, The University of Arizona, Building 90, Room 202, Tucson, AZ, 85721. FAX: (602) 621-6366. Tel: (602) 621-2355.

American Fisheries Society Fish Health Section Annual Meeting and Midwest Fish Disease Workshop,

July 21-23, 1993, Stouffer Concourse Hotel, Denver, CO. first call for papers. Please submit abstracts to Joe Marcino, Minnesota Department of Natural Resources, Box 25, 500 Lafayette Rd., St. Paul, MN 55155-4025 by June 15, 1993.

The 1993 meeting of the Eastern Fish Health Workshop will be held June 29 - July 2 in Falmouth, Massachusetts, and will present contributed papers on all aspects of fish health. The meeting is being co-sponsored by the Northeast Fisheries Science Center of the NOAA-National Marine Fisheries Service and the Marine Biological Laboratory, Laboratory for Marine Animal Health. The NMFS aquarium and the facility for marine animal health studies at MBL will be available for tours. Think about staying the holiday weekend to enjoy New England in summer. For further information and call for papers, contact Sharon A. MacLean, NOAA-NMFS, 28 Tarzwell Drive, Narragansett, RI 02882-1199 (401) 782-3258.

Membership Drive

Membership in the Fish Health Section has declined in recent years to below 1988 levels. Our high point was in 1990 when we had 610 members in the section. The membership committee is initiating a membership drive to boost our numbers and all of you can have a role! For those of you who haven't yet renewed your membership, please do so ASAP. If you are aware of colleagues who are not members (or have not renewed their previous memberships) please discuss with them the benefits of belonging to the Fish Health Section and the American Fisheries Society. Ask them to join, or better yet, offer them a membership application (available in Society publications, from the Bethesda office, or from the membership committee (Pat Chapman, Washington Dept. of Fisheries, P.O. Box 43154, Olympia, WA 98504-3154. 206-902-2668).

This reminder is just the first of several steps the membership committee will be using to boost membership. Look for more in the near future!

Book Review

Techniques in Fish Immunology: Fish Immunology Technical Communications 2. Edited by J.S. Stolen, T.C. Fletcher, D.P. Anderson, S.L. Kaattari, and A.F. Rowley. 1992. SOS Publication, Fair Haven, New Jersey. Price \$45.00. ISBN 0-9625505-3-1.

The sequel to FITC-1 is even better. This second book in the series of Techniques in Fish Immunology broadens the



field of fish immunology with 18 articles showing how recent scientific developments are being integrated into the field and how this particular specialty has potential to benefit human and veterinary immunology and health.

The first article by A. Schots, R. Pomp, and W.B. van Muswinkel from the Netherlands "Production of monoclonal antibodies" describes the techniques used to make monoclonal against fish cell receptors and antibody molecules. These monoclonals are finding important uses in disease diagnosis and in describing molecular functions. "Flow cytometry in fish immunology" to describe fish cells and receptors is written by A. Thuvander, A. Johannisson, and J. Grawe of Sweden. Monoclonal antibodies are recommended for better definition of cell surface antigens. G.A. Davidson, A.E. Ellis, and C.J. Secombes from Scotland wrote "Isolation of leukocytes from mucosa-associated lymphoid tissues." By passing the gut tissue pieces through saline, EDTA/DTT and collagenase/DNase, cells can be finally isolated through a Percoll gradient. Isolated cells could be further characterized, for instance by flow cytometry. The techniques described in "The cryopreservation of fish lymphocytes: by M.F. Tatner and C. Findlay of Scotland give a brief outline for holding the tissues in dimethylsulfoxide-containing media. The storage of cells in liquid nitrogen is further elaborated by M. Faisal, S. Sami, and B. J. Rutan from Virginia in the article "Fish cell lines of leukocyte origin: Maintenance and characterization." This article also describes leukocyte isolation, the lysis to clear red blood cells, adjustment of culture conditions--accounting for fish species, and cytochemical staining.

"Use of homologous salmonid plasma for the improved responsiveness of salmonid leukocyte cultures" by J. DeKonig and S.L. Kaattari shows the importance of holding cells in vitro culture under similar environments and media components as their source. This is particularly true for cell expansion and testing mitogenic responses. Also based on the, M.A. Arkoosh from Washington, and S.L. Kaattari report "Induction of trout antibody-producing cells in microculture." These researchers have done pioneering work in in-vitro cell interactions to show the influences of drugs and pollutants on the fishes immune response. As in other articles, excellent photographs and drawings show materials and outline procedures of these techniques. The in-vitro theme is continued with an article by D.P. Anderson from West Virginia "In vitro immunization of fish spleen sections and NBT, phagocytic, PFC and antibody assay for monitoring the immune response." These techniques have many applications including

testing vaccines for potency and efficacy and showing the effects of immunostimulants and suppressors.

A new technique - "Quantification of antibody secreting cells by the ELISPOT method" - presented by G.A. Davidson and C.J. Secombes from Scotland shows advantages over the hemolytic plaque assay in that complement and heterologous erythrocytes are not necessary to point the secreting cells, and the method is more adaptable to handling large numbers of samples.

"Detection of apoptic killing by trout nonspecific cytotoxic cells using DNA fragmentation assay and agarose gel electrophoresis" by A.R. Greenlee and S.S. Ristow of Washington carefully detail their protocols that show the breakage of DNA from targeted cells by the cytotoxic cells isolated from the anterior kidney. J.T. Zelikoff and N.A. Enane from New York wrote "Assays used to assess the activation state of rainbow trout peritoneal macrophages" to show extensive methods they developed to collect, characterize, and determine functions of these cells. A preliminary step in many fish immunological studies is to isolate antibody molecules from that particular species for use in characterization assays. S.A. Smith from Virginia details methods he used for tilapia studies in "Affinity purification of serum immunoglobulin from fish." Other important blood components include the complement that are important in defense against infections. "Assays of hemolytic complement activity" by T. Yano from Japan shows how these measurements can be standardized. "Identification of Eicosanoids in fish tissues" written by A.F. Rowley from England shows how the prostoglandins and other fatty acid derivatives, which may regulate hormonal effects, can be separated. "Cortisol measurements in fish" by Z. Jeney and G. Jeney from Hungary and A.G. Maule from Washington, details and points on standard methods of radioimmunoassay used for fishes. Unfortunately, as with many immunological assays, they warn of the wide statistical results due to individual fish variations.

The elucidation of fish protozoan life cycles and the hosts they infect may be helped by the brilliant techniques developed by E.J. Noga in North Carolina. "Immune response to ectoparasitic protozoa: The infectivity assay" shows how methods developed for the propagation of *Amloodinium ocellatum* can mimic the life cycle in vitro. "Sandwich enzyme linked immunosorbent assay (ELISA) to detect and quantify bacterial pathogens in fish tissue" by A. Adams in Scotland presents a detailed methodology for diagnosis, which was developed for *Vibro alginolyticus* and can be

used to detect as few as 1000 bacteria per ml of extract. M. Faisal, S.F. Hoegerman, B.J. Rutan and S. Sami of Virginia contributed "Chromosome analysis using fish leukocytes and culture cells" to number and describe the morphology of fish chromosomes.

An interesting appendix describes methods of individual identification and gives commercial sources of the new tags, markers, and transmitters for fish. The new, smaller, coded tags enable researchers to hold large numbers of test and control fish in common tanks and recover individuals with certainty, including individual migrating fish. The appendix also lists sources of chemicals, fish vaccines, and biologics, as this field is rapidly growing. This laboratory workbook is an important addition to the fish immunology field and is recommended to fish biologists, pathologists and students entering fish health studies.

Douglas P. Anderson
National Fish Health Research Laboratory
Kearneysville, WV 25430



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IHN at Lyons Ferry Hatchery: A Case Study of Vertical Transmission

S. D. Roberts, Washington Department of Wildlife, 8411 N. General Grant Way, Spokane, WA 99208

Infectious hematopoietic necrosis (IHN) has occurred on two occasions in steelhead at Lyons Ferry Hatchery despite stringent spawning procedures and rearing the fish on well water. Lyons Ferry Hatchery is located on the north bank of the Snake river in southeastern Washington. The hatchery site includes both Washington Department of Wildlife rainbow trout and steelhead hatchery and Washington Department of Fisheries chinook salmon hatchery. Hatchery operations started in 1982 and steelhead spawning at the hatchery commenced in 1986. The water supply for hatchery is 8 deep wells which can produce 102 cfs of 51° F water. The facility also has a adult steelhead and salmon ladder-trap and broodstock ponds.

The summer steelhead eggs were obtained from steelhead that return to Lyons Ferry Hatchery. Because of the isolation of IHN virus in the steelhead broodstock stringent spawning and incubation procedures were followed. The procedures include individual female spawning, sampling for IHN virus, water hardening 100 ppm iodophor solution for 1 hr, and individual isolation incubation. Eggs were pooled following development to the eyed-egg stage and completion of the viral testing.

In April 1989, IHN occurred in newly feeding steelhead fry being reared in the hatchery building. Initially, IHN virus caused mortality in the fish from the first two egg takes. Later in the summer IHN affected the remaining raceway of Lyons Ferry stock summer steelhead and the disease spread to two adjacent raceways of rainbow trout. The steelhead were progeny of IHN virus positive broodstock (Table 1).

In April 1992, IHN again occurred in newly feeding steelhead fry in the hatchery. The disease was confined to two out five steelhead egg takes. Only the affected fish were immediately destroyed. The fish in the remaining three egg takes are healthy to date. As in 1989, the fish were progeny of IHN virus positive steelhead broodstock (Table 1).

In 1989 and 1992, the source of IHN virus was the summer steelhead broodstock. The well water was not a source since over 4.0 million IHN susceptible rainbow have been reared at Lyons Ferry Hatchery in the past 10 years without any problems. Also, when eggs from IHN virus positive female steelhead broodstock were destroyed in 1986, 1987, and 1990 were destroyed no

IHN occurred in the steelhead reared. Thus, two cases of IHN virus vertical transmission have been seen in summer steelhead at Lyons Ferry Hatchery.

In 1992, IHN outbreaks have not occurred in all egg takes despite high prevalence of IHN virus in broodstock (Table 2). Fish from three of five egg takes have been successfully reared. The unaffected fish have been subjected to a number of stressors including transfer from troughs to deep tanks in the hatchery building, transfer from deep tanks to outside raceways, and adipose fin clipping (removal) and transfer to the rearing pond, yet the disease has not been observed.

Factors effecting the vertical transmission IHN virus need to be examined. Some possible factors include iodophor water hardening, IHN virus titer, and water quality.

Despite the water hardening in 100 ppm iodophor solution for 1 hr, IHN has occurred. In addition in 1992, when the steelhead eggs reached the eyed stage, the eggs were disinfected with 100 ppm iodophor for 10 minutes and IHN occurred. Chapman and Rogers (1992) suggest that current iodophor water hardening may not result in total egg disinfection. Therefore, modification of the water hardening procedure including the use of higher iodophor concentrations, a rinse step and circulation of iodophor solution through the egg mass needs to be explored.

Since past IHN broodstock testing has not examined the IHN virus titer the question of the effect of titer on virus transmission remains. It has been suggested that IHN virus titer may play a role in its transmission (Mulcahy and Pascho, 1985; Meyers et al., 1990). In the future, broodstock IHN virus titer will be determined and their progeny will be separated according to the parental IHN titer.

The effect of water quality on IHN virus transmission also needs to be explored. Electrostatic interactions plays an essential role of binding of vesicular stomatitis virus (similar virus to IHN virus) to membranes of susceptible cells (Conti et al., 1991). Since the Lyons Ferry Hatchery well water has high conductivity, its role in virus transmission may be important. Experiments with deionized water for sperm activation and water hardening will be conducted.

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Table 1. IHN virus broodstock isolation compared to fry outbreaks.

Brood Year	Adult ¹ IHNV +	Fish Reared from IHNV +	Fry IHN
1986	+	No	No
1987	+	No	No
1988	-	-	No
1989	+	Yes	Yes
1990	+	No	No
1991	-	-	No
1992	+	Yes	Yes

¹ 100 % of the female steelhead broodstock were sampled

Table 2. IHN outbreaks compared to broodstock IHN virus prevalence.

Spawn Date	Take No.	Percent IHN Virus Positive	IHN ¹ Days
2/04/92	1	79	None
2/11/92	2	96	48
2/18/92	3	83	None
2/25/92	4	83	48
3/03/92	5	92	None

¹ Days post-fertilization when IHN was first observed

President's Message

Change has caught the attention of many people these last few months and this concept is just as important for the Fish Health Section. The question some of us have is whether our section and its members are prepared to take advantage of the opportunities change provides.

The National Aquatic Animal Health Management Strategy being developed by the Joint Subcommittee on Aquaculture (JSA) promises to challenge all of us and perhaps change the way we do business. During their strategy development process, fish health professionals and regulatory agents will question the validity of transport restrictions for fish health protection. They will challenge the adequacy of current fish certification procedures. They will strive to develop model regulations to standardize interstate live fish transport regulation. They will reexamine the issue of professional standards and make recommendations as to who should be directly involved in certification programs. Should a non-DVM directed laboratory be certifiable for inspection purposes? These are only some of the questions being raised but they provide an opportunity for us, as fish health professionals and as the FHS, to examine our mission and role in fish health management. I suggest these are good initial questions we can use to enable the Fish Health Section to develop short and long range goals.

The science of fish health management itself is also changing and providing new opportunities. In 1992, the Fish Health Section, on behalf of the JSA Quality Assurance Working Group, petitioned the U.S. Food and Drug Administration to rule on the status of several different water treatment chemicals, some of which can be used directly to treat fish diseases. The reason for this petition the dilemma the fish health management community has in regarding the use of compounds traditionally used by aquaculturists but which have recently been challenged by FDA. The FDA conducted a fish farm drug survey in 1989-1990 in which use of unapproved compounds was documented. FDA had since determined (see accompanying letter) that the following compounds will be regarded as new animal drugs of "low regulatory priority (LRP)": acetic acid, calcium chloride, calcium oxide, carbon dioxide gas, garlic (whole form), ice, magnesium sulfate, onion (whole form), potassium chloride, povidone iodine, sodium bicarbonate, sodium chloride, and sodium sulfite. Each compound must be used according to specific requirements. Oxygen will not be regarded as a drug although it meets the definition of a drug. While this ruling does not mean these compounds are approved, it does provide some degree of latitude for their use. Some other compounds also on the FHS petition were not placed on LRP list but will not be regarded as drugs (calcium hydroxide, calcium carbonate, ozone, sodium hydroxide, tris buffer, and Pen/Strep when used in algae culture). All other compounds not currently FDA approved will probably require an Investigative New Animal Drug (INAD) permit from FDA if they are to be used. This will challenge fish health managers to either develop INAD's or develop health management practices that do not rely on certain water treatments or chemicals. This is an opportunity for us to examine our fish health management practices and initiate change.

Randy MacMillan

John R. MacMillan, Ph.D.
Fish Health Section/American Fisheries Society
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Dear Dr. MacMillan:

This is in response to your letters to Dr. Beaulieu (February 21, 1992), and Dr. Homire (May 4, 1992). You have asked the Center for Veterinary Medicine (CVM) to consider 23 aquaculture chemicals as "exempt from regulatory action," or "not requiring an INAD."

We apologize for the delay in responding to your request; however, your questions required an in-depth review of a number of our policies. In addition, each of the compounds you listed has been reviewed by CVM's Division of Toxicology and Environmental Science, the Division of Chemistry Residue Evaluation Branch, and the Divisions of Surveillance and Compliance. Their review and conclusions have been carefully considered by CVM's Aquaculture Coordinating Committee. We have reached the following conclusions regarding your request.

LOW REGULATORY PRIORITY

Each of the following substances will be considered a new animal drug of low regulatory priority for the intended use specified.

Calcium oxide - as an external protozoicide for fingerling to adult fish at a concentration of 200 mg/L for 5 seconds.

Garlic (whole form) - for control of helminth and sea lice infestations of marine species at all life stages.

Ice - to reduce metabolic rate of fish during transport.

Onion (whole form) - to treat external crustacean parasites, and to deter sea lice from infesting external surface of fish at all life stages.

Potassium chloride - as an aid in osmoregulation to relieve stress and prevent shock. Dosages used would be those necessary to increase chloride ion concentration to 10-2000 mg/L.

Calcium chloride - to increase water calcium concentration to insure proper egg hardening. Dosages used would be those necessary to raise calcium concentration to 10-20 ppm CaCO₃.

NOTE: Use of calcium chloride to increase the hardness of water to 150 ppm to enable fish to maintain osmotic balance by preventing electrolyte loss has previously been determined to be of low regulatory priority.

Povidone iodine - as a fish egg disinfectant. This chemical has previously been determined to be of low regulatory priority for this use at the following levels: 100 mg/L for 10 minutes after water hardening, and 50 mg/L for 30 minutes during water hardening.

Magnesium sulfate - to treat external monogenetic trematode infestations and external crustacean infestations in fish at all life stages. Used in all freshwater species. Fish are immersed in a 30,000 mg MgSO₄/L and 7000 NaCl/L solutions for 5 to 10 minutes.

Our enforcement position on the use of these chemicals should be considered neither an approval nor an affirmation of their safety and effectiveness. Based upon the facts available to us at some time in the future, we could come to a different decision on the use of these chemicals.

NOT LOW REGULATORY PRIORITY

The following chemicals are drugs for the intended uses and will NOT be considered of low regulatory priority due to human food safety and/or environmental concerns for their proposed uses.

Benzalkonium chloride and Benzethonium chloride - to treat external bacterial infections (bacterial gill disease).

We have human food safety concerns for the use of benzalkonium chloride and benzethonium chloride in aquaculture. There are not sufficient data available to determine a safe level for these quaternary ammonium compounds in the diet. In addition, there is insufficient information about metabolism and residue accumulation in edible tissue.

In addition, we have only limited information concerning the environmental fate or effects of these compounds and of the potential occupational hazards of their use. The information available indicates that these substances are toxic to aquatic organisms and to humans. The data are not sufficient for us to determine whether the proposed use of benzalkonium chloride and benzethonium chloride could cause a significant environmental impact.

Epinephrine - to induce larval clam and oyster metamorphosis.

We are concerned about user safety and the potential for serious adverse reactions in the individual administering the drug. Epinephrine is a potent cardiostimulant and can cause life-threatening reactions in people, particularly individuals with preexisting cardiovascular disease. Adverse reactions include, but are not limited to, tachycardia, coronary occlusion, cerebral vascular accident (stroke), angina pectoris, and heart failure.

L-Dopa - to induce larval clam and oyster metamorphosis.

We are concerned about user safety and the potential for adverse reactions in the individual administering the drug. Adverse effects include, but are not limited to, cardiac arrhythmias, nausea and vomiting.

Oxytetracycline (by injection and immersion) - to mark fish.

We have human food safety concerns for marking fish with oxytetracycline by immersion or injection, and for treatment regimens that use a larger dose level, longer treatment time, or fish larger than those for which the drug is approved. We do not have the residue chemistry data necessary to establish a safe withdrawal period for these routes of administration and treatment regimens.

We note that use of the approved oxytetracycline product (medicated feed) for marking fish species other than those for which the product is approved constitutes an extralabel use and would require an INAD exemption.

Sodium chlorite - as a bath for controlling bacteria and protozoa.

We have human food safety concerns regarding the drug use of sodium chlorite in aquaculture since no information is available concerning the metabolism, disposition, or depletion of sodium chlorite residues in fish.

There is no information provided on potential occupational or user safety concerns, the maximum concentration expected in effluents, the frequency of releases of these effluents, the types of sites where these releases would occur, or any environmental fate or effects data that may be available.

Cytochalasin B - to induce triploidy in oyster eggs.

This drug has been found to be teratogenic and mutagenic in numerous human and animal toxicity studies. We recognize that for the proposed use of Cytochalasin B in oyster larvae there are factors which significantly decrease the risk of a tissue residue and subsequent human food safety concern. However, the inherent toxicity of this drug precluded CVM from considering it to be of low regulatory priority.

Certain chemicals used in aquaculture, such as benzalkonium chloride, may be registered as pesticides by EPA but have secondary therapeutic benefits in fish. CVM has taken the position that we ordinarily will not object to the concurrent use of EPA registered chemicals for therapeutic purposes in aquaculture if they are used according to the labeling approved by EPA. These chemicals, however, cannot be labeled or promoted for drug use. Nor can they be used for drug purposes if the pesticidal conditions for which the compound is registered do not exist in the treated facility. Any drug use of an EPA registered product which is not consistent with the approved EPA labeling will require an investigational new animal drug (INAD) exemption or an approved new animal drug application (NADA). Of the products listed above, those which are not registered by EPA, and are intended for drug use, can only be used under an INAD exemption or an approved NADA.

NOT LOW PRIORITY - REGULATORY ACTION DEFERRED

We have determined that copper sulfate and potassium permanganate will not be low regulatory priority. However, we are for the time being deferring regulatory action pending further consideration. As you may know, copper sulfate is registered with EPA for use as a pesticide and currently undergoing EPA's reregistration process. Potassium permanganate has also, in the past, been registered as a pesticide by EPA; however, it is no longer registered. We have the following comments regarding these compounds.

Copper sulfate - used as an algicide and as an external protozoan parasiticide or external bactericide and fungicide. (Note: use as an algicide would not normally be considered a drug use).

We do have a human food safety concern based upon the potential for bioaccumulation of copper in treated fish. Large amounts of copper ingested by humans has been associated with sever gastrointestinal disturbances, jaundice, renal damage, and hemolytic anemia. We have no data on copper residues in fish treated with copper sulfate and therefore are unable to assess the level of concern for human food safety. To provide this a study comparing copper levels in treated and untreated fish would be necessary.

The available information indicates that the use of copper sulfate at some sites may cause a significant impact on the environment. We are also concerned that the proposed uses of these copper compounds may significantly increase the quantity of copper introduced into the environment. Assessing the impacts of copper sulfate on the environment is difficult because 1) we do not know the concentrations that would enter the environment, 2) we don't know the frequency of introduction of those concentrations into the environment, 3) the toxicity of copper depends on a number of factors, and 4) the fate of copper in the environment is dependent on a wide variety of physical and chemical factors that are difficult to predict for a given situation. In addition, we have occupational concerns for the use of copper sulfate, including the risk of severe eye damage in the person handling the chemical.

Potassium permanganate - used to oxidize organic matter, as a disinfectant, and as a protozoacide for free swimming stages of various protozoans.

Although the uses you have proposed for potassium permanganate could arguably be considered "non-drug" uses, we are aware that potassium permanganate is widely used in aquaculture as a drug to treat bacterial, fungal, and protozoan diseases of fish at all life stages.

We have a human food safety concern based upon the potential for bioaccumulation of manganese in the treated fish. As with copper sulfate, to address the human food safety concerns we would need a study that compares manganese levels in treated and untreated fish.

We also have environmental concerns for the use of potassium permanganate in aquaculture. The available information indicates that this proposed use could cause a significant environmental impact without proper mitigation.

We will inform you of the regulatory status of copper sulfate and potassium permanganate when this issue has been resolved. In the meantime, we encourage the establishment of INADs for these chemicals. INAD exemptions are granted with the exception that the necessary data for a NADA will be forthcoming.

Questions regarding the INAD process should be directed to Mr. Tom Bell, Office of New Animal Drug Evaluation, Division of Therapeutic Drugs for Food Animals. Mr. Bell can be contacted at the following address:

Food and Drug Administration
Center for Veterinary Medicine, HFV-133
7500 Standish Place
Rockville, MD 20855

NOT DRUG USES

The following compounds, for their intended uses, do not meet the definition of a drug under Section 201(g) of the Federal Food, Drug, and Cosmetic Act (FAD&C Act). These chemicals, for the specific uses described below, are not considered to be new animal drugs. Thus, they will not be regulated as drugs for these uses.

We note that these chemicals would be deemed drugs is used for therapeutic purposes. It is possible that some of these compounds would be of low regulatory priority if they were

intended for drug uses; however, each intended use would require further review to determine such priority.

Calcium hydroxide - to raise water or pond bottom pH to 10 or above.

Calcium carbonate - as a water treatment to alter water pH and total alkalinity.

Sodium hydroxide - to moderate water pH.

Tris buffer - to buffer pH changes in fresh or salt water.

Ozone - to disinfect and remove organic compounds from hatchery water.

Note: Certain uses of ozone may be regulated by the FDA Center for Food Safety and Applied Nutrition (CFSAN) or by the U.S. Environmental Protection Agency (EPA). The agency with primary jurisdiction over the proposed use of ozone in aquaculture systems has not yet been determined. This issue will be reviewed by representatives of the appropriate agencies.

Although the following compounds meet the definition of a drug under Section 201(g)(1)(A) of the FAD&C Act, the proposed uses of these substances do not constitute drug uses. Consequently, we will not regulate these compounds as drugs for the specified intended uses.

Penicillin, Streptomycin - to treat bacterial overgrowth in algal cultures that are subsequently fed to oyster larvae. Stocks of algal cultures are maintained in the laboratory in inoculate growth media to produce feed for shellfish larvae.

Note: This use of penicillin and streptomycin constitutes a pesticide use and is therefore under EPA jurisdiction. Any labeling, promotion, or use as a drug of either of these antibiotics would be regulated by FDA and would NOT be considered of low regulatory priority. Moreover, because the quantities used to treat bacterial overgrowth in algal cultures are so small, FDA will not ordinarily permit the labeling and promotion of penicillin and streptomycin products for that use. We will assume in such instances that the products are intended for drug use.

Oxygen - to maintain saturated dissolved oxygen conditions in water to ensure fish survival.

We caution against a claim that a given chemical is intended for a nondrug use when in fact it is intended for a drug use. In such a case, FDA will regulate the chemical as a drug, requiring an INAD or NADA unless it is found to have low regulatory priority.

We hope this information will be helpful to you. If you have any questions regarding our comments or if we can be of further assistance, please do not hesitate to contact us.

Sincerely Yours,
Richard E. Geyer
Deputy Director
Office of Surveillance and Compliance
Center for Veterinary Medicine

Fish Health Section Newsletter

The Fish Health Section Newsletter is a quarterly publication of the Fish Health Section of the American Fisheries Society. Submissions of any length on a topic of interest to fish health specialists are encouraged with the understanding that material is not peer reviewed. Submissions should be addressed to the editors or to a member of the publications committee.

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Deadline for Next Issue: **April 30, 1993**

FHS/AFS Newsletter
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