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Project Status Report

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Food Safety Research Associated with a Proposed Drug for Fish

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Introduction

Bacterial gill disease (BGD) is a disease of fish cultured in crowded and stressful rearing conditions and is responsible for substantial production losses on federal, state, and commercial hatcheries. The disease is caused by a slow growing, Gram-negative bacterium that most commonly affects fry, but also causes the disease in older fish. The disease is characterized by flared gills, increased respiration, decreased fright response, and reduction in feed intake.

Chloramine-T is a disinfectant that is effective in reducing fish mortalities caused by BGD. Legal use of chloramine-T as a drug in fish culture depends on approval by the U.S. Food and Drug Administration (FDA). An attribute of a drug that must be characterized before the drug is approved is depletion of all the drug's residues from fillet tissue. By characterizing the drug's depletion, the FDA can establish a withdrawal time for fish exposed to chloramine-T ensuring that drug residues reach safe levels before the fillets are consumed by humans.

A metabolite of chloramine-T in fillet tissue, para-toluenesulfonamide (p-TSA), was chosen by the FDA as a marker to represent all chloramine-T residues in fillets. Use of p-TSA as the marker for chloramine-T was based on an early study where the chemistry methods used to determine p-TSA concentrations were time consuming and cumbersome. Since that study, a simple and widely applicable improved method for p-TSA has been developed. This new method, using modern chemistry techniques, was planned for use during chloramine-T depletion studies, but before it could be used, the method had to be tested against FDA criteria for accuracy and precision. In addition, since the selection of p-TSA as the marker for chloramine-T was based on the earlier method, the FDA required that the new method be compared to the earlier method to prove that the methods produced similar results.

Accuracy and Precision of the Modern Method

The new method successfully recovered 86% to 97% of the p-TSA in fillet tissue from channel catfish *Ictalurus punctatus*, rainbow trout *Oncorhynchus mykiss*, and walleye *Stizostedion vitreum vitreum* fortified at 70 to 2,000 ppb. The new method's precision was remarkable with the variability among repeated analyses less than 8%. These data exceed FDA requirements for measuring a marker residue.

Comparing the Earlier and Modern Methods

Comparing two chemistry methods would normally be done by directly comparing each method's results from a split sample, however, since we no longer had the equipment for the earlier method, we could not directly compare the methods. Therefore, the methods were compared by mimicking principal phases of the original chloramine-T depletion study and analyzing fillet tissue from exposed fish with the improved method (Figure). The results from the

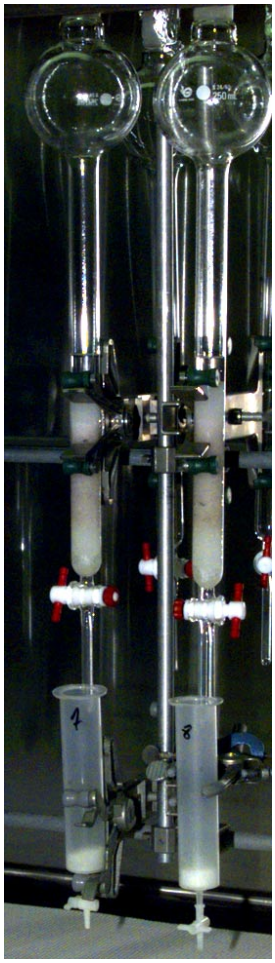

Figure. Extraction of para-toluenesulfonamide from fillet tissue.

new method could then be compared to previously reported results produced by the earlier method.

The most important element for this comparison was to ensure that critical components of the current depletion study were reasonably similar to components from the original depletion study (Table 1). The exposure and sampling techniques for the present study were an exact enactment of the techniques used in the original depletion study.

Concentrations of p-TSA in fillet tissue determined with the new method were similar to concentrations determined with the earlier method (Table 2), i.e., the p-TSA concentrations at each sample time were not statistically different.

Conclusion

An analytical method validated against FDA criteria can now be used in studies critical to the pursuit of allowing public and private hatcheries to use chloramine-T to combat bacterial diseases in fish. ❒

Table 1. Components of the present and original depletion studies where rainbow trout *Oncorhynchus mykiss* were exposed to chloramine-T at a nominal concentration of 20 ppm.

Component	Present study	Original study
Chloramine-T in water (ppm)	19	20
Number of exposed fish	72	72
Exposure duration (min)	60	60
Mean fish weight (g)	51.8	52.7
Fish weight range (g)	37.0 to 64.8	36.1 to 68.3

Table 2. The para-toluenesulfonamide (p-TSA) concentration in fillets from rainbow trout *Oncorhynchus mykiss* exposed to chloramine-T. Data presented from the present depletion study were determined with the new method. Data presented from the original depletion study were determined with the earlier method.

Time after exposure (h)	New method		Earlier method	
	n[a]	Mean p-TSA (ppb)	n	Mean p-TSA (ppb)
1	6	140	6	140
3	6	110	6	130
6	6	100	5	120
12	6	82	6	90

[a]n = the number of fish analyzed from each sample time.

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