



**Modeling the Safety of AQUI-S® to Rainbow Trout *Oncorhynchus mykiss***

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AQUI-S® (50% active isoeugenol) is a synthetic fish sedative manufactured by AQUI-S New Zealand, Ltd. AQUI-S® is approved for use in New Zealand, Australia, and several other countries, where it is classified as a “zero-withdrawal” drug because treated fish can immediately enter the human food chain via harvest from captive-rearing facilities or release into the environment.

In the U.S., AQUI-S® is a candidate for U.S. Food and Drug Administration (FDA) approval for the following indication: *Use at 20 – 60 mg/L (depending on species) to sedate all freshwater fish for handling and management purposes (no withdrawal period required).* To obtain such an approval, data must be generated to show that AQUI-S® is efficacious for its proposed uses; is safe to humans, the environment, and target animals; and can be manufactured consistently from batch-to-batch and year-to-year.

The U.S. Fish and Wildlife (FWS) Aquatic Animal Drug Approval Partnership (AADAP) program is involved in the AQUI-S® approval process. For example, we administer AQUI-S® Investigational New Animal Drug exemption (INAD) 10-541, which allows limited use of AQUI-S® in U.S. aquaculture and fishery management (a 21-day withdrawal period is required). Also, we conduct efficacy and target animal safety research in support of approval. In this bulletin, we summarize a pilot target animal study in which we demonstrated that the safety of AQUI-S® to rainbow trout *Oncorhynchus mykiss* is affected by exposure duration, concentration, and interaction between the two.

**Methods and Materials**

The test article was AQUI-S® (nominal concentrations: 40, 50, 80, and 100 mg/L); test fish were hatchery-reared rainbow trout (mean total length, 51 mm); and test water temperature was 12°C. AQUI-S® concentrations of 40 and 50 mg/L are within the product’s proposed indication range, and 80 and 100 mg/L are concentrations at which fish might easily be accidentally overdosed. Rainbow trout are a representative salmonid, and small rainbow trout are more sensitive to AQUI-S® than large rainbow trout. A water temperature of 12°C is within the range of tem-

peratures (10 – 15°C) at which rainbow trout and other salmonids are commonly reared in the U.S.

*Experimental procedures*—At each of 40, 50, 80, and 100 mg/L AQUI-S®, we established a set of exposure durations (T1<sub>conc</sub> – T6<sub>conc</sub> in minutes) that produced a wide range of mortalities (0 – 100%) for use in modeling probability of mortality via logistic regression. The four sets of exposure durations were different from each other. At each concentration, we exposed one “new” (previously unused) group of fish (14 – 16 fish per group) for T1<sub>conc</sub>, T2<sub>conc</sub>, T3<sub>conc</sub>, T4<sub>conc</sub>, T5<sub>conc</sub>, or T6<sub>conc</sub> in a static bath, at the end of which fish were collectively transferred to a recovery tank of flow-through fresh water. We held fish in the recovery tank for 20 min, after which all fish that showed movement were counted as “lived” and all fish that did not show movement were counted as “died.”

*Data analysis*—Mortality data generated (number “lived” and number “died” per group) were used to fit concentration-specific binary logistic regression models,

$$\text{Logit } P = b_0 + b_1 \text{DUR}$$

in which DUR (exposure duration) was the sole predictor variable (Table 1). From each concentration-specific model, we constructed corresponding probability-of-mortality curves,

$$P(\text{mortality}) = \frac{e^{b_0 + b_1 \text{DUR}}}{1 + e^{b_0 + b_1 \text{DUR}}}$$

which were plotted on one graph to visualize effects of exposure concentration and duration on probability of mortality. Subsequently, we combined all of the mortality data generated into an all-concentrations binary logistic regression model,

$$\text{Logit } P = b_0 + b_1 \text{DUR} + b_2 \text{CON} + b_{12} \text{DUR} \times \text{CON}$$

in which DUR, CON (exposure concentration), and DUR×CON (interaction term) were the predictor variables (Table 1).

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From the all-concentrations model, we constructed an all-concentrations probability-of-mortality equation,

$$P(\text{mortality}) = \frac{e^{b_0 + b_1 \text{DUR} + b_2 \text{CON} + b_3 \text{DUR} \times \text{CON}}}{1 + e^{b_0 + b_1 \text{DUR} + b_2 \text{CON} + b_3 \text{DUR} \times \text{CON}}}$$

which was used to predict mortality for selected combinations of exposure duration (2 - 30 minutes in 0.5-minute increments) and exposure concentration (40 - 100 mg/L). For this bulletin, those combinations of exposure duration and concentration at which predicted mortality was  $\leq 2\%$  were considered “safe” for use on small rainbow trout.

### Results

In this study, probability of mortality (a) increased with AQUI-S<sup>®</sup> exposure duration and exposure concentration and (b) was affected by interaction between duration and concentration (Figure 1). The influence of exposure duration alone is shown by the positive slopes of all four probability-of-mortality curves. The influence of exposure concentration alone is shown by the leftward shift in the probability-of-mortality curves at any given exposure duration. Finally, interaction between exposure duration and concentration is shown by the fact that the slopes of the probability-of-mortality curves become steeper as concentration increases from 40 – 50 to 80 – 100 mg/L. This latter result indicates that the relative influence of exposure duration on probability of mortality increases with exposure concentration.

Based on the all-concentrations probability-of-mortality equation, the combinations of AQUI-S<sup>®</sup> exposure duration and exposure concentration that appeared safe ( $\leq 2\%$  predicted mortality) for use on small rainbow trout ranged from 9 minutes at 40 mg/L AQUI-S<sup>®</sup> to 2 min at 85 mg/L AQUI-S<sup>®</sup> (Figure 2).

### Discussion

In this study, we demonstrated that the longer a fish remains in an AQUI-S<sup>®</sup> solution, the greater its chances of becoming too deeply sedated to recover. Also, we showed that margin for error in exposure duration decreases rapidly as concentration increases. Therefore, as with all experimental aquaculture drugs, fisheries professionals should test AQUI-S<sup>®</sup> on small numbers of fish before treating large numbers and should closely monitor sedated fish to minimize the potential for mortality.

Table 1. Logistic regression models based on mortality data generated.

AQUI-S <sup>®</sup> conc. (mg/L)	Logistic regression equation (Logit P = )
40	-9.008 + 0.572(DUR)
50	-5.848 + 0.577(DUR)
80	-9.269 + 1.998(DUR)
100	-12.744 + 4.161(DUR)
All conc.	-9.687 - 0.676(DUR) + 0.021(CON) + 0.031(DUR×CON)

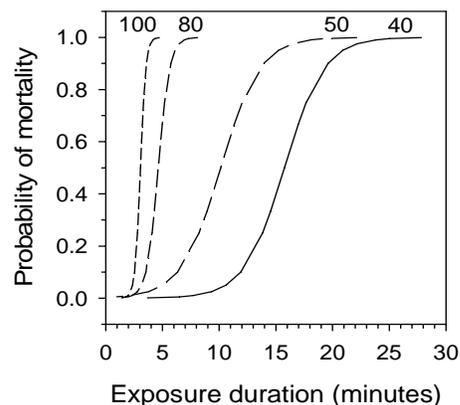


Figure 1. Probability-of-mortality curves for 40, 50, 80, and 100 mg/L AQUI-S<sup>®</sup> constructed from concentration-specific logistic regression models.

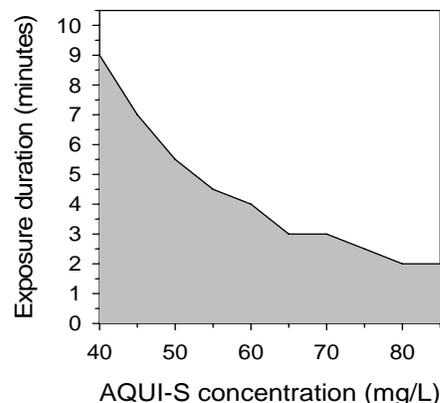


Figure 2. Predicted safe exposure range (gray area indicates  $\leq 2\%$  predicted mortality) based on all-concentrations probability-of-mortality equation.