DRIB No. 59
November 2021

U.S. Fish & Wildlife Service

Aquatic Animal Drug Approval Partnership

DRUG RESEARCH INFORMATION BULLETIN

The Influence of Salinity on Relevant Aquatic Drug Approval Technical Sections

Shane Ramee

U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Road, Bozeman, Montana, 59715, USA

Abstract

Currently, in the U.S., there are no aquatic animal drugs approved by the U.S. Food and Drug Administration (FDA) for use in saltwater. As marine aquaculture expands in the U.S., it is crucial to efficiently approve new drugs for use in saltwater aquaculture. This DRIB briefly reviews literature relevant to how the saltwater environment may affect the Human Food Safety, Effectiveness, and Target Animal Safety major technical sections of a new animal drug approval. Though research on this subject is limited, there are a handful of studies that utilized euryhaline species to isolate salinity as the independent variable. This paper summarizes the patterns that may allow for the use of data from freshwater drug approvals to be transferred to future saltwater approvals. Research suggests that drug residue clearance rates are generally faster in saltwater than in freshwater if other factors are held constant. However, these faster clearance rates may cause a reduction in efficacy in some cases. The effects of saltwater on drug toxicity are inconsistent, possibly highly drug dependent, and deserve further research. This suggests that any future drug approvals in saltwater should begin by addressing the effectiveness technical section, so that the appropriate effective dose is known when addressing the other technical sections.

Introduction

As the U.S. aquaculture industry expands into the marine environment, it must contend with a bare medicine chest, limiting the growth of the industry. As the number of approved drugs in freshwater aquaculture has expanded over the last couple of decades, it is critical the data which lead to the approval of these drugs be efficiently transferred to the saltwater environment. Each approved drug indication requires evidence of effectiveness and safety for that particular species, environment, and pathogen combination. The marine environment presents new combinations of these factors. This may call for novel research on effectiveness, target animal safety, and human food safety in saltwater. However, existing research may be utilized to offer insights and trends on the effectiveness and safety of drugs in the saltwater environment. These data-based trends should be understood and utilized to minimize the number of studies required to approve drugs for saltwater indications. In the following sections, the impact of the saltwater environment on evaluating the efficacy, target animal safety, and human food safety technical sections will be briefly explored with the available scientific literature being used to establish patterns that may help streamline the drug approval process in saltwater.

Human Food Safety

The FDA Center for Veterinary Medicine (CVM) utilizes a risk-based approach to determine the human food safety of a new animal drug. Pharmacokinetics and the clearing rate of drug residues in edible tissues are used to determine a conservative withdrawal time, which may be refined by specific evidence for each species group (FDA's Guidance for Industry 257). The saltwater environment contains a huge diversity of species across a wide temperature range. While the differences in pharmacokinetics among species and temperature groups make it difficult to compare residue depletion rates between saltwater and freshwater, the use of euryhaline species, which can tolerate a range of salinities, may be utilized to reduce the interference of covariates to better understand the effects of salinity on pharmacokinetics. Though research is limited, the pattern of decreased residue depletion time and increased clearing rates at higher salinities compared to freshwater is consistent across a variety of drugs and species.

In studies on Tilapia, the elimination half-lives were significantly shorter in saltwater for all tissues sampled (muscle, liver, and kidney (Feng et al. 2008) and plasma (Rairat et al. 2020)) except bile (Feng et al. 2008). These two studies both found faster drug clearance rates at higher salinities. In addition, Sidhu et al. (2018) found that absorption and elimination of oxytetracycline was faster in saltwater than in brackish and freshwater.

In Atlantic Salmon, flumequine residues were present in blood and muscle for over 8 weeks in freshwater and only 4 days and 2 weeks, respectively, in saltwater (Sohlberg et al. 2002). Uptake of difloxacin by Atlantic Salmon resulted in peak concentrations in freshwater at 24 and 48 h and at 8 and 18 h in saltwater in plasma and muscle, respectively (Elston et al. 1994). Oxolinic acid residues in Rainbow Trout plasma also depleted faster in saltwater, and residues were undetectable after 72 h in saltwater, but were still detectable for at least 244 h in freshwater (Ishida 1992). On the other hand, Abedini et al. (1998) found no significant difference in oxytetracycline absorption and elimination rates between freshwater Rainbow Trout and saltwater Chinook Salmon.

Other species and drug combinations have shown similar results. In Grass Carp, praziquantel elimination half-lives were shorter in the brackish water group in all tissues sampled (Xie et al. 2015). Additionally, in Yellow Belly Pufferfish, the peak enrofloxacin concentrations in all tissues sampled were higher and took less time to peak in the higher salinity treatments. Elimination half-lives were also faster at high salinities in the liver and kidney but slower in the plasma (Ma et al. 2017). Drug depletion rates in muscle tissue are most pertinent to human food safety considerations, since it is the primary edible tissue in fish. Tilapia, Atlantic Salmon, and Common Carp all demonstrated faster drug residue depletion rates in muscle tissue at higher salinities in studies that sampled muscle tissue (Elston et al. 1994, Sohlberg et al. 2002, Xie et al. 2015). On the other hand, in Yellow Bellied Pufferfish, peak enrofloxacin residues and residue levels at all sampling points in the muscle were significantly higher at 20 and 30 ppt than at 10 ppt, but there was no difference in residue half-life in the muscle (Ma et al. 2017).

The mechanism behind the pattern of faster drug clearance rates in saltwater is still debated. While the mechanism is likely affected by the type of drug and species, the differences in osmoregulatory physiology between freshwater and saltwater fish may explain this pattern. In saltwater, a high drinking rate compensates for water lost via osmosis to the saline environment. Monovalent ions are excreted via the gills, and divalent ions are excreted via concentrated urine. It is believed that broad classes of drugs may be excreted along with the divalent ions in the urine. In freshwater, the osmotic gradient causes water to naturally enter the body and salts to naturally escape the body. To deal with this, fish osmoregulate with low drinking rates and high volumes of dilute urine. Physiological changes in the gill prevent excessive losses of salts from the blood plasma. It is hypothesized that these salinity dependent physiological differences affect the excretion of drugs and other xenobiotics.

Overall, the influence of salinity on drug residue depletion curves is consistent among the species, drugs, and tissues for which data is available. The evidence that drug residues in muscle follow similar patterns to that of plasma (which is more easily and commonly collected and tested) expands the data available. This suggests that residue depletion and human food safety should not be a major limiting factor to expanding an aquatic drug approval for freshwater to saltwater, if the dosage is the same.

Drug Efficacy

Pharmacokinetic studies often use indices based on the minimum inhibitory concentration (MIC) as a proxy for efficacy. The MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation (Andrews 2001). Minimum inhibitory concentrations are an in vitro measurement, so applying them to real world efficacy is difficult. For this reason, several different proxy variables have been used to predict efficacy from pharmacokinetic data. The maximum plasma concentration (Cmax) can be compared to the MIC as a proxy for efficacy. A Cmax to MIC ratio of 4:1 or even 8:1 has been considered a threshold to demonstrate real world efficacy (Stramm 1989, Blaser et al. 1987, Rigos & Troisi 2005). Other indices of effectiveness involve the length of time in which the plasma concentration is greater than the MIC (T > MIC), since bacteria re-growth and reinfection can occur when concentrations dip below the MIC. Area under the curve (AUC) is an index that combines the time over the MIC and the magnitude to the tissue concentrations. The mode of action of the drug affects which index best predicts efficacy. These indices can be used to predict changes in efficacy at different salinities.

In Tilapia, the Cmax of oxytetracycline was not significantly affected by salinity but, the AUC in saltwater was about 3 times lower than in freshwater and brackish water, suggesting that the dosing frequency and duration may need to increase to maintain efficacy in saltwater (Sidhu et al. 2018). Researchers also concluded that Nile Tilapia reared in 15 ppt would require a higher dosage of florfenicol to maintain the same level of efficacy as in freshwater (Rairat et al. 2020). In Atlantic Salmon, the Cmax and AUC of difloxacin were significantly greater in fish held in freshwater compared to saltwater (Elston et al. 1994). While this indicates that this antibiotic may not be as effective in saltwater, the tissue concentrations of difloxacin

in saltwater-held fish were still above the reported MIC for Aeromonas salmonicida (Elston et al. 1994). In Yellow Belly Pufferfish, only AUC values in the kidney were lower at the higher salinities, while Cmax/MIC and AUC/MIC ratios were above recognized effective levels in all salinities (Ma et al. 2017).

Though studies assessing the effects of salinity on drug pharmacokinetics are limited, the pattern of faster or equivalent drug clearance rates at higher salinities seems to be conserved across species and drug types. This negatively affects several of the indices typically used to predict drug efficacy. Specifically, T > MIC and AUC/MIC are often negatively affected by increased salinity. For drugs with mechanisms of action that are time dependent (e.g. bacteriostatic), more frequent redosing may be required in saltwater to maintain efficacy. On the other hand, the saltwater environment usually did not affect Cmax. For drugs where Cmax/MIC is most predictive of efficacy (e.g. bactericidal), saltwater may not reduce the efficacy compared to freshwater. Further study of how drug class and chemical properties affect their pharmacokinetics and efficacy in saltwater is also necessary.

As U.S. saltwater aquaculture expands, evidence of drug efficacy will have to be established for the specific pathogens of the marine environment and for real world treatment scenarios. These pivotal efficacy studies will likely need to be the first step in an expansion to saltwater.

Target Animal Safety

There is extremely limited data evaluating the effect of salinity on toxicity or the margin of safety of aquatic animal drugs. The available pharmacokinetic research offers minimal insight into target animal safety. For some commonly used drugs, margin of safety or toxicity studies have been conducted on fish species in fresh and saltwater. However, comparing results across studies on different species can provide only moderate insight due to differences in confounding variables such as temperature and life stage.

Birdsong & Avault (1971) compared the toxicities of acriflavine, copper sulfate, formalin, and potassium permanganate in Pompano at 10, 20, and 30 ppt. Salinity had no effect on acriflavine and formalin toxicities but had a significant effect on copper sulfate and potassium permanganate toxicities. The toxicity of copper sulfate decreased with increased salinity, and potassium permanganate toxicity increased at higher salinities (Birdsong & Avault 1971). However, copper sulfate toxicity is known to be dependent on the hardness and alkalinity of the water (Straus 2017). Since saltwater usually has very high hardness and alkalinity, copper sulfate should not be as toxic to fish in saltwater.

Comparisons of LC50 values for the same drug across species from freshwater and marine environments may offer an opportunity to identify patterns of differential toxicity, but data is still extremely limited, and an extensive amount of data would be required to draw any meaningful conclusions. In saltwater species, such as the Rabbitfish and Bullseye Pufferfish, the 72 h LC50 of formalin was 551.0 and 79 mg/L, respectively (Nasser et al. 2017, Fajer-Ávila et al. 2003) and the 96 h LC50 in American Eel, Atlantic Salmon, Pompano, and Striped Bass was 81, 69, 69.1, and 10.84 mg/L, respectively (Hinton & Eversols 1978, Bills et al. 1977, Birdsong & Avault 1971, Rearborn & Harrell 1990). In freshwater fish, 96-h LC50 values for formalin range from 26 to 1020 mg/L (McKim et al. 1976, Bills et al. 1977, Wellens, 1982). With such wide ranges of LC50 values, it is difficult to derive much of a conclusion other than saltwater species seem to have similarly high formalin tolerances.

Emamectin benzoate is a compound that has safety data for multiple species in both freshwater and saltwater. In freshwater-held Rainbow Trout, no mortalities or signs of toxicity were observed after 14 days of oral treatment at a rate of 150 μg/kg body weight/day (3X the recommended dose and 2X the recommended treatment duration) (Bowker et al. 2013). In saltwater-held Rainbow Trout fed 0, 100, 250, and 500 μg/kg body weight/day (equivalent to 0, 2, 5, and 10X the recommended dose) for 7 days, there were no mortalities during the treatment or post treatment periods (Roy et al. 2000). In the 500 μg/kg dosage treatment group and to a lesser extent in the 250 μg/kg treatment, signs of possible toxicosis included reduced feeding, lethargy, darkened colorization, and anorexia (Roy et al. 2000). However, no histological findings were considered indicative of toxicosis (Roy et al. 2000). Similar findings were found in Atlantic Salmon and Zebrafish in both saltwater and freshwater (Roy et al. 2000, Stone et al. 2002, Collymore et al. 2014).

Overall, information regarding the effects of salinity on target animal safety in fish is extremely limited, and more research is needed. From the information available, it appears that salinity-based patterns will likely be highly drug dependent. As more drug approvals are completed for saltwater indications, more data will become available that may be used to streamline this technical section. A particular drug's chemistry and mode of action will have considerable influence on its safety in the marine environment. For the few drugs with toxicity or margin of safety data available in saltwater, there does not appear to be any added toxicity in saltwater, except with potassium permanganate. While many drugs will likely behave similarly in saltwater and freshwater, there will also be exceptions that will need to be identified. Toxicity information for a particular drug may be used to guide the margin of safety studies in saltwater.

Conclusion

Broad statements are difficult to make regarding aquatic animal drugs. The wide variety of species and drugs means that there are always exceptions. This is especially true when dealing with limited information from which to draw conclusions. However, the pharmacokinetic studies on euryhaline species indicate a consistent pattern of salinity influence on drug residue depletion rates. Faster residue depletion rates in saltwater have been observed in a variety of species, drugs, and tissue types. The faster drug clearance rates in the muscle are especially important for future human food safety in saltwater. It seems unlikely that withdrawal times will increase in saltwater for most drugs approved for similar uses in freshwater. But, to assume that drug clearance will be the same or faster in saltwater, it must be administered at the same dosage as in freshwater. The same pharmacokinetic research that indicates that drug clearance rates are faster in saltwater also suggests that the drugs may not be as effective in saltwater. Therefore, collecting real world effectiveness data should be the first step to confirm the effective dosage. If the effective dosage for saltwater indications is the same as in freshwater, this could minimize the new data needed for the Target Animal Safety and Human Food Safety technical sections. Given the current drug approval paradigm and the fact that no drugs are currently approved in saltwater, it is likely significant research will be required to attain the first saltwater approval. If the research conducted to complete the first few drug approvals in saltwater reinforce the patterns identified here, it is likely the data requirements can be reduced over time, as they have been in freshwater.

Acknowledgments

Thank you to Jon Amberg for serving as an external reviewer. Thank you to the rest of the AADAP team for their guidance and willingness to help with multiple rounds of revisions.

References

Abedini, S., R. Namdari, and F. C. P. Law. 1998. Comparative pharmacokinetics and bioavailability of oxytetracycline in rainbow trout and chinook salmon. Aquaculture 162(1):23-32.

AliAbadi, F. S., and P. Lees. 2000. Antibiotic treatment for animals: Effect on bacterial population and dosage regimen optimization. International Journal of Antimicrobial Agents 14(4):307-313.

Blaser, J., B. B. Stone, M. C. Groner, and S. H. Zinner. 1987. Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. Antimicrobial Agents and Chemotherapy 31(7):1054-1060.

Bowker, J. D., D. Carty, and M. P. Bowman. 2013. The safety of SLICE (0.2% emamectin benzoate) administered in feed to fingerling rainbow trout. North American Journal of Aquaculture 75(4):455-462.

Collymore, C., V. Watral, J. R. White, M. E. Colvin, S. Rasmussen, R. J. Tolwani, and M. L. Kent. 2014. Tolerance and efficacy of emamectin benzoate and ivermectin for the treatment of pseudocapillaria tomentosa in laboratory zebrafish (*danio rerio*). Zebrafish 11(5).

Elston, R. A., A. S. Drum, M. G. Schweitzer, R. A. Rode, and P. R. Bunnell. 1994b. Comparative uptake of orally administered difloxacin in Atlantic Salmon in freshwater and seawater. Journal of Aquatic Animal Health 6(4):341-348.

Fajer-Ávila, E. J., I. Abdo-De La Parra, G. Aguilar-Zarate, R. Contreras-Arce, J. Zaldívar-Ramírez, and M. Betancourt-Lozano. 2003. Toxicity of formalin to bullseye puffer fish (sphoeroides annulatus jenyns, 1843) and its effectiveness to control ectoparasites. Aquaculture 223(1-4):41.

Feng, J., X. Jia, and L. Li. 2008. Tissue distribution and elimination of florfenicol in tilapia (Oreochromis niloticus × O. caureus) after a single oral administration in freshwater and seawater at 28 °C. Aquaculture 276:29-35.

Ishida, N. 1992. Tissue levels of oxolinic acid after oral or intravascular administration to freshwater and seawater rainbow trout. Aquaculture.

Ma, R., L. Yang, T. Ren, Y. Dong, T. Liu, P. Zhuang, W. Fang, X. Yang, and K. Hu. 2017. Enrofloxacin pharmacokinetics in Takifugu flavidus after oral administration at three salinity levels. Aquaculture Research 48(8):4545-4553.

Nasser, N., J. Babikian, S. Monzer, and I. P. Saoud. 2017. Toxicity of four chemotherapeutic agents to RabbitfishSiganus rivulatus. Journal of the World Aquaculture Society 48(6):877-886.

Rairat, T., W. Thongpiam, C. Hsieh, Y. Liu, S. Tunkijjanukij, and C. Chou. 2020b. Salinity-dependent pharmacokinetics of florfenicol in Nile Tilapia (Oreochromis niloticus) and its implication in optimal dosing regimen. Aquaculture 519:734900.

Reardon, I. S., and R. M. Harrell. 1990. Acute toxicity of formalin and copper sulfate to striped bass fingerlings held in varying salinities. Aquaculture 87(3):255-270.

Rigos, G., and G. Troisi. 2005. Antibacterial agents in Mediterranean finfish farming: A synopsis of drug pharmacokinetics in important euryhaline fish species and possible environmental implications. Reviews in Fish Biology and Fisheries 15(1):53-73.

Roy, W. J., I. H. Sutherland, H. D. M. Rodger, and K. J. Varma. 2000. Tolerance of Atlantic salmon, Salmo salar L., and rainbow trout, Oncorhynchus mykiss (Walbaum), to emamectin benzoate, a new orally administered treatment for sea lice. Aquaculture 184:19-29.

Seubert, J. M., and C. J. Kennedy. 2000. Benzo[a]pyrene toxicokinetics in rainbow trout (Oncorhynchus mykiss) acclimated to different salinities. 38:342-349.

Sidhu, P. K., S. A. Smith, C. Mayer, G. Magnin, D. D. Kuhn, M. Jaberi-Douraki, and J. F. Coetzee. 2018b. Comparative pharmacokinetics of oxytetracycline in tilapia (Oreochromis spp.) maintained at three different salinities. Aquaculture 495:675-681.

Sohlberg, S., K. Ingebrigtsen, M. K. Hansen, W. L. Hayton, and T. E. Horsberg. 2002. Flumequine in Atlantic Salmon Salmo salar: Disposition in fish held in sea water versus fresh water. Acronyms, Initialisms & Abbreviations Dictionary 49:39-44.

Stamm, J. M. 1989. In vitro resistance by fish pathogens to aquacultural antibacterials, including the quinolones difloxacin (A-56619) and sarafloxacin (A-56620). Journal of Aquatic Animal Health 1(2):135-141.

Stone, J., W. J. Roy, I. H. Sutherland, H. W. Ferguson, C. Sommerville, and R. Endris. 2002. Safety and efficacy of emamectin benzoate administered in-feed to Atlantic salmon, Salmo salar L., smolts in freshwater, as a preventative treatment against infestations of sea lice, Lepeophtheirus salmonis (Krøyer). Aquaculture 210:21-34.

Straus, D.L. 2017. Copper sulfate toxicity to various fish: role of alkalinity/hardness [abstract]. 6th National Aquaculture Extension Conference, June 6-8, 2017, Boise, Idaho. p. 38.

Tachikawa, M., and R. Sawamura. 1994. The effects of salinity on pentachlorophenol accumulation and elimination by killifish (oryzias latipes). Archives of Environmental Contamination and Toxicology 26(3).

Tachikawa, M., R. Sawamura, S. Okada, and A. Hamanda. 1991. Differences between freshwater and seawater killifish (Oryzias latipes) in the accumulation and elimination of pentachlorophenol. Archives of Environmental Contamination and Toxicology 21:146-151.

Toutain, P. L., Ferran, A., & Bousquet-Mélou, A. 2010. Species differences in pharmacokinetics and pharmacodynamics. Comparative and veterinary pharmacology, 19-48.

Xie, X., Y. Zhao, X. Yang, and K. Hu. 2015. Comparison of praziquantel pharmacokinetics and tissue distribution in fresh and brackish water cultured grass carp (Ctenopharyngodon idellus) after oral administration of single bolus. BMC Veterinary Research 11(84):1-6.