Justification to consider studies that demonstrate drug efficacy on freshwater-reared steelhead trout or rainbow trout be sufficient to satisfy the effectiveness requirements for all freshwater-reared *Oncorhynchus mykiss*.

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Objective

Via material presented in this document, we intend to argue that the demonstration of drug efficacy on either freshwater-reared (i.e., pre-hatchery release) steelhead trout *Oncorhynchus mykiss*, freshwater-reared rainbow trout *O. mykiss*, or any other subspecies or strain of *O. mykiss* should be considered effective for all freshwater-reared *O. mykiss*. This document makes the argument that these subspecies or strains are equivalent (from a drug treatment or approval perspective) and does so by providing sufficient background, nomenclature, life history information, and a brief summary of scientific findings. In addition, we've provided information with respect to the culture of *O. mykiss*, and results from the 2005 federal, state, and tribal aquaculture database that support this assertion.

To summarize, we have cites literature and personal communications from experts in *O. mykiss* culture and fish health to prove there is no genetic difference between subspecies or strains of *O. mykiss* and that during the period that fish are held/reared in freshwater hatcheries, there is virtually no difference between subspecies/strains physiologically or morphologically (that can be detected using taxonomic keys). From this we will claim that all subspecies of *O. mykiss* should be considered equivalent as it relates to drug approval generation.

Background (From Behnke 2002)

Oncorhynchus mykiss is the best-known species of trout in the world. Its renown as a sport fish has led to its introduction worldwide into virtually all suitable cold-water

habitats, and it is by far the most important species of trout raised commercially for markets and restaurant trade. Beautiful iridescent colors, most vivid during spawning times, have given rise to this species' common name.

The different names used for *O. mykiss* can be confusing. The sea-going, or anadromous, form of rainbow trout is known as the steelhead trout. The term redband trout is used to describe distinctive forms of rainbow trout native to particular regions of North America. Redband trout are named for the brick red colors along their flanks, while the sea-going form of rainbow trout are called steelhead because of their metallic silvery color when in salt water. A certain type of redband trout also has a sea-going life history and is called a redband steelhead.

As confusing as this may be for the non-expert, the important consideration is that no matter whether they are called rainbows, redbands, steelhead, or redband steelhead, they are all rainbow trout and are classified as subspecies of the full species *O. mykiss*.

Common and Scientific Names of Fishes (AFS 1991)

Simply put, *O. mykiss* is the scientific name for rainbow trout, and the term steelhead is applied to sea-run rainbow trout and some populations from large lakes (e.g., the U.S. Great Lakes).

Life History

Oncorhynchus mykiss are native to western North America with both resident and anadromous migrating life-history forms found throughout their range (Behnke 1992; 2002). In the case of the anadromous migrating form, they typically spend up to three years in fresh water (the parr stage) before smoltification and migration to the ocean or large lakes (e.g., U.S. Great Lakes). At the time of migration, parr have attained a length of 6 to 8 inches. Steelhead will return to their home rivers to spawn after 15 – 30 months of ocean life (Behnke 2002). Unlike Pacific salmon, steelhead do not necessarily die after spawning and repeat spawning is common. The resident form of *O. mykiss* spends its entire life in fresh water with potential "migration" to nearby freshwater lakes and

streams. Originally, the two life-history forms of *O. mykiss* were classified as two distinct species based on morphology and behavior, but have been reclassified as a single species (Behnke 1992). Consequently, *O. mykiss* populations are now divided into subspecies based primarily upon morphological evidence (Behnke 1992; 2002), many of which each exhibit resident and anadromous migratory life-history patterns of their own.

Scientific Findings

In an effort to address issues surrounding steelhead conservation and the need to define conservation units, research has been focused on the degree of genetic differentiation between steelhead and rainbow trout (i.e., how much gene flow occurs between the two types). The life history differences that distinguish steelhead from stream-resident rainbow trout are so striking that the two forms were once classified as separate species. Whereas stream-raised rainbow trout may complete their life cycle in a limited area of a small stream and attain a length of only 8 inches or so, steelhead may spend half their lives at sea, roaming for thousands of miles (Behnke 2002). However, modern genetic analysis has confirmed not only that steelhead and rainbow trout belong to the same species, but also that the two different life history forms from one river basin are more closely related to each other than they are to similar life history forms in other river basins (McEwan 2001; Behnke 2002; Narum 2004; Olsen 2006). Furthermore, genetic differentiation between similar life-history forms of *O. mykiss* (e.g., resident vs. resident) is greater when those forms occupy different river basins. This indicates that the degree of relatedness among *O. mykiss* populations is generally associated with geographic proximity, not life-history type (Olsen 2006) and suggests that steelhead and rainbow trout are polyphyletic and the result of parallel evolution rather than members of two distinct lineages (Docker et al. 2003). Several taxonomists (Docker et al. 2003; McEwan 2001) have concluded that *O. mykiss* cannot be separated taxonomically at the species or subspecies level by life-history type. Recent research demonstrating that juvenile rainbow trout can adopt a life-history strategy that is different from their parents (i.e., a steelhead can produce non-anadromous migratory progeny and non-anadromous rainbow

trout can produce steelhead progeny) provides further evidence that the two forms are genetically linked (Good et al. 2005; McEwan 2001).

Culture of *O. mykiss*

Oncorhynchus mykiss is the most widely cultured fish worldwide and has been naturalized in all parts of the world where water temperatures are less than about 15^{o}C. Domesticated hatchery strains are used for aquaculture and for stocking (Wedemeyer 2001). The very first propagation of rainbow trout was of coastal rainbow trout from the San Francisco Bay area. From 1880 to 1888, about 2.5 million rainbow trout eggs were shipped to federal and state hatcheries, and broodstock were established in federal hatcheries in Virginia and Michigan, increasing the number of egg sources. From 1895 to 1900, substantial numbers of coastal steelhead were propagated in federal hatcheries (Behnke 1992). Scott et al. (1978) discovered that the first rainbow trout established in New Zealand came from a private hatchery that used steelhead eggs from a tributary to San Francisco Bay. Thus Behnke (1992) concludes the overwhelming majority of hatchery broodstocks of rainbow trout maintained around the world originated mainly from various mixtures of coastal steelhead.

The techniques used to culture young rainbow and steelhead trout are virtually identical (Charlie Smith, USFWS – retired (Director, Bozeman Fish Technology Center) and John Morrison, USFWS –retired (Olympia Fish Health Center), personal communication). Some have observed that steelhead trout may feed less aggressively than rainbow trout because rainbow trout are considered more "domesticated" (Mark Olson, USFWS Hagerman NFH, personal communication). At hatcheries that rear rainbow and steelhead trout, fish are reared in the same water, under the same culture and environmental conditions, and are fed the same feed (Charlie Smith, USFWS – retired (Director Bozeman Fish Technology Center), personal communication). Under such conditions, it is often difficult, if not impossible, to differentiate between the two based on morphology of fry or fingerling (Mark Olson, USFWS Hagerman NFH, personal communication). Steelhead are typically held in the hatchery for <10 - 12 months (they need to be stocked out to make room for the next year class), at which time they may or may not yet begin to

smolt. After hatchery-release to freshwater tributaries, steelhead migrate to the ocean (or to large inland lakes), and return to the hatchery approximately 3 years later ready to spawn.

Changes in steelhead associated with smoltification result in minimal changes with respect to fish culture. During smoltification a variety of morphological, physiological, and behavioral changes prepare juvenile steelhead for migration to, and residence in, the open ocean. Morphologically, body color becomes silvery and weight per unit length (i.e., condition factor) decreases. Physiologically, surges in endocrine hormones stimulate changes in gill epithelial structure and metabolism to allow for the transition from fresh water to salt water. Behaviorally, steelhead are less prone to hold position against the current, and thus downstream movement is initiated. In addition, they become less territorial and more surface oriented (Zabel 2007).

During the smoltification process, steelhead feed consumption may diminish (Behnke 2002). For several reasons, including time of year and water temperature, steelhead trout returning to the hatchery via ladders or gates have "gone off" feed (i.e., do not feed). Adult steelhead kept at the hatchery and "reconditioned" (allowed to spawn over multiple seasons) must be retrained to feed, which is often difficult to accomplish (hence, most fish are killed and disposed of). In the case of summer steelhead (termed summer because of the time of year fish re-enter fresh water), fish may return to the hatchery in May/June but not spawn until December. During this period, fish do not feed (John Morrison, USFWS – retired (Olympia Fish Health Center), personal communication). Although some steelhead continue to feed after entering fresh water, but at much reduced rates (Behnke 2002), there are many instances where fish cease feeding once they re-enter fresh water (Kathy Clemens, USFWS Idaho Fish Health Center, personal communication). During this "waiting" period, fish held for spawning that become infected with systemic bacterial pathogens are treated with antibiotic via injection.

2005 Fish Production Data

Based on results from the 2005 Fish Production Database recently assembled by the USFWS's AADAP Program, with support from the Association of Fish and Wildlife Agencies' Drug Approval Working Group, a total of 118 million *O. mykiss* were cultured in 2005. Of that total, 87 million rainbow trout were produced, including 13 million fry (average size, 1.5 in; range, 0.4 – 3.6 in), 31 million fingerling (average size, 4.6 in; range 1.9 – 8.0 in), 43 million catchable (average size, 10.7 in; range 7.4 – 19.0 in), and 0.07 million broodstock (average size, 17.3 in; range 11.4 – 26.0 in). The remaining 31 million were steelhead trout and comprised 2 million fry (average size, 2.2 in; range, 0.9 – 3.4 in), 17 million fingerlings (average size, 6.3 in; 1.2 – 8.3 in), 12 million catchable (average size, 8.1 in; range 5.6 – 11.2 in), and 0.009 million broodstock (average size, 19.5 in; Note that this broodstock population is maintained by the State of Minnesota and is the result of fish captured at smolt traps (fish size 4.8 – 5.6 inches at capture), transported to the Minnesota DNR French River Coldwater Hatchery, feed trained, and spawned for 2 – 3 yrs before release into Lake Superior; Fred Tureson, Minnesota DNR, personal communication). The term catchable steelhead refers to pre-hatchery release fish that may or may not have undergone smoltification (Note the difference in size of catchable rainbow trout and steelhead trout).

Discussion

The above-noted information supports the presumption that while all life-stages of rainbow trout are reared or held as broodstock and may be subject to treatment with one or more drugs (included medicated feed) during their captivity, virtually no steelhead trout that have returned from the ocean are kept for an extended period of time at a hatchery after they've spawned. Life-stages of steelhead trout commonly reared at hatcheries have yet to fully undergo morphological or physiological changes. Thus, during the time such fish are held in captivity, there are virtually no morphological or physiological differences between steelhead and rainbow trout. We acknowledge that such differences do exist with respect to adult steelhead trout, but point out the difficulty

of holding broodstock steelhead trout of hatchery origin is that, like Pacific salmon, during the return journey to the hatchery they do not actively feed. Steelhead are either killed during the spawning process or are released to outmigrate and return to spawn another year. During the spawning period (at the time when the fish actually enters the hatchery, and in many cases, when the fish enters freshwater), fish do not feed. As a result, in cases where returning steelhead spawners do become infected with systemic bacterial infections such as columnaris, effective treatment with an antibiotic must be administered via injection (John Morrison, USFWS – retired, and Kathy Clemens, USFWS – Idaho Fish Health Center, personal communication).

Conclusions

Based on our experience, information gleaned from the literature, and data from the 2005 Fish Production Database, steelhead trout and rainbow trout are the same species of the genus *Oncorhynchus*, and virtually all such fish reared or held in Federal, State, and Tribal hatcheries are freshwater-reared rainbow or steelhead trout (have yet to migrate to the ocean). Based on this information, one can only hypothesize that *O. mykiss* (regardless of subspecies or strain) be considered equivalent with respect to therapeutic drug treatment. Furthermore, we contend, that because environmental conditions, culture conditions, and culture practices for the various subspecies of *O. mykiss* are nearly identical (life history differences have yet to become evident), the completion of an effectiveness technical section for either freshwater-reared steelhead trout or rainbow trout should satisfy the requirements for all freshwater-reared *O. mykiss*.

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