DRIB No. 01
May 2007

U.S. Fish & Wildlife Service

Aquatic Animal Drug Approval Partnership

DRUG RESEARCH INFORMATION BULLETIN

Buffering Oxytetracycline Hydrochloride Immersion-Marking Solutions with Sodium Phosphate Dibasic

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Oxytetracycline hydrochloride (OTC-HCL) water soluble powder is approved in the U.S. for use in the skeletal marking of finfish fry and fingerlings by immersion at dosages of 200 -700 mg/L active OTC for 2 -6 h. This compound is acidic, and thus most OTC-HCL solutions will likely need to be buffered to prevent or minimize mortality in treated fish due to low pH. Anhydrous sodium phosphate dibasic (SPD) is commonly used as such a buffer, probably because it is relatively safe to humans (Stecher et al. 1968) and its buffering effects are relatively easy to control (Fielder 2002). Our study was designed to show how pH decreases when OTC-HCL is added to various "source waters" to produce 700 mg/L active OTC solutions and how pH increases when SPD is added to buffer such solutions to pH 7.

Methods

Test articles were (a) Oxytetracycline HCl Soluble Powder 343 (343 g active OTC per 454 g premix; Phoenix Scientific, Inc., St. Joseph, MO 64503; FDA-approved in September, 2004) and (b) anhydrous Sodium Phosphate Dibasic (a 99% pure, water soluble powder; E. M. Science, Gibbstown, NJ). Five source waters with different natural buffering capacities were tested (Table 1). Two 2-gal "replicate" water samples were collected from each source water, and each 2-gal replicate was processed as follows: Fourteen 0.5-g aliquots of OTC-HCL were sequentially added to produce a non-buffered, 700 mg/L active OTC solution. Then, 10 1-g aliquots of SPD were sequentially added to buffer the solution to approximately pH 7. The pH of the solution was measured after each aliquot of OTC-HCL or SPD was added and dissolved. Replicate pH measurements were averaged within source water to facilitate comparisons of pH change among source waters.

Results

In all five source waters, pH decreased as aliquots of OTCHCL were added and increased as aliquots of SPD were added (Figure 1). The pH changes observed in the two waters with "high" natural buffering capacity were more gradual and of less overall magnitude than pH changes observed in the two waters with "moderate" natural buffering capacity or in distilled water (no natural buffering capacity). Consequently, the active OTC concentration at which pH was first observed to be unsuitable for aquatic life (pH $\leq$ 6.5 according to the U.S. EPA) was highest (250 mg/L) in the two waters with "high" natural buffering capacity, intermediate (100 -150 mg/L) in the two waters with "moderate" natural buffering capacity, and lowest (50 mg/L) in distilled water. Regardless of source water, 4 -5 g of SPD (a 0.6 -0.7:1 ratio with OTC-HCL) were needed to increase pH to levels suitable for aquatic life (pH > 6.5), and 8 -9 g of SPD were needed to increase pH to 7 (a 1.1 -1.3:1 ratio with OTCHCL). Also, regardless of source water, maximum pH values achieved were never higher than 7.1 — even after all 10 g of SPD had been added to a 700-mg/L active OTC solution.

Discussion

Based on our results, it is clear that the natural buffering capacity of source water affects pattern and magnitude of pH change when OTC-HCL is used to achieve a 700-mg/L active OTC solution and when SPD is used to buffer such a solution to pH 7. According to Bain (1999) and Wurts and Durborow (1992), natural buffering capacity is better estimated by measuring total alkalinity (mg/L CaCO3) rather than total hardness (mg/L CaCO3). Therefore, we recommend that fish culturists measure the total alkalinity of their source water before preparing and buffering an OTC-HCL immersion-marking solution.

In addition, Fielder (2002) recommended that fish culturists measure pH before and during OTC-HCL immersion-marking to ensure that pH is maintained at levels safe to treated fish.

It is also clear from our results that most OTC-HCL immersion-marking solutions will need to be buffered and that such buffering is relatively easy to achieve with SPD. Specifically, both we and Fielder (2002) found that an approximate 1:1 ratio (weight:weight) of SPD to OTC-HCL will buffer almost any 700-mg/L active OTC solution to pH 7 and that addition of "excess" SPD will not increase pH much above 7. Felder (2002) cautioned that "overuse" of SPD can be toxic to treated fish.

Finally, we note that our study was conducted under controlled laboratory conditions with only two test articles, five source waters, and no fish. Therefore, we recommend that fish culturists review Fielder's (2002) OTC-HCL immersion-marking guidelines and conduct pilot tests to determine how various test articles, source waters, and fish interact.

References

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Table 1. Source quality

Source water buffering capacity	Total alkalinity (mg/L $CaCO_3$)	Total hardness (mg/L $CaCO_3$)	Initial pH	Temp (°C)
High	185	200	7.6	6.9
High	165	197	7.8	12.0
Moderate	81	110	7.9	13.4
Moderate	56	74	7.6	13.9
None[2]	0	0	7.1	15.2

[2]distilled water

Figure 1. Observed changes in pH as aliquots of OTC-HCL and SPD were added to source waters.