Attachment 3. Evaluation of ACRs Used to Infer Chronic Toxicity for Delta smelt

Acute-chronic ratios (ACRs) are being used by several investigators, in lieu of chronic toxicity test results, to postulate that ambient concentrations of ammonia in the Delta may be causing chronic toxicity to sensitive species. For example, hypothetical ACRs for rainbow trout were used in a presentation at the Central Valley Regional Water Quality Control Board (CVRWQCB) Ammonia Summit in August 2009 (Werner 2009, slide10), and in recent reports to the CVRWQCB (Werner et al. 2009a,b), to support an argument that chronic exposure to ambient levels of ammonia in the Delta may cause toxicity for Delta smelt. This logic behind the argument can be summarized as follows:

- Chronic toxicity test results are lacking for Delta smelt.
- Delta smelt appear to be as acutely sensitive to ammonia as rainbow trout (*Oncorhynchus mykiss*).
- Therefore, chronic toxicity values for Delta smelt are probably similar to those for rainbow trout.
- Hypothetical ACRs for rainbow trout are alleged to be in the range 14.6-23.5.
- Therefore, one can divide the LC50 for delta smelt (acute value) by hypothetical ACRs for rainbow trout to estimate the concentration of ammonia that would cause chronic toxicity to Delta smelt
- Some ambient ammonia concentrations in the Delta are higher than the values that result from this exercise.

Below we provide information which shows that the hypothetical ACRs for rainbow trout stated above (14.6 and 23.5) rely on information that was excluded by USEPA in 1999 and 2009 for use in developing the chronic criterion and are not based on evidence for chronic effects of ammonia effects on survival, reproduction, or growth of rainbow trout (USEPA 1999, 2009). Consequently, inferences about chronic toxicity for Delta fish species - such as Delta smelt - based on these ACRs are questionable and should be carefully qualified.

USEPA Position on Valid Chronic Endpoints and Chronic Test Design for Fish, and Interpretation of Chronic Data for Rainbow Trout

In 1999, USEPA used explicit criteria to re-evaluate the available chronic toxicity tests for fish and aquatic invertebrates (USEPA 1999). One result of this analysis was a list of acceptable chronic tests. This list appears as Table 5 ("EC20s from Acceptable Chronic Tests") on page 65 of USEPA (1999), along with Species Mean Chronic Values (SMCV) and Genus Mean Chronic Values (GMCV) where it was appropriate to calculate them. Among the criteria for inclusion in this list were (1) the test had to be a flow-through test (except that static renewal is acceptable for daphnids), (2) test conditions had to include acceptable dissolved oxygen concentrations, and (3) the endpoint(s) of the test had to be

Larry Walker Associates
2151 Alessandro Drive, Suite 100
Ventura, CA 93001

survival, growth, and/or reproduction.[1] Where possible, regression analysis was used to generate EC20s for many of the acceptable studies.

In order for a chronic test to be used as part of the basis for a SMCV in USEPA (1999), it had to satisfy the definitions given in the USEPA (1985a) *Guidelines for Deriving Numerical Criteria* for a "life-cycle", "partial-life-cycle", or "early-life-stage" test. These criteria *as they apply to fish* are provided in Table 1 below.

If not meeting the criteria for any of the three test categories in Table 1, USEPA guidelines allow for *potential limited* use of data from two alternative types of tests involving fish:

1. Seven-day tests of survival, reproduction, and/or hatchability, or
2. Ninety-day tests of growth

USEPA requires that such alternative tests using *growth* as an endpoint must last for at least 90 days because reductions in weight gain for fewer than 90 days can be temporary. Per the USEPA (1985a) guidelines, neither of the two alternative types of test above should be used as the basis for a discrete chronic value for a species. However, such tests can be used as evidence for an upper limit for a chronic value (in other words, determinations that the true chronic value is *likely less than* the threshold concentration observed in the test).

The list of acceptable chronic tests for fish and their associated EC20s, and SMCVs and GMCVs (standardized to pH=8 and T=25°C) that resulted from the 1999 vetting process are provided in Table 2 below. Not all of the acceptable chronic tests included in USEPA Table 5 resulted in specific EC20s, or SMCVs. When *none* of the concentrations used in an acceptable chronic test caused significant effects on survival, growth, or reproduction, the highest concentration from the test was entered in USEPA Table 5 as "$>x$" to indicate that underlying (unknown) EC20 was not equivalent to the concentration in the table for that test, but higher than the concentration by an unknown amount. Conversely, if *all* of the concentrations used in an acceptable test caused significant effects on survival, growth, or reproduction (i.e., none of the concentrations were "no-effects concentrations", or NOECs), the lowest concentration from the test was entered in the table as "$<x$" to indicate that the underlying (unknown) EC20 was not equivalent to the concentration in the table for that test, but less than the concentration by an unknown amount. "Less than" or "greater than" qualifiers were also applied to some of the SMCVs and GMCVs calculated by USEPA.

[1] USEPA does not utilize concentrations associated with histopathologic or behavioral endpoints (e.g. swimming speed) for SMCV derivation because they have determined that there is "no justification for equating histopathological effects with effects on survival, growth, and reproduction" (USEPA 1999, p. 45). This position is more fully explained in Appendix 5 in USEPA (1999), and was maintained in the 2009 Draft Update, released on December 30, 2009 (USEPA 2009).

Table 1. USEPA Criteria for Life-Cycle, Partial-Life-Cycle, and Early-Life-Stage Chronic Toxicity Tests for Fish.

Test Type	Fish Test Criteria	Data should Include	Potentially used to Derive:
Life cycle	• Tests must begin with embryos or newly hatched young <48-hrs old • Test must continue through maturation and reproduction • Test should not end less than 90 days after hatching of the next generation (24-hrs for non-salmonids).	• Survival and growth and adults and young • Maturation of males and females • Eggs spawned per female • Embryo viability (salmonids) • Hatchability	Depending on results: • Upper limit for a CV • Lower limit for a CV • CV
Partial life cycle	• Allowed for use with fish that require more than a year to reach sexual maturity. • Test must begin with immature juveniles at least 2 months prior to active gonad development. • Test must continue through maturation and reproduction. • Test should not end less than 90 days after hatching of the next generation (24-hrs for non-salmonids).		
Early life-stage	• Test must begin shortly after fertilization of eggs. • Test must continue through embryonic, larval, and early juvenile development. • Test must continue for 60 day post hatch for salmonids (28-32 days for non-salmonids).	• Survival and growth and adults and young	

Table 2. EC20s and other Toxicity Parameters Accepted by USEPA (1999) from Chronic Tests Meeting USEPA Test Acceptability Criteria for Fish.

Species	EC20s	Species Mean Chronic Value at pH=8 & 25°C (mg N/L total ammonia)	Genus Mean Chronic Value (GMCV) at pH=8 & 25°C (mg N/L total ammonia)	Genus Mean Acute-Chronic Ratio (GMACR)
Pimephales promelas (fathead minnow)	1.97 2.92 5.12	3.09	3.09	10.9
Catostomus commersoni (white sucker)	>4.79	>4.79	>4.79	<8.4
Ictalurus punctatus (channel catfish)	8.38 9.33 <8.7 to <9.9	8.84	8.84	2.7
Lepomis cyanellus (green sunfish)	7.44 4.88	6.03	2.85	7.6
Lepomis macrochirus (bluegill)	1.35	1.35		
Micropterus dolomieu (smallmouth bass)	3.57 4.01 6.5 4.65	4.56	4.56	7.4
Oncorhynchus clarki (cutthroat trout)	<19.7	Not Available: USEPA determined it was inappropriate to calculate SMCVs for *Oncorhynchus* species (see text).	Not Available	Not Available
Oncorhynchus mykiss (rainbow trout)	>5.4[a] <18.7[b] <1.44[c] 1.34[d]			
Oncorhynchus nerka (sockeye salmon)	<4.16			

(a) based on the highest concentration tested by Thurston et al. (1984)
(b) based on LC50s obtained over 42-days by Burkhalter & Kaya (1977)
(c) based on 73-day LC20 obtained by Solbe & Shurben (1989)
(d) based on test results by Calamari et al. (1977, 1981), interpolated by USEPA to estimate a 72-day LC20

USEPA determined that EC20s from five tests using rainbow trout were from acceptable chronic tests. However, as a group, the EC20s for rainbow trout did not meet USEPA standards for further use in calculating SMCVs, or for use in calculating a GMCV for its genus *Oncorhynchus*:

> "Because of the concerns about some of the tests, the differences among the results, and the fact that some of the results are either "greater than" or "less than" values, even though the various results are included in Table 5, a SMCV is not derived for rainbow trout; instead the results of the chronic tests will be used to assess the appropriateness of the CCC". (USEPA 1999; p. 60)

No additional chronic test results for rainbow trout were included in the recently released USEPA Draft 2009 Update for the freshwater ammonia criteria (USEPA 2009), in which USEPA again declined to calculate a GMCV for *Oncorhynchus*.

> "As noted in the 1999 AWQC document, five other studies have reported results of chronic tests conducted with ammonia and other salmonids including *Oncorhynchus mykiss* and *Oncorhynchus nerka*. There is a lack of consistency among the chronic values obtained from these tests, and several tests produced "greater than" and "less than" values (Table 5). Consequently, in keeping with the decision made in the 1999 AWQC document, a GMCV is not derived for *Oncorhynchus*. Instead, the results of the chronic tests were used to assess the appropriateness of the CCC." (USEPA 2009, p. 21)

In Appendix 7 of USEPA (1999), Acute-Chronic Ratios (ACRs) were calculated for all EC20s that *were* used to generate SMCVs (from USEPA 1999, Table 5) *and* which could be paired with comparable acute values (LC50s; see more about pairing criteria below). Then, these ACRs were used to calculate Genus Mean Acute Chronic Ratios (GMACR). This analysis resulted in GMACRs for five genera of fish, which are included in Table 2. ***The USEPA-vetted GMACRs for fish occupy the range 2.7-10.9***.

Origin of Postulated ACRs for Rainbow Trout Being Used to Infer Chronic Toxity for Delta Smelt

At the August 2009 Ammonia Summit, Dr. Inge Werner provided two values as the upper and lower limits for the ACR for rainbow trout (14.6-23.5; Werner 2009a, slide 10). The derivation of these values was not a part of Werner's talk at the Ammonia Summit. The same values were presented in the annual reports for 2008 and 2009 for the UC Davis Aquatic Toxicology Lab's Delta smelt ammonia toxicity tests (Werner et al. 2009a, b) as follows (language is from 2009 report; almost identical passage occurs in 2008 report):

> "Exposure duration is an important factor influencing the toxicity of ammonia. Seven-day toxicity tests, as performed in this study, are unable to detect the potential chronic effects of ammonia/um exposure on delta smelt. Acute-to chronic ratios are one method that has traditionally been used to extrapolate between acute and chronic toxicity when procedures for chronic testing are not available. For fish, the US EPA (1999) reports mean acute-to-chronic ammonia/um ratios for warm water fish that range between 2.7 (channel catfish, *Ictalurus punctatus*) and 10.9 (fathead minnow, *P. promelas*). Cold water species such as rainbow trout, with acute ammonia/um sensitivity similar to delta smelt, have a ratio between 14.6 and 23.5, respectively (US EPA, 1999; Passell et al., 2007). If these safety factors were applied to acute effect concentrations for effluent and delta smelt larvae (7-d LC50: 3.92 mg/L)[2] then the resulting threshold concentrations for total ammonia/um would be 0.27 and 0.17 mg/L for the above safety ratios of 14.6 and 23.6, respectively. These chronic effect thresholds are below long-term average concentrations in the Sacramento River below SRWTP." (Werner et al. 2009b, page 33)

The passage above can be interpreted to mean that rainbow trout ACRs of 14.6 and 23.5 were derived by USEPA or by Passell et al. (2007). However, neither of these references provide ACRs for rainbow trout. As explained above, in 1999 and 2009, USEPA refused

[2]This appears to be a mistake in Werner et al. (2009b). 3.92 mg/L was the 7-day LOEC for this test. The LC50 was 5.40 mg/L (see Werner et al. 2009b, p. 15).

to calculate an ACR for rainbow trout - or for even for the genus *Oncorhynchus* - owing to inadequate data. Chronic toxicity tests were not a part of the original work reported in Passell et al. (2007). As clarification, Dr. Werner explained that she had calculated the ACRs for rainbow trout as follows:

> "I used the chronic values for unionized ammonia provided in Table 3 of Passell et al. (0.031 mg/L and 0.05 mg/L), and the species mean acute value from EPA 1999 (given in total ammonia/um)[3] to calculate the corresponding value for unionized ammonia (0.728 mg/L unionized ammonia), then calculated the ratio between them [which] results in 14.56 and 23.5." (I. Werner, pers. comm., Dec. 22, 2009).

Table 3 in Passell et al. (2007) is a collection of acute and chronic values for several fish species from the literature that was included for discussion purposes in the article. In the table, Thurston et al. (1984) and Burkhalter & Kaya (1977) are cited as the original sources of the 0.031 and 0.05 mg/L un-ionized ammonia-N concentrations, respectively. The original sources of the values are not critically evaluated in the article. Below, we discuss the original studies, and associated information about them in USEPA (1999). The results indicate that the chronic concentrations Dr. Werner used to compute ACRs for rainbow trout did not meet USEPA criteria for such use.

Thurston et al. (1984). Thurston et al. (1984) was a 5-year life cycle test which exposed offspring from one pair of rainbow trout, and their F1 and F2 progeny, to the following mean concentrations of un-ionized ammonia in flow-through troughs: 0.001, 0.013, 0.022, 0.044, 0.063, and 0.074 mg N/L. Regarding this study, USEPA (1999) states that "the important data for each life stage are so variable that it is not possible to discern whether there is a concentration-effect curve" (USEPA 1999; p. 58). According to the original article, there was no significant relationship between ammonia concentration and (1) mortality of all three generations, (2) growth of F1 and F2 progeny[4], or (3) egg production. Because none of the exposure levels used by Thurston et al. (1984) caused significant effects on survival, growth or reproduction, the results of this test fell under the "greater than" category of chronic test results in USEPA (1999). In other words, USEPA concluded that the underlying (unknown) chronic value for rainbow trout must be greater than the highest test concentration used in the study (5.4 mg/L total ammonia-N at pH=8, T=25°C).

Passell et al. (2007) do not explain why they identified 0.031 mg/L un-ionized ammonia-N as an appropriate chronic value from Thurston et al. (1984), or why it merited status as one of only two chronic concentrations for rainbow trout to include in their article. Because *none* of the test concentrations in Thurston et al. (1984) resulted in significant effects on survival, growth, or reproduction for 3 generations of fish, no EC20s (or other effects concentrations) are available from this test for approved endpoints. Earlier USEPA criteria documents (Table 2 in both USEPA 1985b, 1989) list 0.031 as a chronic

[3] The species mean acute value for rainbow trout in USEPA (1999) is 11.23 mg/L total ammonia-N (standardized to pH=8, 25°C).

[4] It was not possible to evaluate growth of the parental fish because they were not weighed at the start of the test.

value for Thurston et al. (1984) which - after comparison of the original article with associated text in USEPA 1984 - appears to have been calculated using a NOEC and LOEC related to epidermal cell changes. However, this interpretation of the results from Thurston et al. (1984), which depends on the use of a non-conventional endpoint, was rejected in both of the most recent USEPA criteria documents (1999, 2009).

Burkhalter & Kaya (1977). Burkhalter & Kaya (1977) did not report EC20s for rainbow trout. Instead, they reported LC50 results from a 42-day exposure of rainbow trout embryos and sac fry. Because the study did not provide EC20s, the results of this test fell under the "less than" category of chronic test values. In other words, USEPA concluded that the underlying (unknown) chronic value would have been less than the LC50 from their study (18.7 mg/L total ammonia-N at pH=8, T=25°C). However, the value of 0.05 mg/L unionized ammonia-N attributed to Burkhalter & Kaya (1977) in Passell et al.'s table (which was ultimately used by Dr. Werner to generate one of her ACRs for rainbow trout) is *not* that associated with the LC50 from their study (which was 0.25 mg/L unionized ammonia-N). The only available explanation for Passell et al.'s identification of 0.05 mg N/L as a chronic value from Burkhalter & Kaya is that 0.05 mg N/L was the lowest exposure concentration they used, which caused "some retardation of early growth and development" (quote from abstract of Burkhalter & Kaya). However differences in growth rate at this low test concentration (0.05) compared to the control were slight, and disappeared after two weeks of exposure. Because of the short duration of Burkhalter & Kaya's test, it was not considered by USEPA in 1999 as an appropriate test to gauge the effects of ammonia on growth on early life stages of rainbow trout.

As indicated above, Thurston et al. (1984) and Burkhalter & Kaya (1977) are discussed in USEPA (1999) and were two of the rainbow trout studies included in the list of acceptable chronic studies (see EC20 values in Table 2 above). However, as explained above, after re-evaluation of these two studies, USEPA interpreted the results of these two studies as evidence for an EC20 *greater than* 5.4 mg/L total ammonia-N (Thurston et al. study) and *less than* 18.7 mg/L total ammonia-N (Burkhalter & Kaya study; both values standardized to pH=8, 25°C). Taken in isolation from other chronic tests, USEPA's upper and lower limits from these two studies imply that the rainbow trout ACR falls somewhere within the range (0.60-2.08)[5] - which is very different than the one proposed by Dr. Werner (14.6 - 23.5).

A recent 90-day chronic test measuring the hatching success of newly fertilized eggs from a wild strain of rainbow trout, and subsequent survival and growth of sac fry and swim-up fry (Brinkman et al. 2009), resulted in a chronic value (the geometric mean of the LOEC and NOEC) of 8.06 mg/L total ammonia-N and a 90-day EC20 (based on biomass) of 5.56 mg/L total ammonia-N (standardized to pH 8). This test appears to meet the USEPA criteria for early-life-stage tests for salmonids outlined in Table 1; an ACR for rainbow trout based on the chronic value from this recent test would be about 1.4. However, even if Brinkman et al. (2009) was added to its list of acceptable chronic

[5] 11.23/18.7 = 0.60; 11.23/5.4=2.08

tests[6], USEPA might still conclude that chronic test data for rainbow trout are too variable, or otherwise insufficient, to calculate SMCVs, or an ACR for the species or the genus.

In general, the approach of pairing acute values and chronic values from different investigations to compute ACRs is not necessarily in agreement with USEPA guidelines. USEPA (1985a), outlines the following steps for producing an ACR from a chronic value:

1. The numerator for the ACR should be the geometric mean of the acute values for that species from all acceptable flow-through acute tests in the same dilution water.
2. For fish, the acute tests should have been conducted with juveniles.
3. The acute tests should have been (a) a part of the same study as the chronic tests, (b) from different studies but from the same laboratory and dilution water, or (c) from studies at different laboratories using the same dilution water.
4. If no such acute tests are available, an ACR should not be calculated.

Conclusion

In summary, *based on the most recent USEPA criteria for chronic test design and endpoints, derivation of ACRs, and interpretation of data from chronic tests for fish*, no information is available to support a proposal that the ACR for rainbow trout occupies the range 14.6-23.5. Derivation of hypothetical ACRs for rainbow trout as high as the ones used at recent meetings and reports is not possible using direct evidence for chronic effects of ammonia on survival, growth, or reproduction and represents a significant departure from current USEPA guidance concerning the use of data from chronic tests for the species. Assertions about chronic toxicity in the Delta that rely on these hypothetical ACRs for rainbow trout should be avoided. At a minimum, such assertions must be carefully qualified as not being based on evidence for population-level effects of ammonia on sensitive fish.

[6] Brinkman et al. (2009) was published after the Feb. 2009 cut-off for the literature review used for the development of the USEPA 2009 Draft Update of the freshwater ammonia criteria.

REFERENCES CITED

Note: References below were provided as pdfs on the CD-ROM which accompanied written testimony submitted to the SWRCB by Sacramento Regional County Sanitation District.

Brinkman, S.F., J.D. Woodling, A.M.Vajda, and D.O. Norris. 2009. *Chronic Toxicity of Ammonia to Early Life Stage Rainbow Trout*. Trans. Amer. Fish. Soc. 138: 433-440.

Burkhalter, D.E., and D.M. Kaya. 1977. *Effects of Prolonged Exposure to Ammonia on Fertilized Eggs and Sac Fry of Rainbow Trout (Salmo gairdneri).* Trans. Amer. Fish. Soc. 106*:* 470-475*.*

Calamari, D., R. Marchetti, & G. Vailati. 1977. *Effects of Prolonged Treatments with Ammonia on Stages of Development of Salmo gairdneri*. Nuovi Ann. Ig. Microbiol. 28: 333-345. (not on CD, but discussed in USEPA 1999)

Calamari, D., R. Marchetti, & G. Vailati. 1981. *Effects of Long-term Exposure to Ammonia on the Developmental Stages of Rainbow Trout (Salmo gairdneri Richardson)*. Rapp. P.-v/ Reun. Cons. Int. Explor. Mer. 178:81-86. (not on CD, but discussed in USEPA 1999)

Passell, H.D., C.N. Dahm, & E.J. Bedrick. 2007. *Ammonia Modeling for Assessing Potential Toxicity to Fish Species in the Rio Grande, 1989-2002*. Ecol. Appl. 17: 2087-2099.

Solbe, J.F.L.G., & D.G. Schurben. 1989. Toxicity of Ammonia to Early Life Stages of Rainbow Trout (*Salmo gairdneri*). Water Res. 23:127-129.

Thurston, R.V., R.C. Russo, R.J. Luedtke, C.E. Smith, E.L. Meyn, C. Chakoumakos, K.C. Wang, & C.J.D. Brown. 1984. *Chronic Toxicity of Ammonia to Rainbow Trout*. Trans. Amer. Fish. Soc. 113:56-73.

USEPA. 1985a. *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*. United States Environmental Protection Agency.

USEPA. 1985b. *Ambient Water Quality Criteria for Ammonia - 1984*. EPA 440/5-85-001. United States Environmental Protection Agency, January 1985.

USEPA. 1989. *Ambient Water Quality Criteria for Ammonia (Saltwater) - 1989*. EPA 440-5-88-004. United States Environmental Protection Agency, April 1989.

USEPA. 1999. *1999 Update of Ambient Water Quality Criteria for Ammonia*. United States Environmental Protection Agency, December 1999. EPA 822-R-99-014

USEPA. 2009. *Draft 2009 Update Aquatic Life Ambient Water Quality Criteria for Ammonia-Freshwater.* EPA 822-D-09-001. United States Environmental Protection Agency, December 2009.

Werner, I. 2009. *Effects of Ammonia/um and Wastewater Effluent Associated Contaminants on Delta Smelt.* Central Valley Regional Water Quality Control Board Ammonia Summit, Sacramento, California, August 18-19, 2009.

Werner , I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2009a. *The Effects of Wastewater Treatment Effluent-Associated Contaminants on Delta Smelt*. Draft Final Report to the Central Valley Regional Water Quality Control Board. January 28, 2009.

Werner , I., L.A. Deanovic, M. Stillway, and D. Markiewicz. 2009b. *Acute toxicity of Ammonia/um and Wastewater Treatment Effluent-Associated Contaminant on Delta Smelt - 2009*. Draft Report to the Central Valley Regional Water Quality Control Board. October 30, 2009.