303(d) Listing for Unknown Toxicity on the Kings River: Evidence in Support of **Not Listing**

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June 11, 2009

Lines of Evidence: Water Flea Ceriodaphnia dubia

- One Incidence of Statistical Mortality in 50 samples
 - 75 percent survival reported Sept. 5, 2006 at Manning Ave ILRP site
- Does Not meet Listing Requirements under Table 3.1 (requires 5 of 50 samples to list)

Lines of Evidence: Fathead Minnow *Pimephales promelas*

- Two Incidences of Statistical Mortality in 50 Samples
 - One at Manning Ave ILRP site on Feb. 23, 2006 showed 88 percent survival
 - One at Lemoore Weir ILRP site on Feb. 23, 2006 showed 55 percent survival
- Does Not meet Listing Requirements under Table 3.1 (requires 5 of 50 samples to list)

- Issues with REDUCED GROWTH since inception of ILRP
- All Algae tests referenced for listing run through same laboratory
- No chemical constituents identified in Phase II testing as cause of "toxicity"
- All samples showed Positive growth, but not at same growth rate as of control

- Investigation
- Regional Board Staff in Fresno Office collected a water sample on same date and location (September 2006) as KRCD and sent it to Fish and Game lab
 - No Significant Differences detected by Fish and Game, but Significant Differences by KRCD contracted lab
- Second Split-Sample study sent samples to KRCD contracted lab and identical samples to Fruit Growers Laboratory (one storm sample, one irrigation sample)
 - Both FGL samples came back as NO SIGNIFICANT DIFFERENCE
 - KRCD samples showed Significant Differences

- Investigation
 - 1. Considerable freedom exists within method leading to inconsistent results from one lab to another (not comparable data)
 - 2. Client (KRCD) not told initially that control water in test could be reformulated to match hardness levels of sample water
 - Control water at primary lab was 6 times higher in both Electrical Conductivity and Hardness than Kings samples

Control vs Sample Water

Constituent	Units	SFL	APPL 02-21-07	APPL 03-01-07	APPL 03-13-07	APPL 04-11-07
EC	umhos/cm	184	31.2	31.5	33.3	35
TDS	mg/L	110	22	26	24	26
Hardness	mg/L	88	10.1	10	12.1	13

- Investigation
 - This fact, according to a USGS researcher familiar with this test, contributes to a "shock effect" on the algae, which delays its growth curve (osmotic shock effects?)
 - A special test run to 8 days (method time is 4 days) confirmed that the sample will statistically match the control sample after the shock effect subsides

- Investigation
 - Tests run May 2009 using hardness matching water as a control sample showed that the river sample actually matched or exceeded the control in algae growth
 - Water sample was collected from a site with no agricultural activity upstream
 - All future algae tests under the new MRP to be run in a similar manner (toxicity will be because of a chemical constituent, not because of the control water)

Conclusion

- All previous tests available to Regional Board Staff (via ILRP reports and SWAMP) were run with Control waters running 6 times (minimum) higher in EC and Hardness than sample water
- Shock effect of placing test organism in "softer, less saline" water temporarily inhibited growth
- Reformulating Control Water to match sample water
 EC and Hardness shows no toxicity effects

Conclusions

- Water Flea and Fathead Minnow data insufficient to list under Table 3.1 of the Listing Policy
- Lab issues with regards to Control Water makeup lead to the statistical differences in sample vs. control tests, primarily due to freedom within prescribed method
 - Said freedom does not allow for comparison between labs for the algae testing (inconsistent application of method)
- This resulted in the "toxicity" seen in the algae tests, not because of an agriculturally related constituent

Conclusions

- Request that 303(d) listing for Unknown
 Toxicity on the Kings River be Rejected or
 Delayed 1-year to reevaluate the impact of the
 method on the results obtained
- This issue is currently before the ILRP TIC, and has been discussed by the labs and staff for the last 2 years