THE USE OF LONG-TERM CHRONIC BIOASSAYS AND BIOMONITORING IN EVALUATING THE ENVIRONMENTAL IMPACT OF A COPPER-BASED ALGAECIDE USED FOR CONTROLLING ALGAL GROWTHS IN A HYDROELECTRIC WATER CONVEYANCE SYSTEM

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Introduction

Historically, copper-based algaecides have been commonly used as an effective means of controlling algal growths in many aquatic environments (Pryfogle, P.A., et al, 1997). However, in recent years the use of these types of algaecides has been of increasing concern to resource agencies in California, due primarily to a perceived long-term negative effect on aquatic ecosystems. Conclusive data on this subject are currently unavailable, especially for site specific applications, and more research is needed to evaluate long-term environmental effects.

The Pacific Gas & Electric Company (PG&E) operates numerous hydroelectric power facilities in California, with over three hundred miles of water conveyance canals. Algal growths in these canals create frequent maintenance problems and reduce the maximum flow through the canal, which directly effects power generation and overall operating costs. As one part of a multi-year study to investigate available algal control technologies, chemical control agents were evaluated for potential use in controlling algal growths in two representative canal systems. These two systems were: the Tiger Creek Canal, which eventually flows into the Mokelumne River; and the Drum Canal, which flows into the Bear River. The overall goal of the study was to develop environmentally sound, cost-effective, long-term management strategies for controlling algae in PG&E water conveyance canals.

Following a preliminary evaluation of numerous algaecide products, a chelated copper sulfate product called EarthTec was chosen for further testing. This product was chosen because of its unique formulation that allows it to be applied at very low concentrations of copper (20-30 ppb), in comparison to other products which are applied at higher concentrations (0.5-1 ppm); and because of its purported effectiveness in waters with low alkalinity, low pH, and low bicarbonates, typical of many high Sierra streams and the water in the Tiger Creek and Drum canals.

Prior to conducting either the field tests or the laboratory tests, the California Department of Fish and Game (CDF&G), California Regional Water Quality Control Board, and the Amador County Agricultural Department were apprised of the proposed algaecide applications. All three of the agencies

participated in reviewing the study design and application procedures for this project.

To ensure that the use of this product was not detrimental to aquatic resources, CDF&G required that: bioassays be conducted to evaluate potential acute and chronic effects of the proposed EarthTec applications on primary fish species occurring in the two river drainages; and that a long-term monitoring program be developed to assess the effects of potential copper accumulation in sediment and aquatic insects resulting from the use of EarthTec in the canals. This report addresses the results of the bioassays that were conducted to allow the use of EarthTec in the canals, and the preliminary results of the field monitoring program.

Methods

Based on the manufacturers recommendations, initial EarthTec applications to the canal were to be made at 22 and 30 ppb for 6- or 12-hour durations, at weekly or biweekly intervals to determine effectiveness in controlling algal growths. A 96-hour acute bioassay was conducted using rainbow trout fry to determine the LC50 of EarthTec in water representative of the canal water. Secondly, to evaluate the potential effects of the proposed treatments on rainbow trout, a bioassay protocol was developed to mimic the actual application procedure to be used in the 17-mile long Tiger Creek Canal.

From March through April, 1996, a series of bioassays were conducted with EarthTec. In these tests, rainbow trout fry were subjected to repeated exposures of 25 ppb for 7-hour periods, and 35 ppb for 7- and 13-hour periods, on 7- and 14-day intervals. To provide a conservative estimate of the effects that might be encountered during actual canal applications of 22 and 30 ppb, slightly higher concentrations of 25 and 35 ppb were chosen for testing. Following exposure, rainbow trout fry were transferred to freshwater (with the same water quality characteristics as that found in the Tiger Creek Canal) until the next treatment. In each of the test scenarios, rainbow trout fry were exposed to EarthTec concentrations three to five times at either 7- or 13-hour treatment durations, at 7- and 14-day intervals. A summary of the test scenarios are provided in Table 1. A total of 40 trout (20 trout in two replicate aquaria) was used for all test scenarios and for controls.

Table 1. Test scenarios for the March-April, 1996 bioassays.

EarthTec Concentration	Chemical Exposure Duration	Duration in Freshwater from One Treatment to the Next	# Days After Initial Exposure that Fish were Analyzed for Copper	Total # of Treatments
25 ppb	7 Hours	7 Days	7, 14, 21, and 28	5
25 ppb	7 Hours	14 Days	14, 28, and 42	3
35 ppb	7 Hours	7 Days	7, 14, 21, and 28	5
35 ppb	7 Hours	14 Days	14, 28, and 42	3
35 ppb	13 Hours	7 Days	7, 14, 21, and 28	5
35 ppb	13 Hours	14 Days	14, 28, and 42	3
Control	----	Continuous	7, 14, 21, 28, and 42[1]	----

[1] Control fish were analyzed on these days, but were never exposed to copper treatments.

Fresh EarthTec concentrations of 25 and 35 ppb were prepared each week just prior to treatment. Fish from both replicate aquaria of each test scenario were carefully transferred from their freshwater tanks into aquaria containing the fresh EarthTec solutions. Following treatment, the fish were transferred back to their freshwater tanks. Control fish were transferred to new freshwater tanks every two to three weeks so that any handling effects would be consistent for all fish.

Water changes were conducted on a weekly basis during periods in freshwater to maintain water quality parameters similar to that found in the Tiger Creek Canal. These parameters were: pH = 6.8-7.2; alkalinity = 10-15 mg/l $CaCO_3$; and hardness = 10-15 mg/l $CaCO_3$. Control water, dilution water, and water used in weekly water changes were created using deionized water and reagent grade chemicals. All aquaria were aerated throughout the test period. Dissolved oxygen, pH, temperature, percent survival, and any unusual behavior was recorded every other day for all test aquaria throughout the test period.

Whole body copper analyses were conducted periodically, on both test and control fish to monitor copper body burden levels. Percent survival and copper body burdens for trout in each of the test scenarios, were the primary end results determined during the testing period. A body burden analysis was performed rather than a gill tissue, or liver analysis, due to the small size of the fish.

Test and control fish to be used for whole body copper analysis were randomly obtained from each replicate aquarium at the end of the required period in freshwater, just prior to the

next EarthTec treatment. Three rainbow trout fry were removed from each of the two replicate aquaria of each test group prior to re-treatment. After removal from the aquaria, fish to be used for copper analysis, were placed in carbonated water until dead. The fish were then placed in plastic zip-lock bags, labeled, and quickly frozen for transport to PG&E's Technical & Ecological Services Analytical Chemistry Unit in San Ramon, for processing. The three rainbow trout fry from each replicate aquaria were composited separately to obtain two copper body burden values for each treatment scenario. Fish samples were prepared for analysis using a nitric acid/hydrogen peroxide digestion performed in Teflon pressure vessels with microwave assisted heating. This method is a variation on the Association of Official Analytical Chemists (AOAC) closed vessel digestion method 974.14 for mercury in fish. This variation is documented in the CEM microwave digestion system methods manual. The digested samples were subsequently analyzed using atomic absorption spectrophotometry. Preparation blanks were used during each set of analyses.

Because the results of the field tests to control algae in Tiger Creek Canal during the summer and fall of 1996 were only partially successful, a second series of new field tests were scheduled for the summer of 1997.

The proposed Tiger Creek Canal treatment procedures for 1997 involved the application of EarthTec at the same concentration of 30 ppb, but for significantly longer periods. Consequently, new long-term bioassay tests were needed to provide the necessary data to support these new application rates.

To determine the effect of the proposed 1997 EarthTec applications on resident trout species, three application durations at one concentration (30 ppb) were proposed for testing. These durations were 48-, 72-, and 96-hours, with a period of one to three weeks between treatments. These application scenarios were designed to allow for flexibility in the canal treatment schedule, depending on the effectiveness of a particular treatment duration. Again, the primary goal was to determine the shortest treatment duration with the longest period between treatments necessary to maintain control of algal growths in the canal. Because the new proposed treatment durations were significantly longer than previous applications, CDF&G requested that the bioassay testing also include brown trout, which occur in the Mokelumne River drainage. Consequently, new bioassay test procedures were designed to replicate the new proposed canal treatments to determine the effects of each EarthTec treatment scenario on rainbow trout fry and brown trout fingerlings.

The second series of bioassays was conducted from October, 1996, through January, 1997, for a period of 28-84 days, depending on the treatment duration and the length of time in freshwater between treatments. Rainbow trout fry and brown trout fingerlings were exposed to 35 ppb EarthTec for all of the proposed treatment scenarios. Again, a slightly higher

concentration of 35 ppb was used to provide a conservative estimate of the effects. The same general procedures described previously for the March-April bioassays were used in these tests. A total of 24 brown trout fingerlings (12 trout in replicate aquaria), and 40 rainbow trout fry (20 trout in replicate aquaria) were used for all test scenarios and for the controls. A summary of the new test scenarios are provided in Table 2.

Table 2. Test scenarios for the October, 1996 through January, 1997 bioassays.

Fish Species	EarthTec Concen-tration	Chemical Exposure Duration	Duration in Freshwater from One Treatment to the Next	# Days After Initial Exposure that Trout were Analyzed for Copper	Total # of Treatments
Brown Trout	35 ppb	48 Hours	5 Days	14, 21 and 28	3
Brown Trout	35 ppb	48 Hours	12 Days	14, 28, 42 and 56	4
Brown Trout	35 ppb	72 Hours	11 Days	14, 28, 42 and 56	4
Brown Trout	35 ppb	72 Hours	18 Days	21, 42, 63 and 84	4
Brown Trout	35 ppb	96 Hours	10 Days	14, 28, 42 and 56	4
Brown Trout	35 ppb	96 Hours	17 Days	21, 42, 63 and 84	4
Brown Trout	Control	----	Continuous	14, 21, 28, 42, 56, 63 and 84[1]	----
Rainbow Trout	35 ppb	48 Hours	5 Days	14, 21 and 28	3
Rainbow Trout	35 ppb	48 Hours	12 Days	14, 28, 42 and 56	4
Rainbow Trout	35 ppb	72 Hours	11 Days	14, 28, 42 and 56	4
Rainbow Trout	35 ppb	72 Hours	18 Days	21, 42, 63 and 84	4
Rainbow Trout	35 ppb	96 Hours	10 Days	14, 28, 42 and 56	4
Rainbow Trout	35 ppb	96 Hours	17 Days	21, 42, 63 and 84	4
Rainbow Trout	Control	-----	Continuous	14, 21, 28, 42, 56, 63 and 84[1]	----

[1] Control fish were analyzed on these days, but were never exposed to copper treatments.

Test trout were exposed to the 35-ppb EarthTec solution for varying treatment durations (i.e., 48-, 72-, and 96-hours) for a minimum of four treatments. The fish were then transferred back to the freshwater tanks for a 5- to 17-day period, depending on the treatment duration (e.g., 48-hour treatment/5 days freshwater, 72-hour treatment/17 days freshwater, etc.). The

number of days in freshwater between treatments was established so applications would always fall on the same day of the week.

Once again, test and control fish to be used for whole body copper analysis were randomly obtained from each replicate aquaria at the end of the required period in freshwater, just prior to the next EarthTec treatment. However, during this test two rainbow trout fry and one brown trout fingerling were removed from each of the two replicate aquaria of each test group prior to re-treatment. The two rainbow trout fry from each replicate aquaria were composited separately to obtain two copper body burden values for each treatment scenario. The brown trout fingerlings were analyzed individually.

Field Monitoring

As part of the overall monitoring plan for EarthTec applications on the Tiger Creek Canal, CDF&G required PG&E to develop a monitoring plan for evaluating the long-term effects of copper on downstream aquatic habitats potentially affected by the algaecide. The monitoring plan was initiated in 1996 at the beginning of the algal season with the collection of baseline samples from the Tiger Creek Canal system, and from Tiger Creek, which is linked with the canal. Monitoring involved the collection of sediment samples from five stations within the Tiger Creek Regulator Reservoir, and sediment and aquatic insect samples from two stations on Tiger Creek, which flows into and out of the regulator. The control station was located on Tiger Creek above the regulator, which is not affected by algaecide applications to the Tiger Creek Canal. Figure 1 provides details of the canal system and copper monitoring stations.

The monitoring protocol required that samples be collected each year just prior to the first use of EarthTec, a second set of samples to be collected midway through the summer, and a third sampling effort after the last application of the year. The data would then be analyzed and evaluated by PG&E and CDF&G prior to the next year's applications. The results would be used to determine if the EarthTec applications were causing a net increase in copper levels in either sediment or aquatic insects, during the year, or from one year to the next.

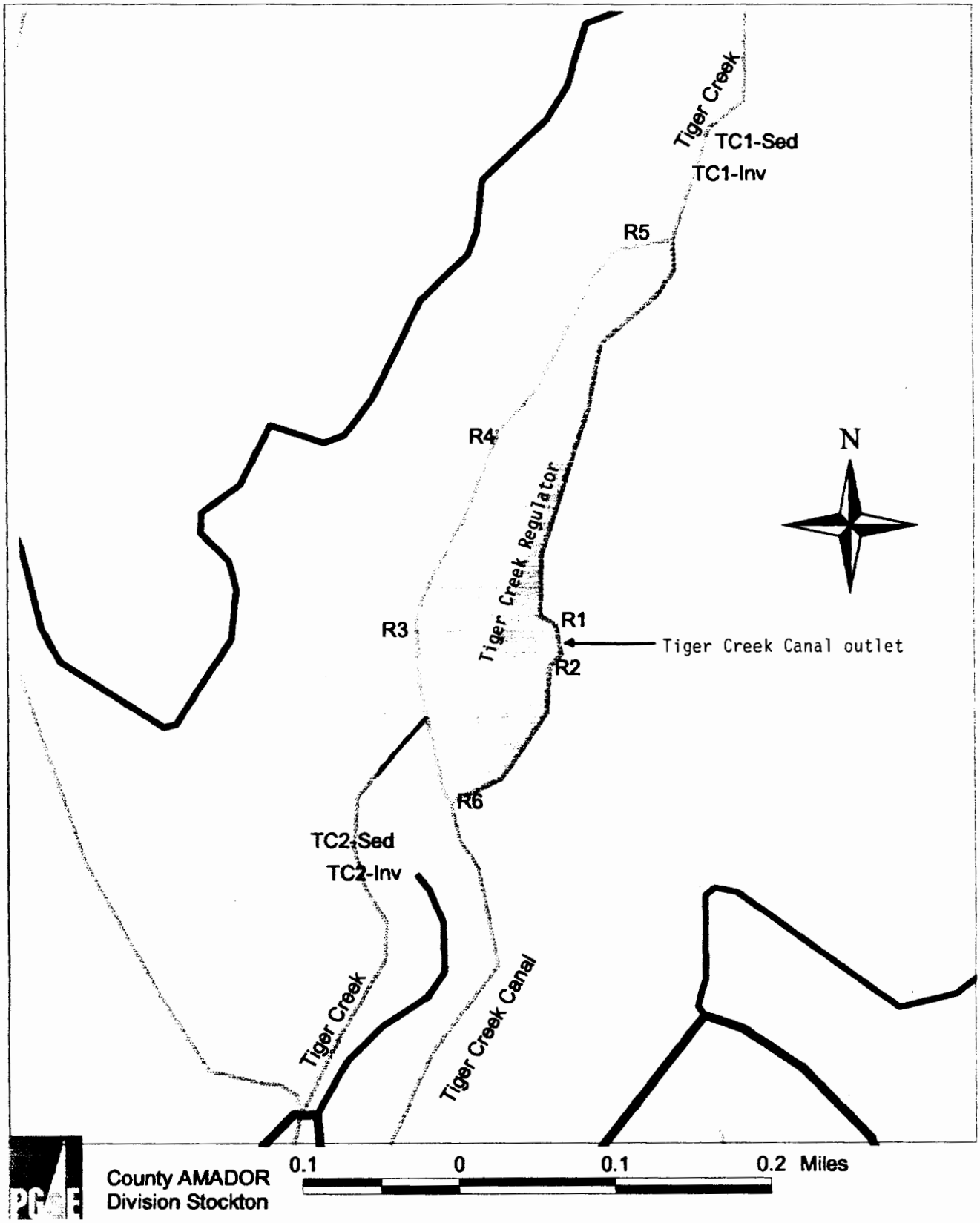

Figure 1. Copper Monitoring Stations for Sediment and Aquatic Insects.

Results

Series 1 Bioassays, March-April, 1996

In the 42-day test period from March to April 1996, rainbow trout fry survival was 100 percent for all test scenarios, and for the controls. The results of the whole body copper analyses showed little difference in copper concentrations between test fish and control fish over the duration of the test period. Table 3 provides a summary of the results. In addition, trout growth rates in all test aquaria were similar to that of control fish.

Table 3. Rainbow trout fry whole body copper analyses from the March-April, 1996 bioassays.

Test Scenario	EarthTec Concentration	Total Treatments	Results of Whole Body Copper Analyses in ppm (mg/kg Cu)[1,2]				
			3/19	3/26	4/3	4/11	4/18
7 Hr. Treatment/ 7 Days Freshwater	25 ppb	5	0.85		1.18		0.98
7 Hr. Treatment/ 14 Days Freshwater	25 ppb	3		0.83		1.54	
7 Hr. Treatment/ 7 Days Freshwater	35 ppb	5	0.83		1.00		0.84
7 Hr. Treatment/ 14 Days Freshwater	35 ppb	3		0.79		0.81	
13 Hr. Treatment/ 7 Days Freshwater	35 ppb	5	0.95		0.92		0.98
13 Hr. Treatment/ 14 Days Freshwater	35 ppb	3		0.82		0.91	
Control	-----	-----	0.85	0.77	0.83	0.82	0.89

[1] Three rainbow trout fry were composited for each analysis.
[2] The detection limit for all samples ranged from 0.1 to 0.2 mg/kg Cu.

In addition to the copper present in the EarthTec, the rainbow trout chow contained 9.9 mg/kg copper, which contributed to the body burden levels found in all of the fish. Even the test fish, when originally obtained from the hatchery, contained low levels of copper. Three replicate samples of rainbow trout fry obtained from the hatchery had a mean copper level of 1.80 mg/kg prior to being subjected to test conditions, which was similar to values obtained for fish analyzed at the end of the test period after four applications. Based on this information, copper levels found in the control rainbow trout, which were similar to the levels found in the test trout, appear to represent a baseline condition for all trout in the test.

Based on the positive results of the 1996 bioassays, and the results of the initial field applications on the Drum Canal, EarthTec was used to control algae in the Tiger Creek Canal during the summer and fall of 1996. These operational

applications to the canal were generally successful; however, the maximum application rate and duration (30 ppb for 12 hours) was only partially effective in controlling the growth of algae in the canal. Consequently, significantly longer treatment durations were proposed for the 1997 algae season.

Series 2 Bioassays, October, 1996 through January, 1997

During the 84-day test period from October, 1996 through January, 1997, all six test scenarios for both brown and rainbow trout were successfully completed. Survival rates for both the rainbow trout fry and brown trout fingerlings were generally in the 90 - 100% range at the end of the test period. This is an extremely high survival rate considering that the tests were conducted in static aquaria over an extended period of time (28 to 84 days), and that fish were handled repeatedly throughout the test period.

With the exception of the rainbow trout 96-hour/17 day freshwater test, no unusual problems occurred in any of the test scenarios throughout the duration of the study. During the last eight days of the test (days 76-84), 13 rainbow trout fry died in the two replicate aquaria of the 96-hour/17 day test. Water quality parameters in the two tanks were similar to all other test and control aquaria. There was no evident reason for the mortalities; however, the fish were somewhat lethargic and may have developed a disease. It is unclear whether the 96-hour treatments weakened the fish and predisposed them to disease or whether the mortalities were due to some other extraneous factor. However, since the other 96-hour test scenario (96-hour/10 days freshwater) had more frequent treatments with 100% survival, it seems unlikely that the treatments caused the mortalities.

Based on the high survival rates for all test scenarios (except the rainbow trout 96-hour/17 day freshwater treatment) repeated long duration (48- to 96-hour) EarthTec exposures did not negatively affect either brown trout fingerlings or rainbow trout fry. Growth rates did not appear to be negatively affected by any of the EarthTec treatment scenarios. The mean length of test fish was similar to that of control fish for both rainbow trout fry and brown trout fingerlings.

Whole body copper analyses were conducted on both rainbow trout fry and brown trout fingerlings after each EarthTec treatment and the required period in freshwater, except for the 48-hour/5 day freshwater scenario, which started after the second treatment period. Control fish were also analyzed each time test fish were analyzed. The results of the whole body copper analyses showed little difference in copper concentrations between test fish and control fish for both rainbow and brown trout throughout the duration of the test period.

Rainbow trout and brown trout copper body burdens did not change significantly from one EarthTec treatment to the next,

regardless of exposure time or the duration in freshwater. In addition, the copper levels in the test fish were not significantly different from the copper levels in the control fish. Tables 4 and 5 provide a summary of the rainbow trout fry and brown trout fingerlings analyses results. An Analysis of Variance (ANOVA) showed no significant difference between treated and control fish for any of the test scenarios. Results of the copper analyses indicate that, for the scenarios tested, the periods in freshwater between the EarthTec treatments was sufficient to allow depuration of copper from the bodies of both rainbow and brown trout.

Table 4. Rainbow trout fry whole body copper analyses from the October, 1996 through January, 1997 bioassays.

Test Scenario[1]	Total # of Treatments	Results of Whole Body Copper Analyses in ppm (mg/kg Cu) for Replicate Aquaria[2,3]						
		10/28	11/4	11/11	11/25	12/9	12/16	1/6
48 Hr. Treatment/	3	0.81[4]	1.15	---[5]				
5 Days Freshwater		0.96[4]	1.10	---[5]				
48 Hr. Treatment/	4	0.84		1.22	1.97	1.10		
12 Days Freshwater		0.78		1.00	1.16	0.96		
72 Hr. Treatment/	4	0.73		0.75	1.06	1.07		
11 Days Freshwater		0.68		1.13	1.11	1.15		
72 Hr. Treatment/	4		0.86		0.86		1.14	0.99
18 Days Freshwater			0.82		1.00		1.14	1.09
96 Hr. Treatment/	4	0.68		0.94	1.36	1.04		
10 Days Freshwater		0.88		1.35	1.27	1.26		
96 Hr. Treatment/	4		1.32		1.02		1.26	1.65
17 Days Freshwater			0.99		0.77		1.15	1.47
Control	----	1.05	0.81	0.99	1.31	1.52	1.08	1.12
		0.75	1.07	0.83	1.04	1.15	1.30	1.15

[1] All test scenarios were conducted at EarthTec concentrations of 35 ppb.
[2] Two rainbow trout fry were composited from each aquarium for analysis.
[3] The detection limit for all samples ranged from 0.2 to 0.3 mg/kg Cu.
[4] Fish with weekly treatments were initially analyzed for copper after the second chemical treatment, and after each subsequent treatment. In other treatment scenarios, fish were analyzed for copper after all treatments.
[5] The fish removed from the 48-hr. treatment on 11/11 were misplaced at the laboratory and consequently were not analyzed.

Table 5. Brown trout fingerlings whole body copper analyses from the October, 1996 through January, 1997 bioassays.

Test Scenario[1]	Total # of Treatments	Results of Whole Body Copper Analyses in ppm (mg/kg Cu) for Replicate Aquaria[2,3]							
		10/21	10/28	11/4	11/18	11/25	12/2	12/9	12/30
48 Hr. Treatment/	3	1.39[4]	0.81	2.08					
5 Days Freshwater		0.93[4]	0.96	1.62					
48 Hr. Treatment/	4	1.27		1.24	1.32		1.47		
12 Days Freshwater		1.14		1.79	1.33		1.81		
72 Hr. Treatment/	4	0.76		1.09	1.08		1.47		
11 Days Freshwater		1.04		1.51	1.03		1.60		
72 Hr. Treatment/	4		1.20		1.86			1.32	1.87
18 Days Freshwater			1.11		0.83			1.16	1.59
96 Hr. Treatment/	4	1.28		1.84	1.11		1.38		
10 Days Freshwater		1.58		1.31	1.49		1.66		
96 Hr. Treatment/	4		1.62		1.29			1.52	1.21
17 Days Freshwater			1.85		1.62			1.41	0.92
Control	----	0.84	0.73	2.09	1.40	1.15	1.43	1.56	1.52
		1.17	1.21	1.83	1.70	0.87	1.11	1.19	1.46

[1] All test scenarios were conducted at EarthTec concentrations of 35 ppb.
[2] The detection limit for all samples was 0.1 mg/kg Cu or less.
[3] Brown trout fingerling were analyzed individually.
[4] Fish with weekly treatments were initially analyzed for copper after the second chemical treatment, and after each subsequent treatment. In other treatment scenarios, fish were analyzed for copper after all treatments.

The rainbow trout chow contained similar levels of copper as described in the results for the March-April, 1996 bioassays. The brown trout chow also contained copper (approximately 12.5 mg/kg), which contributed to the body burden levels found in all of the fish. The test fish, obtained from the hatchery, also had low levels of copper. Based on this information, copper levels found in the control brown trout, which were similar to the levels found in the test trout, appear to represent a baseline condition for all trout in the test.

Field Monitoring

The results of the 1996 and 1997 sediment and aquatic insect monitoring program indicate that EarthTec applications to the Tiger Creek Canal have not caused a net increase in copper in either sediment or aquatic insects at any station. Table 6 provides a summary of the results obtained through 1997. The monitoring program is scheduled to continue through the year 2000. If at the end of this period, results still show no increasing trend in overall copper levels, the monitoring program may be terminated after consultation with CDF&G.

Table 6. Copper monitoring data for sediment and aquatic insects for Tiger Creek and the Tiger Creek Regulator.

Station Location	Sampling Dates and Copper Analysis Results in ppm (mg/kg)					
	7/11/96	9/6/96	11/26/96	4/3/97	8/7/97	11/18/97
Tiger Creek Regulator R1 - Sediment	21	17	49	38	64[2]	25
Tiger Creek Regulator R2 - Sediment	36	35	21	22	16	24
Tiger Creek Regulator R3 - Sediment	20	17	12	26	62[2]	24
Tiger Creek Regulator R4 - Sediment	21	17	35	21	20	31
Tiger Creek Regulator R5 - Sediment	8.1	6.7	7.3	6.2	8.3	6.6
Tiger Creek Regulator R6 - Sediment	---[1]	9.4	17	14	22	15
Tiger Cr. above Regulator TC1 - Sediment	6.6	4.6	10	8.1	67[2]	5.4
TC1 - Insects	5.3	4.4	5.3	5.2	8.3	8.2
Tiger Cr. below Regulator TC2 - Sediment	---[1]	---[1]	21	33	62[2]	28
TC2 - Insects	12	12	12	7.8	13	17

[1] These samples were originally not required as part of the monitoring plan, but were added later to augment information from the other stations.

[2] Data are highly suspect; extremely high values may be due to contamination at the laboratory.

Conclusions

Considering the high rate of survival in both series of bioassays, and the results of the whole body copper analyses, none of the test scenarios appeared to have a negative effect on either the rainbow trout fry or brown trout fingerlings. Based on these findings, EarthTec applications on the Tiger Creek or Drum canals of 30 ppb for 48- to 96-hour durations (every two to three weeks) does not appear to have an acute or chronic effect on either rainbow trout or brown trout.

Based on the overall results of both series of bioassays, CDF&G approved the continued use of EarthTec on the Tiger Creek and Drum canals at the application rates that were tested.

The results of the 1996 and 1997 sediment and aquatic insect monitoring program show no increasing trend in copper in either the sediments, or in aquatic insects. Based on these data, PG&E will continue EarthTec treatments in 1998.