EEB File

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

NOV 26 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Submission of Chlorfenpyr (Pirate® AC 303,630 3SC) Sediment Toxicity Test Protocol

FROM: William Evans, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

THRU: Ann Stavola, Supervisory Biologist 11/26/96
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

THRU: Norman Cook, Acting Branch Chief 11/26/96
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Dennis Edwards, Branch Chief
Insecticide/Miticide Branch
Registration Division (7505C)

The EEB has completed a review of a protocol submitted by the American Cyanamid Company to satisify the sediment toxicity test requirements for Chlorfenpyr. The protocol was transmitted to EEB under DP Barcode #:D227768.

As explained in the submission, the EEB requested that two acute sediment toxicity tests be conducted according to procedures as outlined in the EPA publications EPA/600R-94/024 and EPA/600R-94/025. However, the registrant requested that a 28-day exposure sediment toxicity test which was being conducted be substituted for the required acute tests.

The major problem, as pointed out by the registrant, is that in the test system proposed by the registrant the test substance is added directly into the water column instead of the sediment. In order to test the toxicity to benthic organisms, it is imperative that

2051575

the sediment be spiked directly. Spiking the sediment will ensure uniform mixing of the test substance into the sediment. If added into the water column, partitioning will occur as the test substance descends into the sediment, and adequate mixing into the sediment will not occur.

The registrant also explains that mixing the test substance in the water column can better estimate the concentrations in bodies of water and can therefore be easily incorporated into an ecological risk assessment. While there may be some validity to this reasoning in terms of exposure modeling, the purpose of this test is to measure the toxicity of chlorfenpyr to sediment dwelling organisms. The EEB must therefore maintain that the sediment toxicity tests be conducted as outlined in the EPA protocols.

If there are any questions concerning this review, please contact Bill Evans on 703-3005-6754.

STUDY PROTOCOL EVALUATION RECORD
FIELD STUDY - AVIAN CENSUS

1. **CHEMICAL:** **PIRATE™** PC Code No.: 129093
4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile

2. **TEST MATERIAL:** AC 303,630 Purity: N/A

3. **CITATION**

Study Director: Dr. James A. Gagne
Title: A Characterization of Avian Species On and Around Cotton Fields in the Cotton Belt of the Southern United States.
Projected Study Date: 1 June 1995
Laboratory: Wildlife International, Ltd.
Sponsor: American Cyanamid Company, Princeton NJ
Laboratory Report ID: Wildl. Intern. No. 130-172
DP Barcode: D216644

4. **REVIEWED BY:** John Eisemann, Wildlife Biologist, EEB, EFED

Signature: John D. Eisemann **Date:** Sept 18, 1995

5. **APPROVED BY:** Ann Stavola, Head of Section (5), EEB, EFED

Signature: Ann Stavola **Date:** 9/18/95

6. **OBJECTIVES:**

PRIMARY: To determine the abundance and composition of avian species found in and around cotton fields throughout the growing season.

SECONDARY: To monitor the activities of birds while they are within a cotton field.

7. **CONCLUSIONS:** The purpose of this study is unclear. A major portion will repeat work previously conducted. Since PIRATE is known to affect reproduction in some species, nest monitoring would be a valuable addition to provide background information to determine suitable focal species for investigation when PIRATE is actually applied.

Study fields should be larger than 20 acres. Fields of 20 acres will necessitate the placement of census plots in close proximity to each other and will result in interdependence, thereby violating accepted statistical practices. Census plot sample size will probably be inadequate. Sample size determinations should be conducted prior to beginning this study.

8. **METHODS:**

Study Site: Study sites are located in the cotton producing regions of Alabama/Mississippi, Texas and Arizona.

Site Selection: Ten to twelve cotton fields $\geq$ 20 acres will be selected in each region, divided between irrigation regimen and adjacent habitat types. Site determination will be chosen from aerial photographs and/or ground surveys.

Site Description: A map or aerial photo encompassing a distance of 100 meters beyond the field edge will be made once during the study. Adjacent habitat features will be described in detail to include the name of the habitat type, dominant plant species and structural characteristics. In addition proximity to bird feeders and houses and census station locations will be identified. Detailed descriptions of the cotton fields and census plots will also be made.

Sampling Methods: The avian census technique proposed utilizes fixed circular plot sampling stations. Six plots, 50 meters in radius, will be established at each site. Plot centers will be permanently marked and situated a minimum of 100 meters apart along the edge of the field where the cotton meets the adjacent habitat. After arriving at the plot center a two minutes waiting period will be observed. During the following 8 minute period all birds sighted or heard within the plot will be recorded.

Time-activity surveys will be conducted to obtain information on bird activities while in cotton fields. Biologists working in pairs will record the activities of focal birds for a period of 1 hour. A technique know as focal switching will be employed to account for the time periods when focal birds move out of sight.

Avian census and time-activity observations will occur three times during the growing season (early, middle and late season). On each sampling period, biologists will conduct three fixed circle plot censuses (morning) and two time-activity surveys (one morning and one evening).

Observations of wildlife use, including visual and aural cues, scat, tracks and others signs will also be recorded.

Statistical Analysis: Data will be summarized by grouping observations over all test sites within each region and by test site within each region. Plot census data will

be summarized for the total number of birds, mean abundance, percent relative abundance, frequency of occurrence, species diversity and percent of observations in cotton fields. Time-activity data will be summarized as the percent of observations which birds were exhibiting various behaviors. Diversity calculations for each site and region will be calculated using the Brillouin Index and the Shannon-Weaver Index, respectively.

GLP: Guidelines as specified by the U.S. EPA's Good Laboratory Practice Standards 40 CFR Part 160 will be followed.

Quality Assurance: EPA GLP Standards and SOP's of the test facility will be followed. The Quality Assurance Unit of the test facility will conduct inspection and provide timely reports to the Study Director and the Study Director's management.

References:

Edwards, D.K., G.L. Dorsey, and J.A. Crawford. 1981. A Comparison of Three Avian Census Methods. Studies in Avian Biology No. 6, 170-176.

Fite, E. C., L. W. Turner, N. J. Cook, and C. Stunkard. 1988. Guidance Document for Conducting Terrestrial Field Studies. U. S. Environmental Protection Agency Report EPA 540/09-88-109.

Morrison, M. L., R. W. Mannan, and G.L. Dorsey. 1981. Effects of number of circular plots on estimates of avian density and species richness. Pp. 405-408. *In* C. J. Ralph and J. M. Scott (eds). Estimating the numbers of terrestrial birds. Stud. Avian. Bio. 6.

Tilmann, K., S. Gallagher, D. Palmer, and J. Sullivan. 1994. A characterization of avian species on cotton fields in the cotton fields of the southern Unites States. American Cyanimid Report Number ECO 93-151. 335pp.

REVIEWER COMMENTS:

Although study objectives were listed, it is unclear why this study is being conducted. Is this study designed to investigate additional sites in preparation for a future field study in which PIRATE will be applied? With the exception of time activity monitoring of Red-winged blackbirds, it appears this study duplicates work done previously by Tilmann (1994). Will results of a duplicate census justify the effort required?

The main issue which needs addressed in a field study is, is PIRATE bioavailable to terrestrial species? We know the chemical will be

present in the environment. We know it will be available to the target invertebrate pests. But is it available to non-target species? The assumption is yes it is. Work done previously shows PIRATE is present in bluegill tissues when the chemical is in the water but once the fish are presented clean water the chemical is quickly transformed into the debrominated form. Is this the case with birds? No data supports this relationship. If the chemical is available to primary consumers does it bioaccumulate and present a problem to predatory species? Again, no data negate this. To address bioaccumulation in raptors a large scale field project would be required. In this case a laboratory study would be a logical first step.

There are both acute and chronic exposure effects associated with PIRATE. Acute effects can be assessed by conducting carcass searches and techniques have been proposed to evaluate this in another study protocol. Chronic exposure is a major concern for species establishing territories and breeding near cotton fields. It is known that small amounts of PIRATE can significantly affect reproduction in some avian species. Efforts such as nest surveys and monitoring would provide evidence of (or lack of) population effects. Rather than repeat the avian census, a survey of those species nesting within the study sites would provide information for choosing a focal species for the upcoming field study.

When choosing study fields careful attention should be given to the irrigation regimen of the field, the proximity of open water and the type and structure of the adjacent habitat. Because of the potential for the active ingredient to bioaccumulate, raptor, owl and predatory mammal populations in the area are of concern. The habitat adjacent to cotton fields should be similarly structured to provide equal foraging opportunities. Additionally, waterfowl exposure is of concern due to their sensitivity to the active ingredient and use of irrigation practices of cotton in sometimes arid environments.

Detailed methods were listed for the census of diurnal species. Methods should be incorporated into this study to estimate nocturnal species abundance. Nocturnal avian surveys should be designed to detect the presence of owls and common night hawks. This study is structured to determine avian use, but incidental observations will be recorded on non-avian species (ie. visual and aural cues, scat, tracks and other signs). Valuable information could be obtained on non-avian use of these habitats by the establishment of predator scent post stations or other stations designed to yield clear tracks and the use of pitfall traps to estimate amphibian and reptile use.

In addition to using Morrison's (1981) recommended sample size for fixed circular plots, sample size determinations should be conducted following the methods outlined in the EPA Guidance Document for Conducting Avian Field Studies (Fite et al. 1988) to insure the number of plots surveyed will provide meaningful statistical comparisons. Previous census work in Arizona (Tilmann

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OCT 31 1996

MEMORANDUM

SUBJECT: Screen of Chlorfenpyr (Pirate® AC 303,630 3SC) Microcosm

FROM: William Evans, Biologist
Ecological Effects Branch
Environmental Fate and Effects Division

THRU: Ann Stavola, Supervisory Biologist 6/30/96
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

THRU: Daniel Rieder, Acting Branch Chief 10-31-96
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Dennis Edwards, Branch Chief
Insecticide/Miticide Branch
Registration Division (7505C)

The EEB has completed a screen of the microcosm submitted by American Cyanamid Company. The study was transmitted to EEB under DP Barcode #:D210808 (MRID # 434928-23). Since there is no EPA protocol or guidance documents for performing or reviewing microcosms, the results from this study can only be used as supplemental information only.

Based on the limited data summarized in the study, the reviewer concludes that it is highly probable that significant drift from aerial applications will result in fish mortalities. 90% and 100% mortalities were observed at nominal concentrations of 300 and 30 µg ai/L (221.32 and 11.33 µg ai/L measured concentrations). Therefore, the LOEC for fish is the nominal concentration of 30 µg ai/L (11.33 µg ai/L measured). Significant test substance related effects for rotifers, copepod naupalii, and insects are also highly likely, but statistical significance can not be examined due to the fact that no replicates were used in any test levels throughout the study.

For questions concerning this review you may contact William Evans at 305-6754.

ECOLOGICAL EFFECTS BRANCH
MICROCOSM SCREEN

1. **Chemical:** Chlorfenpyr; PIRATE®; AC 303,630; 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile, 94.5% a.i.

2. **Study:**

Type: Aquatic microcosm using 30,900 L tanks; system included aquatic invertebrates and bluegill sunfish.

Citation: Rand, Gary M. and Wisk, Joseph D. 1994. "Evaluation of the Fate and Effects of AC 303,630 3SC in an Outdoor Microcosm System; A Pilot Study". Toxikon Environmental Sciences, 106 Coastal Way Jupiter, Florida 33477. Study sponsored by American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ 08543-0400. Project # J9303003. Cyanamid Study #: 954-93-149. EPA MRID #: 434928-23.

3. **General Information on Chemical:**

Pirate (AC 303,630) is a new broad spectrum insecticide with no currently registered uses. According to the submission from American Cyanamid Company, Pirate which belongs to a new class of pesticides called pyrrole, offers a unique mode of action for efficacious pest control. The manufacturer purports that the mode of action uncouples the oxidative phosphorylation process by disrupting the electrochemical gradient in the mitochondria. It inhibits the ability to produce energy.

Pirate is formulated as an insecticide-miticide as two products. PIRATE™/AC 303,630 3SC is a suspension concentrate which contains 3.0 lbs a.i./ gallon = 30.83% a.i. and is applied east of the Rocky Mountains. ALERT™/AC 303,630 2SC is a suspension concentrate which contains 2.0 lbs a.i./gallon = 21.44% a.i. and is applied west of the Rocky Mountains.

Pirate (303,630) can be applied using ground and aerial equipment. The pounds of active ingredient per acre are pest dependent with a minimum of 0.06 lb a.i./A to a maximum of 0.35 lb a.i./A. Application is as required based on scouting and is repeated as necessary to maintain control. Pirate is not to be applied through any irrigation system and unprotected personnel are not permitted to enter until spray has dried. The maximum amount which can be applied per year is 1.05 lb ai/A.

In addition to a FIFRA Section 3 registration on cotton, American Cyanamid Company has applied for a number of Section 18 and experimental use permits and a temporary tolerance (0.5 ppm) for ALERT™/AC 303,630 2SC insecticide-miticide. Uses include cotton, greenhouse and ornamentals, citrus, cabbage,

lettuce, and tomatoes.

4. Dosage and Dates:

The test was conducted during the summer of 1993 using 5 unreplicated treatments and 1 untreated control (regression design). Treatments simulated either spray drift, surface runoff, or a combination of both. For spray drift simulation, nominal concentrations of 30 and 300 µg ai/L were applied directly to tanks 1 and 2 on September 29. The highest treatment level of 300 µg ai/L was based on the maximum proposed label rate of 0.4 lb ai/A. The 30 µg ai/L level was based on a 10% spray drift into a 6-inch deep pond.

To simulate surface runoff 30 µg ai/L of test substance was applied to soil on September 29. The soil was held outdoors in glass trays and exposed to sunlight, then applied to tank 6 on September 30. The 30 µg ai/L test level was based on 1% surface runoff from a 10 acre field into 6-inch deep 1-acre pond. To simulate a combination of spray drift and surface runoff tanks 3 and 4 received both applications. Tank 3 received 45 µg ai/L (15µg ai/L based on 5% spray drift of maximum application rate and 30 µg ai/L based on 1% surface runoff from a 10 acre field into 6-inch deep 1-acre pond). Tank 4 received 3.7 µg ai/L (1.5 µg ai/L based on 5% spray drift and 2.5 µg ai/L based on 1% runoff from a 10 acre field into a 6 feet deep 1 acre pond). Tank 5 functioned as an untreated control and consisted of dilution water followed by untreated soil 25 hours later.

5. Purpose of Study:

The purpose of this study was to evaluate the effects of AC 303,630 on aquatic organisms under conditions more representative of an actual environmental application. This study was undertaken due to the high acute and chronic toxicity to aquatic organisms as well as the fate properties exhibited in laboratory studies. This study was submitted to fulfill guideline requirement 72-7, a simulated aquatic field study.

The general objectives of the study were:

- To establish a series of small experimental aquatic communities;
- to monitor biological, chemical and physical changes in the microcosm to evaluate potential impact on populations of aquatic organisms following direct application (in water and/or soil) of AC 303,630 3SC at different concentrations (treatments); and
- to compare treated to untreated microcosms.

6. Test Description:

The test was conducted during the summer of 1993. The microcosm tanks (6.7 x 1.9 x 2.43 m = 30.9 m^3) were filled to a depth of 1.5 m with water and sediment taken from the on site pond on June 14, and were allowed to age for four weeks.

Twenty juvenile bluegill sunfish were added to each tank on July 15. A ten week pre-treatment phase included monitoring and sampling to establish base line biotic and abiotic conditions. Pond treatment begun on September 29 as detailed in 4. above. A 14-day post-treatment period which monitored population trends and residue concentrations of the test substance followed pond treatment.

7. Endpoints:

Meteorological data were collected daily on-site, and included air temperature, relative humidity, barometric pressure, solar radiation, and total rainfall and evaporation.

Sediment characteristics including % organic matter, pH, phosphorous, potassium, magnesium, calcium, sodium, % sand, % silt, % clay, textural classification and bulk density were analyzed. Dissolved oxygen, pH, conductivity, and temperature were monitored daily. Every two weeks alkalinity, hardness, total suspended solids (TSS), total dissolved solids (TDS), turbidity, dissolved organic carbon (DOC), total organic carbon (TOC), total nitrogen, NO_2, NO_3, total Kjeldahl nitrogen (TKN), total phosphorus, and ammonia were measured during the treatment and post treatment period. Additionally, microcosm water was analyzed for physico-chemical characteristics and organochlorine and organophosphate pesticide residues and metal screen.

To determine the effect of the test substance the following biotic components were monitored.

Phytoplankton - Chlorophyll a and phaeophytin densities concentrations were used to estimate alga biomass. Also populations of phytoplankton species were reported as number of organisms/mL.
Zooplankton - Abundance was measured using the densities (no. organisms/L) of four major taxa (Cladocera, Copepoda, Ostracoda, and Rotifera).
Macroinvertebrates - Abundance was measured as no. organisms/m^2/wk among the following taxa (Hydracarina, Insecta, Annelida, and Nematoda)
Fish - Mortality was observed during pretreatment, treatment, and post-treatment phases. Individual length and weight data was recorded at the beginning and end of study.

8. Reported Results:

Concerning meteorological data it was noted that it rained several times during the post-application period. Alkalinity measurements revealed a wide range of measurements. Dissolved oxygen was at a low of 7.0 - 7.5 mg/L at the beginning of the study and increased to a high of 9.2 - 9.5 mg/L at the end of the observation period. pH ranged from 7.9 - 9.2, and the temperature ranged from 28 to 30°C.

Residue concentrations of AC 303,630 in pond water was not detected in the control tank during the 14-day post-treatment period. The two highest spray treatments (300 and 30 µg ai/L) detected concentrations ranging from 221.32 and 23.57 µg ai/L at two hours after treatment to 103.33 and 11.33 µg ai/L after 14 days post-treatment. In the 15 µg ai/L spray, 30 µg ai/L runoff treatment detected residues after two hours were 13.87 µg ai/L and 9.7 µg ai/L after 14-days. In the 1.2 µg ai/L spray, 2.5 µg ai/L runoff treatment no residues were detected in the spray treatment after two hours. However, the concentration after the runoff application ranged from 2.56 µg ai/L after two hours to 2.84 µg ai/L after 14 days. The 30 ai/L runoff only treatment ranged from 2.84 to 3.64 µg ai/L throughout the post-treatment period (see Table 12 attached).

AC 303,630 was not detected during the 14-day post-treatment period in the sediment in the control tank. The two highest spray treatments (300 and 30 µg ai/L) did not have detectable concentrations after two hours. However, concentrations were detected at 24 hours and peaked to measured concentrations of 488.35 and 24.17 µg ai/L respectively after 3 days and declined to 271.03 and 12.9 µg ai/L after 14 days. In the 15 µg ai/L spray, 30 µg ai/L runoff treatment there was no detected residues in the sediment two hours after the spray treatment. However, 2 hours after the runoff treatment 131 µg ai/L was detected and concentrations continually increased to 201.3 µg ai/L after 14 days. In the 1.2 µg ai/L spray, 2.5 µg ai/L runoff treatment residues were detected only in the 24-hour and 7-day post-spray/runoff treatment. For the 30 µg ai/L runoff only treatment no residues were measured one day after treatment. However, residues were measured at 43 µg ai/L 2 hours after treatment and 292 µg ai/L 14-day post-treatment (see Table 13 attached).

For phytoplankton, the different chlorophyte groups showed significant decreases in population abundance at various treatment levels, however, regression analysis did not show strong correlations for effects due to treatments. Either the largest change in abundances were found in the control or the decreases in abundances were not high enough to draw any conclusions (see Table 19 attached).

For zooplankton, regression analysis showed no strong correlations for effects due to treatments. However, differences in abundance for treatments indicate subtle effects to copepods and copepod nauplii (see Table 16 attached).

Macroinvertebrate abundance was primarily divided among the Insecta, Annelida, and Nematoda taxa. It was concluded that a consistent decrease in abundance of Insecta occurred after treatment with AC 303,630 SC. Regression analysis indicated a subtle effect of spray applications on insects and nematodes

(see Table 22 attached).

For fish, minimal fish mortality occurred during the pretreatment phase, and all were replaced. In the highest spray treatment (300 µg ai/L) 18 of the 20 fish died within 24 hours of application. The remaining 2 fish died within 6 days. In the next highest spray treatment (30 µg ai/L) 100% of the fish died. The greatest mortality occurred during the second week post application. In the 15 µg ai/L spray, and the 30 µg ai/L runoff treatment no fish mortality occurred with the exception of 4 mortalities between 18 - 20 days after treatment while tanks were being drained. No other mortalities occurred in other treatment levels throughout the post-treatment period. The length and weight data collected for all fish showed that fish length and weight were similar from tank to tank (see Tables 24 and 25 attached).

9. Reported Conclusions:

The study authors concluded that residues of AC 303,630 3SC applied directly to the test systems remained elevated longer than would have been predicted from the laboratory studies.

In the 300 and 30 µg ai/L direct application treatments and the 45 µg ai/L spray/runoff treatment, the water concentrations exceeded the laboratory acute LC_{50} for bluegill (11.6 µg ai/L). However, the authors maintain that no fish mortalities were observed in the 45 µg ai/L treatment throughout the 14-day post-treatment phase. A 100% fish mortality was observed in the 300 and 30 µg ai/L direct application treatments, and a 20% mortality in the 45 µg ai/L spray/runoff treatment between two and three weeks post application. In addition, the authors conclude that AC 303,630 3SC was less toxic to bluegill when introduced into the tanks by the soil application.

AC 303,630 3SC eventually accumulated in the sediment, regardless of method of introduction, and there was no evidence of significant degradation during the two-week post treatment period.

Although there were no statically significant effects of AC 303,360 3SC on phytoplankton, zooplankton, and macroinvertebrates, there were negative trends in some taxa when mean abundances from samples collected during the two-week pre-application period were compared with samples collected during the two-week post-application period. The taxa which showed abundance decreases were cryptophyta, rotifers, copepod nauplii, and insects.

10. Reviewer's Conclusions:

Aquatic microcosm studies are not substituted as guideline requirements for aquatic mesocosm studies. Hence, there is no

EPA protocol or guidance documents for performing or reviewing microcosms. The results from this study will therefore be used as supplemental information only.

It should first be noted that no replicates were run at any test level. Therefore, the power of the test may be quite limited. Measured residue concentrations of AC 303,630 in pond water which ranged from 221.32 µg ai/L two hours after direct spray treatment to 103.33 µg ai/L after 14 days, suggest that residues remain in pond water at levels higher than the bluegill laboratory LC_{50} of 11.6 µg ai/L. The 90% bluegill mortalities observed in the study within 24 hours reconfirm the presence of the residues at lethal concentrations to fish. Additionally, a 100% bluegill mortality was observed during the 14-day post-treatment period at the nominal 30 µg ai/L (11.33 µg ai/L measured) direct spray treatment. The results of these fish mortalities confirm that it is highly probable that significant drift from aerial applications will result in fish mortalities. Therefore, the LOEC for fish is the nominal concentration of 30 µg ai/L (11.33 µg ai/L measured).

Notable effects (decreases in abundance) were also observed among phytoplankton and zooplankton. However, due to the variation among the various test levels as well as the control and lack of replications, it can not be decisively concluded that the effects observed were due to the treatment.

Among the macroinvertebrates, rotifers, copepod nauplii, and insects showed significant decreases in abundance. Although the authors conclude that there were no statistically significant test substance related effects on macroinvertebrates, it is clear that consistent decreases in insect abundance occur.

Reviewed By:
William Evans
Biologist, EEB/EFED Date:

Approved By:
Ann Stavola 10/30/96
Section Chief, EEB/EFED Date:

Table 12. Concentrations of AC 303,630 in Water 14 Days Post-Treatment in Microcosm Tanks (n=6)[a]

Tank	AC303,630 (µg/L) Observed in Pond Water					
	Event					
	1	2	3	4	5	6
1	221.32	159.13	152.37	113.04	105.11	103.33
2	23.57	19.72	19.38	16.27	15.55	11.33
3	13.87	11.93	12.52	12.81	11.29	9.97
4	< 1 ppb	2.56	1.39	1.61	2.84	1.20
5	< 1 ppb	< 1 ppb	< 1 ppb	< 1 ppb	< 1 ppb	< 1 ppb
6	< 1 ppb	2.84	3.71	3.52	3.85	3.64
QC low	1.21	1.56	< 1 ppb	< 1 ppb	6.29	1.08
QC med.	10.67	9.92	8.69	10.02	10.73	9.24
QC high	107.73	89.88	92.89	95.07	89.74	90.94

[a] Tank 1; 300 µg/L spray
Tank 2; 30 µg/L spray
Tank 3; 15 µg/L spray/30 µg/L runoff
Tank 4; 1.2 µg/L spray/2.5 µg/L runoff
Tank 5; control
Tank 6; 30 µg/L runoff

Event 1, two hours after day-0 spray treatment
Event 2, two hours after day-1 runoff treatment
Event 3, one day after day-1 runoff treatment
Event 4, three days after day-1 runoff treatment
Event 5, seven days after day-1 runoff treatment
Event 6, fourteen days after day-1 runoff treatment

NOTE: Nominal concentrations for the low, medium, and high QC samples were 1.00, 10.0, and 100 µg/L, respectively.

Table 13. Concentrations of AC 303,630 in Sediment 14 Days Post-Treatment in Microcosm Tanks (n=6)[a]

Tank	AC303,630 (µg/Kg) Observed in Pond Sediment					
	Event					
	1	2	3	4	5	6
1	< 10 ppb	61.62	279.65	488.35	354.72	271.03
2	< 10 ppb	13.74	20.88	24.17	13.19	12.90
3	< 10 ppb	131.60	61.16	72.39	67.80	201.30
4	< 10 ppb	< 10 ppb	33.19	< 10 ppb	24.20	< 10 ppb
5	< 10 ppb	< 10 ppb	< 10 ppb	< 10 ppb	< 10 ppb	< 10 ppb
6	< 10 ppb	43.03	< 10 ppb	16.51	12.19	292.10
QC low	21.80	21.27	14.17	15.40	11.88	< 10 ppb
QC med.	102.89	97.63	101.53	112.58	94.50	94.81
QC high	858.33	807.36	926.82	910.41	1251.95	910.04

[a] Tank 1; 300 µg/L spray
Tank 2; 30 µg/L spray
Tank 3; 15 µg/L spray/30 µg/L runoff
Tank 4; 1.2 µg/L spray/2.5 µg/L runoff
Tank 5; control
Tank 6; 30 µg/L runoff

Event 1, two hours after day-0 spray treatment
Event 2, two hours after day-1 runoff treatment
Event 3, one day after day-1 runoff treatment
Event 4, three days after day-1 runoff treatment
Event 5, seven days after day-1 runoff treatment
Event 6, fourteen days after day-1 runoff treatmentTable 13.

NOTE: Nominal concentrations for the low, medium, and high QC samples were 10, 100, and 1000 µg/Kg, respectively.

Table 16. Comparison of the Average Abundance of Zooplankton (Mean #/L) Between the Two Weeks Prior to Application and During the Two-Week Post-Application Period for Each Major Taxonomic Group

Tank #/ Treatment	Cladocera	Copepoda	Ostracod	C.Nauplii	Rotifer	Total
1) 300 ppb (direct)						
Pre-Treat	3.0 ± 1.5	5.9 ± 6.8	1.9 ± 2.3	58 ± 50	417 ± 228	486 ± 187
Post-Treat	0.1 ± 0.2	0.1 ± 0.1	0.03 ± 0.1	2.0 ± 3.3	81 ± 61	84 ± 62
2) 30 ppb (direct)						
Pre-Treat	2.4 ± 1.3	5.1 ± 4.3	1.5 ± 0.9	44 ± 34	328 ± 105	381 ± 85
Post-Treat	0.00	1.1 ± 0.8	0.00	4.7 ± 5.3	1240 ± 492	1246 ± 488
3) 45 ppb (comb.)						
Pre-Treat	1.3 ± 1.2	3.0 ± 2.6	3.6 ± 2.3	50 ± 31	362 ± 237	420 ± 226
Post-Treat	0.00	1.1 ± 1.1	0.00	13 ± 13	742 ± 219	755 ± 227
6) 30 ppb (soil)						
Pre-Treat	3.1 ± 3.6	14.7 ± 6.8	1.4 ± 1.9	50 ± 31	755 ± 448	824 ± 472
Post-Treat	0.3 ± 0.2	11.1 ± 8.9	0.1 ± 0.2	35 ± 20	327 ± 214	374 ± 229
4) 3.7 ppb (comb.)						
Pre-Treat	2.0 ± 1.0	6.5 ± 4.0	1.0 ± 1.4	86 ± 26	680 ± 436	775 ± 430
Post-Treat	0.7 ± 0.6	6.9 ± 3.3	0.2 ± 0.2	105 ± 33	439 ± 201	552 ± 173
5) Control						
Pre-Treat	0.7 ± 0.5	44 ± 48	0.4 ± 0.6	34 ± 29	615 ± 554	694 ± 539
Post-Treat	0.2 ± 0.3	19 ± 16	0.00	46 ± 16	563 ± 195	628 ± 205

Table 19. Comparison of the Average Abundance of Phytoplankton (Mean #/mL) Between the Two Weeks Prior to Application and During the Two-Week Post-Application Period for Each Major Taxonomic Group

Treatment	Bacillariophyta	Chlorophyta	Cryptophyta	Cyanophyta	Euglenophyta	Pyrrhophyta	Total Phytoplankton
1) 300 ppb (direct)							
Pre-Treat	18 ± 21	110 ± 30	73 ± 32	5 ± 8	2 ± 4	35 ± 25	241 ± 86
Post-Treat	0.0	76 ± 30	2 ± 4	2 ± 4	2 ± 4	23 ± 12	103 ± 34
2) 30 ppb (direct)							
Pre-Treat	0.0	134 ± 52	32 ± 39	10 ± 9	2 ± 4	40 ± 18	218 ± 64
Post-Treat	0.0	65 ± 27	19 ± 17	3 ± 5	2 ± 4	113 ± 46	2547 ± 898*
3) 45 ppb (comb.)							
Pre-Treat	13 ± 17	216 ± 166	27 ± 30	10 ± 9	0.0	11 ± 9	278 ± 209
Post-Treat	3 ± 5	39 ± 31	11 ± 11	0.0	0.0	26 ± 16	79 ± 36
6) 30 ppb (soil)							
Pre-Treat	2 ± 4	158 ± 46	18 ± 7	10 ± 12	0.0	15 ± 10	203 ± 62
Post-Treat	2 ± 4	148 ± 72	5 ± 8	2 ± 4	0.0	11 ± 11	168 ± 74
4) 3.7 ppb (comb.)							
Pre-Treat	5 ± 12	160 ± 74	45 ± 29	6 ± 8	0.0	42 ± 39	258 ± 97
Post-Treat	2 ± 4	144 ± 49	31 ± 35	2 ± 4	0.0	27 ± 14	205 ± 90
5) Control							
Pre-Treat	6 ± 8	148 ± 108	16 ± 17	16 ± 16	0.0	18 ± 21	205 ± 101
Post-Treat	2 ± 4	60 ± 28	11 ± 16	3 ± 5	0.0	11 ± 11	87 ± 47

*Total phytoplankton in these samples contained an average of 2346 ± 878 organisms/mL of an unknown unicellular flagelate.

Table 22. Comparison of the Average Abundance of Macroinvertebrates (Mean #/Meter Squared/Week) Between the Pre-Application and Post-Application Periods for Each Major Taxonomic Group

Tank #/ Treatment	Insecta	Annelida	Nematoda	Total Macroinvertebrate
1) 300 ppb (direct)				
Pre-Treat	707 ± 222	99 ± 60	105 ± 96	911 ± 297
Post-Treat	4 ± 7	81 ± 61	2 ± 5	88 ± 64
2) 30 ppb (direct)				
Pre-Treat	391 ± 138	233 ± 60	37 ± 64	667 ± 177
Post-Treat	76 ± 62	488 ± 282	4 ± 11	568 ± 229
3) 45 ppb (comb.)				
Pre-Treat	970 ± 1109	452 ± 229	140 ± 87	1561 ± 1379
Post-Treat	79 ± 24	306 ± 129	44 ± 39	428 ± 140
6) 30 ppb (soil)				
Pre-Treat	283 ± 226	85 ± 109	6 ± 9	374 ± 261
Post-Treat	35 ± 31	104 ± 82	11 ± 13	149 ± 75
4) 3.7 ppb (comb.)				
Pre-Treat	589 ± 312	78 ± 54	22 ± 14	689 ± 329
Post-Treat	147 ± 58	150 ± 50	0.00	297 ± 62
5) Control				
Pre-Treat	436 ± 148	228 ± 168	65 ± 77	728 ± 317
Post-Treat	451 ± 179	180 ± 57	26 ± 28	657 ± 140

Table 24. Fish[a] Mortality Record for Tanks (n=6) After Application(s) of AC 303,630 3SC

Tank	Application: Spray 9/29	Runoff 9/30	10/1	10/2	10/3	10/4	10/5	10/6	10/7	10/8	10/9	10/10	10/11	10/14
1 (300 µg/L spray, no runoff)	0	18[b]	1	0	0	0	1	0	→					
2 (30 µg/L spray, no runoff)	0	0	1	0	1	0	0	2	4	1	0	8	3	
3[c] (15 µg/L spray, 30 µg/L run-off)	0	→												
4 (1.2 µg/L spray, 2.5 µg/L run-off)	0	→												
0 (control)	0	→												
6 (no spray, 30 µg/L runoff)	0	→												

[a] Fish; n = 20 at beginning of application
[b] Fish collected on 9/30/94 died within 24 hours of the first application on 9/29/94.
[c] Tank 3; fish died after termination of study on 10/14 - 10/18 (1 death), 10/19 (1 death), 10/20 (2 deaths)

Table 25. Fish Length and Weight Measurements at Termination of Microcosm Study

TANK 1 *			TANK 2 *			TANK 3		
DATE	STANDARD LENGTH (cm)	WET WEIGHT (g)	DATE	STANDARD LENGTH (cm)	WET WEIGHT (g)	DATE	STANDARD LENGTH (cm)	WET WEIGHT (g)
30-Sep-93	4.50	2.40	01-Oct-93	4.60	2.91	*18-Oct-93	4.70	2.49
30-Sep-93	4.20	2.03	03-Oct-93	4.50	2.73	*19-Oct-93	4.20	2.04
30-Sep-93	4.10	2.13	06-Oct-93	4.60	2.47	*20-Oct-93	4.20	1.87
30-Sep-93	4.10	2.02	06-Oct-93	4.60	2.57	20-Oct-93	4.80	2.93
30-Sep-93	5.30	4.38	07-Oct-93	4.70	2.71	20-Oct-93	5.70	4.84
30-Sep-93	4.20	2.04	07-Oct-93	4.30	2.18	20-Oct-93	4.70	3.14
30-Sep-93	5.60	5.71	07-Oct-93	4.40	2.59	20-Oct-93	4.60	2.90
30-Sep-93	5.10	3.90	07-Oct-93	3.90	1.85	20-Oct-93	4.50	2.49
30-Sep-93	4.50	2.51	08-Oct-93	4.70	2.72	20-Oct-93	4.60	2.52
30-Sep-93	4.50	2.38	10-Oct-93	5.40	4.18	20-Oct-93	4.90	3.24
30-Sep-93	4.60	2.92	10-Oct-93	4.30	2.14	20-Oct-93	4.50	2.65
30-Sep-93	4.70	3.22	10-Oct-93	4.40	2.28	20-Oct-93	4.20	2.20
30-Sep-93	4.60	2.85	10-Oct-93	4.10	1.87	20-Oct-93	4.60	2.82
30-Sep-93	3.90	1.83	10-Oct-93	5.10	2.75	20-Oct-93	4.60	2.86
30-Sep-93	4.40	2.69	10-Oct-93	4.20	1.88	20-Oct-93	4.40	2.50
30-Sep-93	4.80	3.41	10-Oct-93	4.10	1.89	20-Oct-93	4.70	2.96
30-Sep-93	4.10	2.46	10-Oct-93	4.20	1.69	20-Oct-93	4.20	2.06
30-Sep-93	4.50	2.92	11-Oct-93	4.20	1.96	20-Oct-93	4.50	2.43
01-Oct-93	4.30	1.92	11-Oct-93	4.40	2.39	20-Oct-93	4.00	2.03
05-Oct-93	4.40	**	11-Oct-93	4.20	2.16	20-Oct-93	N/A	N/A
AVERAGE:	4.52	2.83	AVERAGE:	4.45	2.40	AVERAGE:	4.56	2.68
STD:	0.43	0.98	STD:	0.35	0.56	STD:	0.37	0.68

* FISH WERE DEAD PRIOR TO MEASUREMENTS
** FISH WAS TOO DECOMPOSED TO TAKE AN ACTUAL WEIGHT
N/A FISH WAS NOT COLLECTED

Table 25. Fish Length and Weight Measurements at Termination of Microcosm Study, (Cont'd)

TANK 4			TANK 5			TANK 6		
DATE	STANDARD LENGTH (cm)	WET WEIGHT (g)	DATE	STANDARD LENGTH (cm)	WET WEIGHT (g)	DATE	STANDARD LENGTH (cm)	WET WEIGHT (g)
18-Oct-93	5.60	5.56	15-Oct-93	4.90	3.12	19-Oct-93	4.60	2.52
18-Oct-93	4.30	2.27	15-Oct-93	4.40	2.41	19-Oct-93	4.70	2.75
18-Oct-93	4.60	2.86	15-Oct-93	4.40	2.22	19-Oct-93	4.60	2.32
18-Oct-93	4.40	2.35	15-Oct-93	4.70	2.89	19-Oct-93	4.10	1.80
18-Oct-93	4.50	2.64	15-Oct-93	4.60	2.46	19-Oct-93	4.50	2.49
18-Oct-93	4.60	2.81	15-Oct-93	4.50	2.47	19-Oct-93	4.60	2.60
18-Oct-93	4.60	2.69	15-Oct-93	4.60	2.38	19-Oct-93	4.40	2.39
18-Oct-93	4.50	2.34	15-Oct-93	4.70	2.97	19-Oct-93	4.60	2.75
18-Oct-93	5.40	4.03	15-Oct-93	5.20	3.41	19-Oct-93	4.60	2.75
18-Oct-93	4.50	2.27	15-Oct-93	4.20	1.91	19-Oct-93	5.40	4.39
18-Oct-93	4.10	2.15	15-Oct-93	4.40	2.14	19-Oct-93	4.50	2.43
18-Oct-93	4.50	2.43	15-Oct-93	5.00	3.10	19-Oct-93	4.40	2.36
18-Oct-93	5.30	4.01	15-Oct-93	4.40	2.23	19-Oct-93	4.70	2.96
18-Oct-93	4.40	2.43	15-Oct-93	4.50	2.24	19-Oct-93	4.40	2.14
18-Oct-93	4.10	2.15	15-Oct-93	4.60	2.44	19-Oct-93	4.20	2.06
18-Oct-93	4.40	2.43	15-Oct-93	4.70	2.65	19-Oct-93	4.10	1.93
18-Oct-93	4.50	2.36	15-Oct-93	4.40	1.61	19-Oct-93	4.60	2.69
18-Oct-93	5.20	3.07	15-Oct-93	4.60	2.64	19-Oct-93	4.40	2.13
18-Oct-93	4.50	2.51	15-Oct-93	4.60	2.42	19-Oct-93	4.50	2.27
18-Oct-93	4.00	1.78	15-Oct-93	4.50	2.17	19-Oct-93	4.10	1.96
AVERAGE:	4.60	2.76	AVERAGE:	4.60	2.50	AVERAGE:	4.51	2.48
STD:	0.44	0.87	STD:	0.23	0.42	STD:	0.29	0.55