

Toxicity of Anionic Polyacrylamide Formulations when Used for Erosion Control in Agriculture

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Addition of anionic polyacrylamide (PAM) to agricultural irrigation water can dramatically reduce erosion of soils. However, the toxicity of PAM to aquatic life, while often claimed to be low, has not been thoroughly evaluated. Five PAM formulations, including two oil-based products, one water-based product, one granular product and one tablet product, were evaluated for acute and/or chronic toxicity to five species commonly used for freshwater toxicity testing [*Hyalella azteca* (Saussure), *Chironomus dilutus* (Shobanov et al.), *Ceriodaphnia dubia* (Richard), *Pimephales promelas* (Rafinesque), and *Selenastrum capricornutum* (Printz)]. When applied as an oil-based product, acute toxicity was seen to four of the five species at concentrations less than the 10 mg/L that is often used for erosion control. Toxicity was diminished, but still remained, after passage of the irrigation water across an agricultural field, indicating a potential impact to nearby surface waters. Results from the non-oil-based products indicated minimal toxicity associated with PAM even at concentrations 10 times those used in agriculture when applied in the granular form, as a tablet, or in a water-based liquid. These data suggest that other agents in the oil-based products, such as surfactants or emulsifiers, rather than the PAM itself, contribute to the toxicity. Care is required in selecting an appropriate PAM formulation when the potential exists for entry of tailwater to nearby surface waters.

ANIONIC polyacrylamide polymers, when added to irrigation water, have been shown to be extremely effective at reducing soil erosion (see Sojka et al. [2007] for review). The PAM used in agriculture consists of high molecular weight polymers (12–15 megagrams per mole) containing >150,000 acrylamide monomer units. These long, linear, negatively-charged molecules stabilize the soil surface, inhibit resuspension of sediment, and flocculate those few particles that are resuspended. The PAM can be applied as a liquid metered into the irrigation water (typically at 1–10 mg/L), or as a solid tablet or granule placed in the bottom of the furrow. The solid forms dissolve in the water as the furrow stream passes over them. In most cases, the use of PAM results in a 75 to 95% reduction in the loss of soil via the runoff (Aase et al., 1998; Lentz and Sojka, 1994, 2000; Goodson et al., 2006).

Cationic and neutral PAM polymers, as well as the acrylamide monomer (a micro-contaminant found in PAM products), are all recognized to have significant toxicity issues associated with them, but the anionic forms used in agriculture are typically portrayed as safe for aquatic life. Several publications have discussed aquatic toxicity of anionic PAM (Barvenik, 1994; Seybold, 1994; Entry et al., 2002; Sojka et al., 2000, 2007). These papers have discounted its toxicity potential, based largely on theoretical grounds such as the expectation that toxicity would be mitigated by complexation with dissolved organic matter (Goodrich et al., 1991), because any PAM leaving the field would be rapidly adsorbed to soils (Lentz et al., 2002), because the size of the molecule would prevent passage across biological membranes (Stephens, 1991), or because of its use in food products and the low toxicity that implies. Yet despite these prior claims of low aquatic toxicity potential, there are remarkably little published quantitative data on toxicity thresholds for those

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Abbreviations: CI, confidence interval; EPA, U.S. Environmental Protection Agency; IC50, median inhibitory concentration; LC50, median lethal concentration; LOEC, lowest observed effects concentration; PAM, polyacrylamide; TSS, total suspended solids.

species commonly used in the United States for freshwater toxicity testing. Quantitative data are limited to a couple older publications with scant description of methods or vague toxicity endpoints (McCullister et al., 1965; Beim and Beim, 1994).

Definitive data on the aquatic toxicity of anionic PAM is desirable to ensure that its use to avoid the adverse effects of sedimentation on water quality does not, in itself, contribute to water quality degradation through acute toxicity. Moreover, it is important to determine if the use of PAM would cause a toxic response in any of the species normally used for regulatory monitoring purposes in waters downgradient of agricultural lands. Given the paucity of quantitative data confirming the presumption of the low toxicity of anionic PAM, experiments were conducted to test the toxicity of a variety of PAM formulations to species most commonly used for water and sediment toxicity testing. Laboratory exposures were conducted with solid form, oil-based and water-based PAM formulations to determine whether concentrations commonly used in agriculture present a risk of acute or chronic toxicity to the standard toxicity testing species of *Hyalella azteca*, *Chironomus dilutus* (formerly *C. tentans* Fabricius), *Ceriodaphnia dubia*, *Pimephales promelas*, and *Selenastrum capricornutum* (renamed *Pseudokirchneriella subcapitata* Korshikov). Finally, field trials with two oil-based PAM formulations were done to determine if passage of water over the length of the field would eliminate toxicity of PAM-treated irrigation water, by testing runoff samples with *C. dubia* and sediment samples with *H. azteca*.

Materials and Methods

Preparation of Polyacrylamide Solutions

Five PAM formulations were tested. First, a granular product was evaluated (Soilfloc 100D, Hydrosorb, Orange, CA). Unlike the other products evaluated, the granular material contains few ingredients other than PAM (97% purity). Second, a tablet product was evaluated, containing 40% PAM (Soilfloc Clearwater Tablet, Hydrosorb). Third, a liquid product consisting of 37% PAM in an oil-based emulsion was tested (Soilfloc 300E, Hydrosorb). Fourth, a similar oil-based product, but containing 50% PAM, was tested (Soilfix, Ciba, Basel, Switzerland). Finally, a water-based liquid formulation was evaluated (PAM25, Terawet, San Diego, CA). This product consists of 25% finely ground PAM particles (<150 μm), 65% water, and 10% unspecified humectants. Though the products varied in their PAM content, the data presented adjusts for these differences by expressing all toxicity results in terms of PAM concentration, since this is likely to be the defining variable in determining field application rates.

All products were first dissolved in water to create working stock solutions ranging from 500 to 1500 mg/L. The PAM was added to the water while the solution was stirred vigorously on a magnetic stirrer. Approximately 4 h was provided for dissolution of the granular PAM, and 1 h for the tablet PAM that had previously been ground to fine particles with a mortar and pestle. All other products were used within a few minutes of creating the stock solutions. The stock solutions were diluted

in the test water to achieve the intended nominal PAM concentrations. The test solution concentrations varied by a factor of two (e.g., 0.18, 0.37, 0.75, 1.5, 3, 6 mg/L PAM) with any given test employing seven to eight concentration steps. The range of concentrations tested usually spanned from 0.18 to 25 mg/L, though when preliminary data for particular products suggested less toxicity, a range of 1.5 to 100 mg/L was used. The test solutions in all tests were replaced daily during the course of the exposures with freshly prepared PAM solutions. Water samples were taken at the initiation of nearly all tests to analytically confirm the nominal PAM concentration.

Toxicity Testing

Hyalella azteca Tests

Tests with the freshwater amphipod, *H. azteca* were done at the University of California, Berkeley, and followed standard sediment-testing protocols (USEPA, 2000). When testing field-collected sediments, approximately 75 mL of sediment was placed in 400 mL glass beakers, creating a bed of sediment approximately 2 cm deep. Approximately 250 mL of overlying water was added with the appropriate PAM concentration. The test water consisted of deionized water reconstituted to moderately hard by addition of salts (USEPA, 2000). Ten individuals of *H. azteca* were added to each of five replicate beakers. The tests were maintained at 23°C, with a 16:8 light/dark cycle, and with addition of 1 mL yeast-cerophyll-trout food to each beaker daily. Conductivity, pH, alkalinity, and hardness were measured at the beginning and end of the test; temperature and dissolved oxygen were measured regularly during the test. After a 10-d exposure, the animals were sieved from the sediment with a 425- μm screen and survivors counted.

The toxicity of the various PAM formulations was evaluated using *H. azteca* in 96-h water-only exposures (i.e., no bed sediment in the exposure containers). For these tests the 400 mL beakers contained only the PAM solution at the appropriate concentration, and a small square of nylon screen to provide a substrate for the amphipods. There were three replicate beakers per concentration, each with 10 amphipods. The environmental conditions and feeding schedule were identical to those described for the sediment tests. The PAM solutions were renewed daily by replacing approximately 80% of the water with freshly prepared solutions. Survivors were enumerated after a 96-h exposure.

When conducting laboratory tests of the oil-based Soilfloc 300E, *H. azteca* median lethal concentration (LC50) determinations were made in parallel tests with and without the presence of sediment. Preliminary testing had indicated toxicity issues were of greatest concern with the oil-based products, thus it was important to establish if sediment, as would be present in field situations, would mitigate the toxicity. The treatments differed only in the presence of 75 mL of soil from Salinas, CA that had previously been determined to be nontoxic. Both tests with and without sediment used three replicate beakers at each of six concentration steps (0.18–6 mg/L PAM at 2 \times concentration steps). These tests were of 96-h duration with daily replacement of approximately 80% of the overlying PAM solutions.

Finally, a sediment toxicity test was done to determine if sediment previously exposed to PAM solutions retained any PAM residues capable of causing acute toxicity. In this experiment, 0.8-L solutions of Soilfloc 300E were prepared, ranging from 1.5 to 200 mg/L PAM. A lab control sediment from Salinas, CA, previously verified to be nontoxic to *Hyalella azteca*, was added to each PAM solution (270 g wet weight; approximately 150 g dry), and vigorously mixed on a platform shaker at 300 rpm for 2 h. The soil/water suspension was allowed to settle for 48 h, and the supernatant discarded. These sediments were tested using the 10-d *H. azteca* sediment testing protocol described above, except that three replicates per concentration were used to derive a concentration/response relationship. The overlying water was moderately hard water containing no PAM, and the only PAM exposure would have been through any residues of the compound remaining in the spiked sediments.

Chironomus dilutus Tests

The acute toxicity of PAM formulations to *C. dilutus* was performed at Southern Illinois University and evaluated using 96-h static toxicity tests with a 16:8 light/dark photoperiod (USEPA, 2000). Both water-only and sediment tests were performed for the oil-based Soilfloc 300E, while water-only tests were performed for the water-based and granular PAM. Each desired treatment concentration was made by mixing the PAM formulation stock into moderately hard reconstituted water. Ten third instar midge larvae were transferred to 1-L jars containing 800 mL of moderately hard reconstituted water. Because of the cannibalistic nature of the midge larvae, a fine layer of sand substrate (approximately 15 g) was added to each test beaker for the water-only tests. The sand substrate has been often used for testing of other contaminants and shown not to affect their toxicokinetics or toxicity (Lydy et al., 1990). Approximately 50 g (wet weight) of Salinas soil were placed into the sediment replicates for the Soilfloc 300E sediment test. Each exposure consisted of seven concentrations with three replicates at each concentration, and the tests were done at 23°C. Negative controls were conducted with each test. The organisms were fed 1 mL of a liquid Tetrafin (Tetra Holding, Blacksburg, VA) formulation (6 g/L) once a day. Daily one-third water changes were performed, replacing the removed water with freshly made test solutions. Water chemistry parameters including temperature, pH, conductivity, and dissolved oxygen were measured daily in the controls. After 96 h the tests were scored for survival.

Ceriodaphnia dubia Tests

The PAM toxicity to the cladoceran, *C. dubia*, was assessed at Pacific EcoRisk (Fairfield, CA) using EPA chronic testing protocols (USEPA, 2002a). Test water was a mixture of commercial spring waters (80% Arrowhead and 20% Evian). Ten replicates per concentration were used, with each replicate consisting of 15 mL of test solution in a 30-mL plastic cup, containing one neonate (<24-h old). The test was maintained at 25°C, with a 16:8 photoperiod. The original individual was transferred to fresh solution in a new cup every day, and fed with the alga *S. capricornutum* and yeast-cerophyll-trout food.

At each daily transfer, survival of the original individual was determined, as well as the number of offspring produced, and the test continued until 60% of the individuals in the lab control produced three broods of offspring (typically 6–8 d). Water quality measurements were taken at the beginning and end of each 24 h exposure period. The test endpoints were survival and reproductive output.

Field-collected water samples were tested using the same chronic, 6 to 8 d *C. dubia* test as described above for the PAM-spiked solutions, but were also tested with an acute, 48-h test with survival as the endpoint (USEPA, 2002b). Test conditions were identical to the chronic *C. dubia* except that temperature was 20°C, there were four replicates per test each with five neonates, and the animals were not fed during the test. The absence of food limits the duration of the acute test (48 h), but also avoids the possibility that food particles could reduce the bioavailability and toxicity of the toxicant.

Fathead Minnow Tests

Tests for toxicity of PAM to fathead minnows (*P. promelas*) followed standard protocols (USEPA, 2002a), and were performed by Pacific EcoRisk. Four replicates at each concentration step were used, with each replicate consisting of 400 mL of moderately hard water in a 600-mL glass beaker. Ten larval fathead minnows (<48-h old) were added to each beaker, and the beakers held at 25°C, with a 16:8 photoperiod, and with twice daily additions of brine shrimp nauplii. Approximately 80% replacement of the PAM solutions were made daily, and water quality measurements were taken at the beginning and end of each 24 h period. After a 7-d exposure, survivors were counted, and the fish were then dried at 100°C overnight and weighed. The total weight was divided by the number of initial fish per replicate (10) to determine a biomass value (as per EPA protocol).

Selenastrum capricornutum Tests

Selenastrum capricornutum testing was done by Pacific EcoRisk. Test water consisted of 0.45- μ m filtered deionized water amended with nutrients following EPA guidelines (USEPA, 2002b). Four replicates were used at each PAM concentration, with each consisting of a 250-mL Erlenmeyer flask containing 100 mL of test solution. An additional replicate at each concentration was established for water quality measurements during the test and at termination. Each flask was inoculated with *S. capricornutum* to an initial cell density of 10,000 cells/mL. The flasks were held at 25°C under continuous cool-white fluorescent lights, and gently shaken a minimum of three times per day. After 96 h the algal cell density was determined by spectrophotometric analysis, except in tests with the water-based PAM when the cloudiness of the solutions at higher concentrations required counting under a microscope using a hemacytometer slide.

Toxicity Test Data Analysis

The probit LC50 in the *H. azteca* test was determined using Toxcalc software (Tidepool Scientific, 1994). Log-probit analysis was used to estimate the LC50 toxic endpoint concentrations for *C. dilutus* using SAS 9.1 software (SAS Institute,

2008). Toxicity statistics for the *C. dubia*, fathead minnow, and *S. capricornutum* were determined using CETIS software (Tidepool Scientific, 2007). Dunnett's multiple comparison test was used to identify those concentration steps at which the response was different than the control (e.g., lowest observed effects concentration [LOEC]).

When testing field-collected samples for acute toxicity to *C. dubia*, differences in survival relative to the lab control were tested by the equal variance *t* test if parametric assumptions were met, or by the Wilcoxon rank sum two-sample test if they were not. The *C. dubia* chronic test used a Fisher exact test for the survival endpoint, and either an equal variance *t* test or Wilcoxon rank sum two-sample test for the reproductive endpoint. Differences in *H. azteca* survival between the sediments from the various field plots were tested by equal variance *t* tests.

Field Studies

While the laboratory tests were useful in determining if the various PAM formulations could cause aquatic toxicity at the concentrations at which they are applied for erosion control, these tests would not incorporate any change in toxicity that could occur as the irrigation water flowed down the furrows and off the field. Since it is the toxicity of the tailwater and its effects on nearby surface water that is of primary concern, field trials were performed to evaluate runoff quality as a function of the PAM formulation applied.

Trials were conducted at the U.S. Department of Agriculture's Spence Research Farm in Salinas, CA. Soil type was a Chualar sandy loam (fine-loamy, mixed, superactive, thermic Typic Argixerolls). The field had been planted in lettuce (*Lactuca sativa* L.), though the crop had not yet germinated. Four adjacent plots were established, each 140 m long, with 26 1-m wide beds. The field was sprinkler irrigated using groundwater, with a conductivity of 530 $\mu\text{S}/\text{cm}$ and a sodium adsorption ratio of 2. No pesticides were used in the current crop cycle, and at least 9 mo would have elapsed since any previous pesticide use on prior crops grown on the plots.

An initial irrigation in all plots was done without PAM to establish the absence of sediment or runoff toxicity in all plots. Water application rate in this and subsequent irrigations was approximately 1.5 cm/h for the 3 to 4 h duration of the irrigation. Following this pretreatment irrigation, two more irrigations were made with PAM continuously applied in the irrigation water at a concentration of 5 mg/L, with approximately 4 d separating all the irrigations. One plot was allocated for use of water-based PAM25, and it received this product in both of the two PAM irrigations. Two plots were allocated for oil-based PAM products. They both received Soilfix in the first PAM irrigation, and they both received Soilfloc 300E in the second PAM irrigation. Finally, one plot was a no-PAM control that received unamended groundwater in all irrigations. This approach yielded four treatments (three with PAM, one without), each tested in two replicate plots.

Water samples were taken from the incoming sprinkler irrigation water after addition of PAM and from the tailwater at the foot of the furrows. The irrigation events lasted 3 to 4 h, during which

three samples of the incoming water to each plot and the runoff from each plot were collected in 4-L glass bottles at approximately hourly intervals. Samples collected in the three intervals during an irrigation were composited to create the sample used for analysis. A portion of the composited runoff samples slated for PAM analysis were centrifuged (3800 \times g, 10 min) within 4 h of collection to remove suspended sediment and stabilize dissolved PAM concentrations (Lentz et al., 1996). The supernatant was then held for PAM analysis. The remaining uncentrifuged composite sample was held at 4°C and allowed to settle overnight, after which the overlying water was set aside for *C. dubia* acute and chronic toxicity testing initiated within 36 h of sample collection. The settling step was done to reduce the high concentration of suspended sediment that would have made it difficult or impossible to observe performance of the test organisms.

Though the primary emphasis of this study was not on efficacy of the various PAM formulations in reducing sprinkler irrigation-induced erosion, runoff samples for total suspended solids (TSS) analysis were collected at approximately 30-min intervals throughout the irrigations, yielding four to six samples for each plot in each irrigation event. These samples were processed by filtering the water on a tared 934-AH glass fiber filter (Whatman, Florham Park, NJ), and then the samples were weighed after drying overnight at approximately 105°C.

Bed sediment deposited from runoff water at the foot of each plot was collected at the conclusion of each irrigation event. The upper 1 cm of material was skimmed from the deposit with a stainless steel scoop, compositing approximately 20 such scoops in to a single sample. Bed sediment samples were held at 4°C and used for *H. azteca* toxicity testing, following the general procedures for a 10-d sediment test as described above.

Polyacrylamide Analyses

In nearly all the toxicity tests with lab-prepared PAM solutions, an aliquot of the 6 mg/L concentration solution was set aside for analytical chemistry to determine if the actual PAM concentration was comparable to the nominal concentration. In addition, PAM-treated irrigation water applied to the plots during the field trial, and the runoff leaving the plots, were analyzed for PAM concentration using the composite samples described above.

Analysis was conducted at U.S. Department of Agriculture-Agricultural Research Service Northwest Irrigation and Soils Research Laboratory in Kimberly, ID. Dissolved PAM concentrations in irrigation and laboratory water samples were determined using size exclusion chromatography (Beazley, 1985; Lu et al., 2003) on a Waters (Milford, MA) 1525 high performance liquid chromatograph (HPLC) equipped with a 717 autosampler and 2487 UV/visible absorbance detector. A 750 μL volume of the water sample was mixed with 250 μL 0.2 M KH_2PO_4 solution and placed in a 1000- μL autosampler vial. This sample was eluted with an aqueous solution of 0.05 M KH_2PO_4 through a TSK-Gel GMPWXL polymeric gel column (Tosoh Biosep Corp., Montgomeryville, PA), which separates the PAM from interfering solutes. Quantitation was done by UV detection at 195 nm.

Results

Confirmation of Nominal Concentrations

During most of the toxicity tests the nominal PAM concentration was confirmed by size exclusion chromatography. Arbitrarily, the 6 mg/L concentration step was used for this purpose, and immediately after preparation of the solution, an aliquot was set aside for analysis of PAM concentration. Samples from 15 of the 18 toxicity tests were evaluated in this manner.

Actual PAM concentrations were usually (73% of the occasions) slightly less than nominal values. However, deviations from nominal concentrations were never unacceptably large. The difference from nominal values ranged from 2 to 49%, with a median difference of 12%. Therefore, all data reported below, based on the nominal concentrations, is believed to accurately reflect actual concentrations achieved.

Granular Polyacrylamide

All five test species were exposed to granular PAM dissolved in test water at concentrations up to 100 mg/L (Table 1). *Hyalella azteca* and *C. dilutus* showed no evidence of toxicity at the highest concentration used, with 93 and 100% survival at 100 mg/L for *H. azteca* and *C. dilutus*, respectively. Thus, the LC50s for these species could not be determined, but were in excess of 100 mg/L.

Chironomus dubia showed no statistically significant reduction in survival relative to the control up to 25 mg/L, but there was no survival in the 50 and 100 mg/L treatments. The LC50 was calculated as 28.7 mg/L. Reproductive output was reduced at every concentration tested (1.6 mg/L through 100 mg/L), with the inhibitory concentration for a 50% reduction in reproduction (IC50) of 5.1 mg/L.

Fathead minnows showed no indication of granular PAM toxicity, with 95% survival at the highest test concentration of 100 mg/L, compared to 100% survival in the controls. The LC50, therefore, could not be determined but was in excess of 100 mg/L. Similarly, the biomass IC50 was also in excess of 100 mg/L as the biomass at this PAM concentration (0.33 mg) was not significantly different than that of the control (0.35 mg).

Finally, *S. capricornutum* showed no significant reduction in cell density at any concentration, with the density at 100 mg/L 89% that of the control. Thus, the IC50 was in excess of 100 mg/L.

The PAM concentrations used for erosion control are typically in the range of 1 to 10 mg/L (Sojka et al., 2007). Therefore, even if these concentrations were maintained for an extended period (4–8 d in the various toxicity tests employed) most of the lethal or sublethal endpoints indicated no potential for toxicity at concentrations at least 10 times higher than those likely to be used. *Ceriodaphnia dubia* was the most sensitive to granular PAM, with acute toxicity appearing at a concentration at least three times that likely to be used, but with reproductive impairment possible within the range of concentrations employed in agriculture.

Tablet Polyacrylamide

Only *H. azteca* was tested with the tablet formulation. The species showed no evidence of toxicity with 97% survival in the

highest concentration tested, thus its LC50 exceeds 100 mg/L PAM. Control performance in the same test was 93% survival.

Soilfloc Oil-based Polyacrylamide

The Soilfloc 300E product exhibited toxicity to most of the test species at concentrations within or below the range of likely use (Table 2). Toxicity to *H. azteca* in a water-only exposure was evaluated in two independent tests, using different batches of product purchased in different regions of California on two separate occasions. The LC50 was 2.1 mg/L PAM in the first trial, and 0.8 mg/L in the second trial. We view this two-fold variation to be within the normal range of variation of multiple tests. Toxicity to *C. dilutus* in water-only exposure was comparable to that of *H. azteca*, with an LC50 of 3.0 mg/L PAM.

Ceriodaphnia dubia was the most sensitive of all test species to the oil-based Soilfloc product, with an LC50 of 0.3 mg/L and with complete mortality observed at concentrations of 0.75 mg/L or greater. Reproductive output was diminished by 24% at the lowest concentration tested of 0.18 mg/L, and the IC50 was determined to be 0.25 mg/L.

Fathead minnows showed statistically significant mortality at 1.5 mg/L, with an LC50 of 16.6 mg/L. Biomass was significantly reduced (47% reduction) at the highest concentration tested of 25 mg/L, but the IC50 was undeterminable and in excess of this concentration.

Selenastrum capricornutum showed no reduction in cell density at the highest concentration tested (25 mg/L PAM).

Hyalella azteca and *C. dilutus* were also tested with a layer of soil from a Salinas, CA farm in the exposure beakers to establish if bed sediment, as would normally be present in a field situation, altered the toxicity of PAM in comparison to all tests discussed above done in water-only systems. The sediment tests were done concurrently with the water-only exposures to the Soilfloc 300E product, and differed only in the presence of the sediment. The presence of sediment appeared to slightly increase the toxicity of oil-based PAM to *H. azteca*, with an LC50 of 0.48 mg/L (95% confidence interval (CI) = 0.37–0.58 mg/L; LOEC = 0.37 mg/L) with sediment, compared to 2.1 mg/L (CI = 1.2–2.8 mg/L; LOEC = 0.75 mg/L) without sediment. Sediment reduced the toxicity of oil-based PAM to *C. dilutus*, with an LC50 of 6.6 mg/L (CI = 4.9–8.8 mg/L; LOEC = 6.0 mg/L) with sediment, compared to 3.0 mg/L (CI = 2.4–3.7 mg/L; LOEC = 3.0 mg/L) without sediment.

Finally, *H. azteca* was tested with sediment that had previously been exposed to Soilfloc oil-based PAM concentrations of 1.5 to 200 mg/L, though the sediment was tested after removing the overlying PAM solution and using only lab water for the 10-d duration of the test. There was evidence of toxicity due to PAM residues in the sediment only at the highest PAM concentration (200 mg/L). Control survival (no PAM) was 98%, and survival in the 100 mg/L treatment was 97%. However survival decreased to 57% in the 200 mg/L treatment, though this concentration is at least 20 times higher than is likely to be used in agriculture.

Table 1. Toxicity of granular polyacrylamide (PAM) in water-only exposures with the five species tested and the exposure durations shown. Concentrations in mg/L PAM. The 95% confidence interval of the point estimates are shown in parentheses. IC50s refer to the concentrations causing 50% reduction in reproductive output (*C. dubia*), biomass (fathead minnow), or cell density (*S. capricornutum*). Lowest observed effects concentrations are shown for survival (LOEC_s), reproduction (LOEC_r), biomass (LOEC_b), or cell density (LOEC_d). Control performance is shown to help assess the validity of the tests.

	<i>Hyalella azteca</i> (96 h)	<i>Chironomus dilutus</i> (96 h)	<i>Ceriodaphnia dubia</i> (6–8 d)	Fathead minnow (7 d)	<i>Selenastrum capricornutum</i> (96 h)
Granular PAM	LC50 > 100 LOEC _s > 100	LC50 > 100 LOEC _s > 100	LC50 = 28.7 (23.5–35.1) LOEC _s = 50 IC50 = 5.1 (4.7–5.6) LOEC _r = 1.6	LC50 > 100 LOEC _s > 100 IC50 > 100 LOEC _b > 100	IC50 > 100 LOEC _d > 100
Control	97% survival	96% survival	100% survival 24.6 neonates per female	100% survival 0.35 mg biomass	2.820,000 cells/ml

Soilfix Oil-based Polyacrylamide

The Soilfix product was only evaluated with *H. azteca* in a 96-h water exposure. The LC50 was found to be 9.1 mg/L PAM (CI = 6.9–12.4 mg/L), a value indicating less toxicity than the other oil-based product (0.8–2.1 mg/L Soilfloc PAM), but still within the range of concentrations likely to be applied to agricultural fields. The LOEC was 6 mg/L.

Up to this point all toxicity data have been expressed on the basis of concentration of active ingredient (PAM) to allow comparisons between the diverse formulations evaluated and because a target PAM concentration would be the defining parameter in dictating the amount of product applied in actual use. However, when comparing the oil-based products that vary in PAM content, it may be helpful to consider toxicity in terms of the concentration of formulated product, particularly if a product constituent other than PAM is the toxic agent. When toxicity is expressed in this manner, the LC50 of the Soilfix product (50% PAM) equates to 18.2 mg/L product, and that of the Soilfloc product (37% PAM) ranges from 2.2 to 5.7 mg/L product.

PAM25 Water-based Polyacrylamide

The water-based PAM formulation showed very limited evidence of toxicity up to concentrations of 100 mg/L PAM (Table 3). The *H. azteca* LC50 was in excess of 100 mg/L with 77% survival at that concentration. Similarly, *C. dilutus* survival at the highest concentration used of 100 mg/L was 68%, indicating the LC50 was at a still higher concentration.

There was complete survival of *C. dubia* at the highest concentration, though with a statistically significant, but modest, 25% reproductive impairment at 100 mg/L. Fathead minnows showed good survival (95%) at 100 mg/L, and a statistically significant, but minimal, 16% reduction in biomass at this concentration.

Only *S. capricornutum* showed other than minor effects over the range of PAM concentrations tested. There was a significant reduction in cell density at all concentration steps equal to or above 6 mg/L, and the IC50 was 14.6 mg/L PAM.

Field Trials

Field trials in Salinas, CA provided an opportunity for comparison of the toxicity and effectiveness of the two oil-based products (Soilfix, Soilfloc 300E) and the water-based PAM25, all added to irrigation water to attain a PAM concentration of 5 mg/L. Size exclusion chromatography of the composite samples taken throughout the irrigations indicated the nominal concentration of injected PAM was achieved reasonably well. Actual concentrations of the nominal 5 mg/L treatments were 5.1 (for Soilfix), 3.9 (for Soilfloc), and 5.5 and 6.3 (for the two replicate treatments of PAM25). There was some adsorption of PAM to the soils as the water moved down the furrows, but approximately half of the original PAM concentration remained in the tail-water. Average concentration reductions were 25, 54, and 49% for the Soilfix, Soilfloc, and PAM25 treatments, respectively.

Before any use of PAM on the field a sprinkler irrigation with unamended water (groundwater) was done to be certain there

Table 2. Toxicity of oil-based Soilfloc 300E polyacrylamide (PAM) in water-only exposures with the five species tested and the exposure durations shown. Concentrations in mg/L PAM. The 95% confidence interval of the point estimates are shown in parentheses. IC50s refer to the concentrations causing 50% reduction in reproductive output (*C. dubia*), biomass (fathead minnow) or cell density (*S. capricornutum*). Lowest observed effects concentrations are shown for survival (LOEC_s), reproduction (LOEC_r), biomass (LOEC_b), or cell density (LOEC_d). Control performance is shown to help assess the validity of the tests.

	<i>Hyalella azteca</i> (96 h)	<i>Chironomus dilutus</i> (96 h)	<i>Ceriodaphnia dubia</i> (6–8 d)	Fathead minnow (7 d)	<i>Selenastrum capricornutum</i> (96 h)
Soilfloc PAM	First trial LC50 = 2.1 (1.2–2.8) LOEC _s = 0.75	LC50 = 3.0 (2.4–3.7) LOEC _s = 3.0	LC50 = 0.30 (0.25–0.36) LOEC _s = 0.37 IC50 = 0.25 (0.22–0.27) LOEC _r = 0.18	LC50 = 16.6 (10.6–31.9) LOEC _s = 1.5 IC50 > 25 LOEC _b > 25	IC50 > 25 LOEC _d > 25
Second trial	LC50 = 0.8 (0.5–1.1) LOEC _s = 0.37				
Control	83–93% survival	100% survival	100% survival 19.0 neonates per female	100% survival 0.49 mg biomass	802,000 cells/mL

Table 3. Toxicity of water-based polyacrylamide25 (PAM25) in water-only exposures with the five species tested, and the exposure durations shown. Concentrations in mg/L PAM. The 95% confidence interval of the point estimates are shown in parentheses. IC50s refer to the concentrations causing 50% reduction in reproductive output (*C. dubia*), biomass (fathead minnow) or cell density (*S. capricornutum*). Lowest observed effects concentrations are shown for survival (LOEC_s), reproduction (LOEC_r), biomass (LOEC_b), or cell density (LOEC_d). Control performance is shown to help assess the validity of the tests.

	<i>Hyalella azteca</i> (96 h)	<i>Chironomus dilutus</i> (96 h)	<i>Ceriodaphnia dubia</i> (6–8 d)	Fathead minnow (7 d)	<i>Selenastrum capricornutum</i> (96 h)
Water-based PAM	LC50 > 100 LOEC _s = 100	LC50 > 100 LOEC _s = 100	LC50 > 100 LOEC _s > 100 IC50 > 100 LOEC _r = 100	LC50 > 100 LOECs > 100 IC50 > 100 LOEC _b = 100	IC50 = 14.6 (10.5–18.2) LOEC _d = 6.3
Control	98% survival	96% survival	100% survival 25.6 neonates per female	100% survival 0.37 mg biomass	3,720,000 cells/mL

was no toxicity in the runoff due to substances that may have been left in the soil due to uses of the field in past years. Runoff from all plots in this preliminary irrigation showed 100% survival of *C. dubia* in both the acute (48 h) and chronic (6–8 d) test. A single plot (one later used as a no-PAM control plot) showed a statistically significant but slight reproductive impairment, with production of neonates only 82% that of the lab control water.

When testing the various PAM formulations, each of the four treatments (three with PAM, one without) was tested in two replicate plots. Water samples were taken from the incoming sprinkler irrigation water after addition of PAM and from the tailwater at the foot of the furrows, and tested by both acute and chronic tests with *C. dubia*. Water samples from the two plots that received no PAM caused no mortality or reproductive impairment to *C. dubia*, either in the irrigation water or tailwater (Table 4). Similarly, samples from the two plots that received water-based PAM25 showed 100% survival and reproductive output slightly greater than that of the lab control water.

However, toxicity was apparent in water samples taken from the oil-based PAM plots, particularly those that received Soilfloc 300E. Only 23% of the *C. dubia* individuals survived a 48-h exposure to the incoming irrigation water, and none survived a 6 to 8 d exposure. There was also no production of neonates when the irrigation water was tested. There was some improvement in quality of the water as it moved down the furrows, with acute toxicity of tailwater evident in only one of the two Soilfloc plots, but in the chronic tests of the tailwater there was no *C. dubia* survival or reproduction.

The other oil-based product, Soilfix, caused no acute mortality in either the sprinkler irrigation water or tailwater, though the organisms exposed to the irrigation water were not swimming normally, and largely lay on the bottom of the exposure container. This response was not seen in the tailwater samples. Nevertheless, tailwater from one of the two Soilfix plots diminished reproductive output, as did the incoming irrigation water.

Sediment samples were taken from the tailwater ditch at the foot of each plot, and tested for 10-d survival of *H. azteca* (Table 5). After a preliminary irrigation, before any PAM use, sediments from all plots caused no acute mortality, with survival rates ranging from 88 to 96%, comparable to the control at 94%. In the two subsequent irrigations, the control plot that received no PAM treatment continued to show good survival of *H. azteca* (86–90%). There was statistically significant mortality in both of the Soilfloc 300E plots (54 and 70% survival),

one of the Soilfix plots (74% survival), and one of the PAM25 plots (80% survival). Toxicity in both the Soilfloc plots suggests further evaluation of sediment toxicity from this product would be warranted. Results are less clear for Soilfix and PAM25, both because of the low magnitude of mortality and the fact that toxicity was not seen in both replicates.

Total suspended solid samples were taken from the tailwater throughout all irrigation events. In the two plots that received no PAM, the tailwater contained mean TSS concentrations over the course of an irrigation event of 333 and 560 mg/L (Fig. 1). All PAM treatments, regardless of formulation, substantially reduced the suspended sediment load with average TSS concentrations throughout the irrigation of typically about 100 mg/L.

Discussion

Our results indicate that the toxicity of anionic PAM products to aquatic life varies as a function of product formulation. In tests with the granular product that is nearly pure PAM, four of the five species tested showed no evidence of toxicity at even the highest concentration tested of 100 mg/L. Only *C. dubia* showed an effect from granular PAM, with a reproductive IC50 of 5 mg/L and a LC50 of 29 mg/L. It is difficult to compare these toxicity thresholds with the concentrations that may be achieved in agricultural use. The granular product would typically be spread on the ground at the head of the furrow so that the PAM could dissolve in to the overlying irrigation water as it passed, but this procedure does not allow precise control of the concentration achieved. If it is assumed sufficient granular material is used to achieve the same PAM concentration desired when using liquid formulations (1–10 mg/L PAM), then it appears no acute toxicity to any of the test species would be expected, and at most there may be impairment of *C. dubia* reproductive ability within this concentration range.

The low toxicity of the tablet and water-based product further supports the presumed low toxicity of PAM. When the PAM was provided in a tablet, there was no indication of toxicity up to at least 100 mg/L to the one species tested (*H. azteca*). In a water-based form, there was no indication of toxicity to most species for all but the highest concentration tested of 100 mg/L. Only *S. capricornutum* showed appreciable effects, and even for this species, its IC50 of 14.6 mg/L slightly exceeded the maximum concentration likely to be applied to a field of 10 mg/L.

Table 4. Percent survival or reproductive output of *Ceriodaphnia dubia* exposed to runoff from a control treatment without polyacrylamide (PAM) and three treatments with various PAM formulations added to the irrigation water. The incoming irrigation water and the tailwater at the foot of the field were sampled in two replicate plots for each treatment. When both plots within a treatment shared a common water source, only one sample of the irrigation water was collected. Toxicity results statistically different from lab control water are indicated by bold type and an asterisk.

Endpoint	No PAM		Soilfloc 300E		Soilfix		PAM25		
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2	
Acute test (48 h) survival (percent of individuals)†									
Irrigation water	100	80		23*		100‡		100	100
Tailwater	95	80	33*	90	100	100	100	100	100
Chronic test (6–8 d) survival (percent of individuals)§									
Irrigation water	100	100		0*		70		100	100
Tailwater	100	100	0*	0*	100	100	100	100	100
Chronic test (6–8 d) reproductive output as percentage of neonates produced in lab control water¶									
Irrigation water	129	114		0*		5*		145	111
Tailwater	119	95	0*	0*	62*	91		133	128

† Lab control water survival in the acute test ranged from 95 to 100%.

‡ At 48 h the individuals in this test were alive, but were on the bottom of the container and not swimming normally.

§ Lab control water survival in the chronic test was always 100%.

¶ Lab control water reproductive output ranged from 15.4 to 21.2 neonates.

Table 5. Survival of *Hyalella azteca* in 10-d exposures to sediment collected at the foot of field plots from a control treatment without polyacrylamide (PAM) and three treatments with various PAM formulations, each with two replicate plots. Laboratory control sediments, tested concurrently with the toxicity tests from which the data are presented in the table, had survival of 86 to 94%. Results statistically different from lab control sediments as determined by equal variance t tests are indicated by bold type and an asterisk.

Endpoint	No PAM		Soilfloc 300E		Soilfix		PAM25	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Mean survival (%) and standard deviation	86 (± 9)	90 (± 7)	54* (± 25)	70* (± 22)	96 (± 5)	74* (± 17)	80* (± 10)	84 (± 11)

However, when PAM was provided in an oil-based formulation, a very different picture emerged. The concentration of the Soilfloc product that is likely to be applied exceeded the LC50 for all three of the invertebrates (*H. azteca*, *C. dilutus*, *C. dubia*). The fathead minnow LC50 was only slightly above the concentrations used, and its LOEC was within the typical concentration range. Only the alga, *S. capricornutum*, appeared relatively insensitive to the oil-based PAM products. Though only *H. azteca* was tested with the Soilfix oil-based product, the difference in toxicity between the two oil-based products was not great, suggesting the toxicity concerns may extend to oil-based PAM formulations in general. Since the granular, tablet, and water-based PAM proved to be relatively innocuous, the toxicity of these oil-based products must be due to the oil or other “inert” ingredients in the formulation. In addition to the oil, these products contain proprietary emulsifiers and surfactants.

Results of the field trial were consistent with the laboratory toxicity studies, indicating the oil-based PAM products have greater toxicity to aquatic life than the water-based formulation. But more importantly, the field trial allowed consideration of tailwater toxicity, in addition to that of the PAM solutions as applied. The toxicity of oil-based PAM did appear to be mitigated after passage of the water down the furrow, but the effect was insufficient to eliminate that toxicity. Tailwater coming off the plots still impaired *C. dubia* reproduction (both Soilfix and Soilfloc 300E) and caused acute and chronic mortality to that species (Soilfloc 300E).

A reduction in survival or impairment of one of the sublethal endpoints, has, to this point, been referred to as toxicity, but given the physical attributes of PAM solutions it is

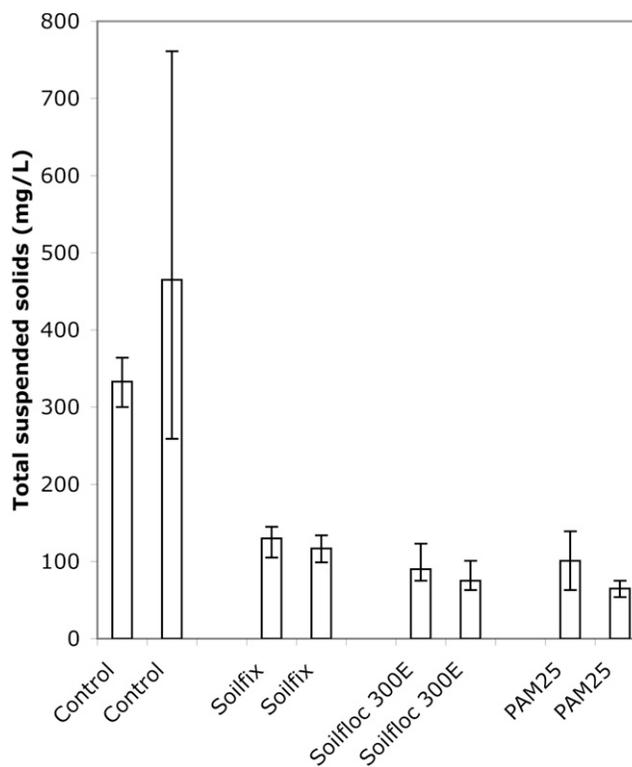


Fig. 1. Means and ranges of total suspended solids (TSS) concentrations in tailwater from plots receiving either unamended irrigation water or any of three polyacrylamide (PAM) formulations. Each bar represents a single irrigation, with four to six TSS samples collected.

possible that the effects may, in some cases, be physical rather than chemical. Polyacrylamide increases the viscosity of the solution, even to the point that when terminating the *H. azteca* tests, it was noted that passage of the PAM-containing water through a 425- μ m screen was noticeably slowed at 50 mg/L or greater. This high viscosity may have exerted a considerable stress on the test organisms, particularly small organisms that swim in the water column such as *C. dubia*. In addition, PAM acts as a flocculent, and caused notable flocculation of the yeast-cerophyll-trout food mixture used as food in some of the tests. This flocculation may reduce the cell density of algal species such as *S. capricornutum*. While it is not possible to be certain of the mechanism underlying the effects seen, it is suspected that physical impacts may be responsible for the effects on *C. dubia* seen with the granular PAM and the effects on *S. capricornutum* seen with the water-based PAM. Actual toxicity is more likely to be responsible for the effects seen with the oil-based products since they were noted at such low concentrations (as little as 0.3 mg/L), below concentrations at which obvious physical changes in the solution occur.

While these data do suggest potential effects of PAM usage on aquatic life, particularly for the oil-based products, it should be recognized that the laboratory testing represents worst-case conditions for several reasons. First, the duration of the water toxicity tests ranged from 2 to 7 d, and in most cases was dictated by standard testing protocols for the particular species tested. In many agricultural situations, exposure of aquatic life to PAM-containing tailwater may be considerably shorter if limited to a single irrigation event. In many areas it is customary for a grower to irrigate only a portion of the total acreage of a field, and then irrigate other portions in succession. Each individual irrigation event in a given portion of the field may last about 24 h, but several days may be required to complete the entire field. Thus, the duration of the laboratory tests could represent a worst-case condition when a downstream water body received PAM-containing tailwater over many days, but in many cases actual exposure is likely to be much shorter.

Second, it is also possible that the grower may not use PAM throughout the entire irrigation. The National Resource Conservation Service recommends a concentration of 10 mg/L PAM only as the water front advances down the furrow, with PAM addition terminated when runoff begins (Sojka et al., 2007). Thus, exposure of organisms in the receiving water body may be brief, though at relatively high concentrations. Other investigators have used a lower PAM concentration (e.g., 1–2.5 mg/L; McCutchan et al., 1993; Lentz et al., 2002), but have applied it continuously throughout the entire irrigation.

Third, there may be further reduction in toxicity as the tailwater moves from the edge of the field to the nearest surface water body. This study demonstrated some reduction as the water moved down 140-m furrows. (As the water was introduced by sprinklers throughout the field, not all the PAM-treated water would have traveled the full 140 m.) The length of travel necessary to completely eliminate acute toxicity, if even feasible, was not determined. It should be noted that while PAM adsorbs to soils and can be lost from tailwater in a relatively short distance

after leaving the field (Lentz et al., 2002), the toxicant in the oil-based products tested in this study appears to be an ingredient other than PAM, thus the rate of PAM adsorption to soils is not related to the rate at which toxicity is lost.

The data from this study are equivocal as to whether PAM treatment leaves toxic residues in the sediment. Theoretically, the fact that there is little or no desorption of PAM from soil particles (Nadler et al., 1992), would suggest minimal bioavailability. The field trials occasionally showed low to moderate *H. azteca* mortality in sediments from PAM-treated plots, but the effects were not consistent among replicates of a given PAM treatment. Also despite the lack of any toxicity in a pretreatment set of samples, it is possible that the toxicity was due to other substances in the soil remaining from uses of the field in past years. The laboratory trial controlled for such variables, and showed no residual toxicity in sediment that had been exposed to 100 mg/L PAM in the water, but there was some toxicity when exposed to 200 mg/L. These concentrations are far in excess of those used in agricultural systems, but it is difficult to equate the concentration in a static sediment:water laboratory suspension to field soils over which a PAM solution may flow for a considerable time.

Polyacrylamide can be extremely effective at mitigating off-field transport of suspended sediment, with benefits to the grower and nearby surface waters. There are a wide variety of formulations available, and many factors are likely to determine a grower's choice. Paramount among these is efficacy. The limited data collected under this study suggests the oil-based and water-based products are comparably effective in reducing erosion, and granular PAM is comparable in effectiveness to the liquid formulations in most instances (Sojka et al., 2007). Product cost, which is greater for the water-based product, is also likely to be a significant consideration. Ease of application is also an important factor, for example, granular or tablet formulations are useful in furrow irrigation but not practical in sprinkler irrigation. However, strictly from the standpoint of aquatic toxicity, it appears that use of the solid form and water-based PAM products are preferable over the oil-based products. The latter show evidence of acute and chronic toxicity to aquatic life at environmentally realistic concentrations due to non-PAM ingredients in their formulations, and passage of the water down the length of the furrow reduces but does not eliminate that toxicity. Use of solid and water-based forms of PAM appear to provide the environmental quality benefits of PAM, such as reduced sediment transport to surface waters (Lentz and Sojka, 2000) and reduced off-site movement of nutrients (Entry and Sojka, 2003; Lentz et al., 2001), pesticides (Singh et al., 1996), and microorganisms (Sojka and Entry, 2000), with minimal toxicity concerns associated with use of the products themselves.

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