United States
Environmental Protection
Agency

Environmental Monitoring Systems
Laboratory
Las Vegas NV 89114

Research and Development

EPA-600/S4-84-087 Jan. 1985

EPA

Project Summary

Toxicity Persistence in Prickly Pear Creek, Montana

John R. Baker and Barry P. Baldigo

Instream toxicity tests using the larval fathead minnow *Pimephales promelas* and the cladoceran *Ceriodaphnia reticulata* were conducted on Prickly Pear Creek, Montana, waters to study toxicity persistence in a stream. The toxicity source was Spring Creek, a tributary of Prickly Pear Creek. Gold mining tailing and settling ponds in the Spring Creek drainage release zinc, copper and cadmium to Prickly Pear Creek. Flow regimes, water quality, and biotic conditions were characterized in conjunction with toxicity testing. The study objectives were to: (1) develop a data base for validating a toxicity persistence model; (2) assess the applicability of data from the Prickly Pear Creek study relative to model assumptions; and (3) assess field techniques for acquiring model input data.

Toxicity to the test organisms was primarily due to zinc and copper in Spring Creek waters. Changes in Prickly Pear Creek toxicity downstream from the Spring Creek confluence were primarily due to dilution and complied with model assumptions. However, other unidentified toxicants were present in other tributary waters, and Spring Creek was not the sole source of toxicity in Prickly Pear Creek waters. *C. reticulata* was highly sensitive to toxicity in Spring Creek waters and provided model input data. *P. promelas* had a higher tolerance, and bioassay data from these organisms could not be used for model input. In the field, test organism nutritional problems were encountered using procedures described in bioassay protocols for both of these organisms, suggesting that either a quantitative food regime should be developed or a non-feeding test be used in the future.

This Project Summary was developed by EPA's Environmental Monitoring Systems Laboratory, Las Vegas, NV, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The U.S. Environmental Protection Agency's (EPA) Office of Water Regulations and Standards, Monitoring and Data Support Division (MDSD), is examining persistence and degradation rates of industrial and municipal toxic wastes discharged to streams. MDSD is seeking to identify methods most suitable for assessing instream persistence of whole effluent toxicity in receiving waters. Methods are required for site specific assessment of effluent toxicities, both acute and chronic, prior to discharge, at the discharge point, and at downstream locations where dilution, degradation, and partitioning to other compartments result in reduced toxicant concentrations. Particular interest centers on validation of toxicity models designed to predict instream toxicity persistence and validation of methods for acquiring input data for these models.

Instream toxicity testing has recently been conducted at several sites by EPA's Environmental Monitoring Systems Laboratory-Las Vegas (EMSL-LV) and EPA's Environmental Research Laboratory-Duluth. Model validation will be based on results from these investigations. A stream dilution model is presently being assessed. Model assumptions are: (1) toxic chemicals and toxicity itself follow conservative (not enhanced or degraded) mixing behavior; (2) physical, chemical, and biological interactions do not substantially alter toxicity at the point of complete mixing; and (3) variations in effluent toxicity are reflected in varying toxicity of the receiving waters and can be described by mass balance relationships.

In order to provide additional instream toxicity persistence data to MDSD, the EMSL-LV conducted a stream toxicity study in fall 1983 at Prickly Pear Creek, Montana. The objectives of this study were to: (1) develop a data base to be used for model validation; (2) assess the applicability of data from Prickly Pear Creek relative to model assumptions; and (3) assess field techniques for acquiring model input data. The study consisted of short-term acute and chronic toxicity tests using two test organisms, and stream survey characterization of flow regimes, water quality, and biotic conditions. Data for model validation are given in Appendices of the parent report.

Prickly Pear Creek forms its headwaters in the Elkhorn Mountains approximately 32 km southeast of Helena, Montana. The stream flows north for 64 km before entering Lake Helena and the Missouri River. Gold mining in the Corbin and Spring Creek drainage basin (draining into Prickly Pear Creek) began in the early 1860s. Tailing and settling ponds remain as prominant features within these drainages and release high concentrations of zinc, copper, and cadmium, which are carried into Prickly Pear Creek via Spring Creek. Areas along Prickly Pear Creek were also subjected to extensive mining operations in the early 1900s. Over 75 percent of Prickly Pear Creek was subjected to stream bed modifications and dredging during the mining process.

Methods

Spring Creek toxicity and instream toxicity persistence in Prickly Pear Creek were determined using static renewal bioassays designed to measure both acute and chronic toxicity. Test organisms were the cladoceran *Ceriodaphnia reticulata* and the larval fathead minnow *Pimephales promelas*. Toxicity tests were conducted with water collected from September 30 through October 9, 1983. Prickly Pear Creek stations were 011, a control station located 1.1 km upstream from the Spring Creek confluence; 013 and 014 were biological impact stations located 300 m and 3.8 km, respectively, downstream from the Spring Creek confluence; and 018, a biological recovery station, was located 12 km downstream from the Spring Creek confluence. Water quality and hydrological parameters were also measured as part of the study. For toxicity tests, control test waters were collected from the upstream Prickly Pear Creek station 011. These waters were diluted with Spring Creek water (the toxicity source) to obtain dilution test volumes with varying metals concentrations for comparison to ambient Prickly Pear Creek water toxicity.

Results and Discussion

Metal Concentrations

Spring Creek metal contributions caused significant increases in concentrations of metal in Prickly Pear Creek water. There was a consistent decline in downstream Prickly Pear Creek metal concentrations, with approximately a two-fold decrease between stations 013 and 018, due primarily to other tributary inflow dilution. Dissolved metal concentrations were low in all of these tributary streams.

Total recoverable cadmium, zinc, and copper concentrations in Spring Creek and Prickly Pear Creek samples consistently exceeded EPA-recommended acute criteria for aquatic life during the toxicity testing period. Concentrations of arsenic and lead were below the aquatic life criteria at all stations. Silver exceeded the acute criteria on October 6 at station 013 but was well below the acute criteria for all other dates and stations. Although cadmium exceeded the acute criteria, concentrations were below reported toxic levels for *C. reticulata* and larval fathead minnows. Toxicity in test organisms was attributed to zinc and copper, but *C. reticulata* bioassays indicated that another unidentified toxicant was present. Zinc and copper concentrations in Spring Creek were variable over the 10-day testing period with peak total recoverable concentrations on test days 1 and 5 (test numbers 0 through 9 refer to dates, September 30 - October 9). A small rainstorm occurred on September 30 and resulted in the October 1 (test 1) concentration peak. The cause of the October 5 peak in total recoverable concentrations was not determined.

Toxicity Tests

Ceriodaphnia reticulata

Acute and Chronic Toxicity in Dilution Tests—Dilution tests with Spring Creek water produced acute effects (LC-50s) to *C. reticulata* at dilution volumes varying from approximately 5 to 20 percent. There were no significant differences in Spring Creek acute toxicity in tests 2 through 5, 8, and 9, but toxicity was significantly higher in tests 1, 6, and 7. Higher toxicity in test 1 corresponded to high total recoverable and dissolved concentrations of zinc and copper in Spring Creek on October 1. An increase in total recoverable concentrations of these metals on October 5 had no apparent effect on toxicity. Metal concentrations generally declined on October 6 and 7 and the increase in toxicity in tests 6 and 7 was not due to an increase in any of the metals analyzed in this investigation. There was no mortality in the control organisms for tests 6 and 7, indicating that mortality in the Spring Creek dilution tests was due to toxicity. Chemical analyses for other parameters were not possible and the toxicant was not identified.

Chronic toxicity, resulting in reduced neonate production, was only evident in tests 5 through 8 and occurred at dilution volumes of 5 to 10 percent Spring Creek water. Reduced neonate production in tests 1 through 4 and in test 9 was in part or totally due to mortality, and chronic effects were not evident. Spring Creek toxicity, resulting in chronic effects, was greatest in tests 5 through 7 with significantly lower neonate production at dilution volumes of 5 percent Spring Creek water. Greater chronic toxicity in tests 6 and 7 was associated with greater acute toxicity, as previously stated, and was due to the unidentified toxicant. The increased toxicity resulting in chronic effects in test 5 was due to either the initial occurrence of the unidentified toxicant or to the increase in total recoverable zinc and copper on that day. Overall, the relationship between toxicity and metal concentrations was poor. This was primarily due to the occurrence of the unidentified toxicant.

Control water toxicity was also evident in tests 1 through 3 with significantly lower neonate production in the control tests relative to the culture water tests. Bioassays conducted on water collected from the tributary streams on October 16 revealed a potential source of control water toxicity due to Copper Creek inflow, located 100 m upstream from control station 011. Copper Creek water was chronically toxic, resulting in low neonate production, and therefore may have been a source of control water toxicity. A significant difference in neonate production was also found in test 5, but this was probably due to nutritional differences in the culture water and control treatments, and not to control water toxicity. The culture water supported high concentrations of algae (*Closterium*) and bacteria, and provided additional food for *C. reticulata* in the culture water treatments. This resulted in higher neonate production in the culture water treatments for almost all tests.

Downstream Station and Dilution Test Comparisons—Prickly Pear Creek was toxic to *C. reticulata* at the downstream stations. Toxicity in the Spring Creek dilution tests and in the downstream Prickly Pear Creek tests was compared to determine if downstream changes in toxicity were due strictly to inflow of Spring Creek water. Validity of test comparisons was based on dilution volumes of Spring Creek water in the dilution and downstream tests. Dilution volumes of Spring Creek water at the downstream stations 013, 014, and 018 were 17.3, 7.2, and 2.4 percent, respectively, and were similar to dilution volumes of Spring Creek water used in the *C. reticulata* dilution tests (20, 10, and 2.5 percent). Mortality in dilution and downstream tests having comparable Spring Creek dilution volumes showed a high degree of similarity. However, higher mortality in some downstream tests indicated that there was an additional downstream source of toxicity and that the toxicant may have been similar in nature to the unidentified toxicant in Spring Creek. Neonate production in dilution and downstream test comparisons also showed no significant difference in a majority of the tests. However, there was a trend for lower neonate production in the downstream tests in most tests. This trend in higher toxicity in the downstream tests resulted from either additional downstream sources of toxicity or downstream enhancement of Spring Creek toxicity.

Although one or both of the above processes may have occurred, differences in test comparisons were minimal and did not refute that variations in Prickly Pear Creek toxicity were primarily due to downstream dilution of the Spring Creek inflow. Downstream toxicity persistence, therefore, did appear to follow a conservative behavior.

Pimephales promelas

Larval fathead minnows were more tolerant to Spring Creek toxicity than were *C. reticulata*. Estimated LC-50s for fathead minnows were at control dilution volumes greater than 25 percent Spring Creek water, which were greater than dilution volumes found for downstream Prickly Pear Creek stations. This was also reflected in the downstream station tests which showed little or no mortality.

Fathead minnow LC-50s indicated that Spring Creek toxicity was highly variable for this species. Minimal mortality occurred in tests 2, 8, and 9, and acute effects were not evident for those days. There was a significant decline in toxicity in tests 6 and 7 indicating that the unidentified toxicant resulting in toxicity to *C. reticulata* was not at toxic concentrations for fathead minnows. Higher toxicity in tests 0, 1, and 5 did correspond to higher total recoverable concentrations of zinc and copper; however, a strong relationship for these metal concentrations and toxicity was not clearly evident.

Part of the variability found in the fathead minnow test was probably not inherently related to Spring Creek toxicity. High control mortality occurred after the third or fourth day and at test termination, mortality was greater than 30 percent in six of the 10 tests (0, 1, 2, 4, 7, and 8). High control mortalities are usually indicative of procedural problems; however, mortality declined in the lower dilution treatments with little or no mortality at either 12.5 or 25 percent in all tests. The consistent decline in lower dilution treatment mortality relative to high control mortality strongly suggested that Spring Creek water was ameliorating conditions in the control water. This mortality decline may have been due either to dilution of control water toxicity or to the addition of some factor enhancing survival. Control water toxicity was evident in *C. reticulata* bioassays; however, reconstituted water controls included in fathead minnow tests 6 and 7 suggested that control mortality was not due to toxicity.

The inherent growth variability in fathead minnows precluded demonstrating chronic effects. Growth was significantly increased with increased feeding in a separate feeding experiment, indicating test fish were probably underfed and that a quantitative food regime should be developed for future tests. Nevertheless, fathead minnows raised at the Las Vegas Laboratory from identical egg batches showed variations in length approaching 400 percent after 30 days. This kind of growth variability would highly influence test results and a nonfeeding lethality test may be more appropriate for field testing.

Stream Survey

Water Quality

Nonmetal water quality parameters measured in the stream survey did not reveal any other sources of toxicity or toxicants. Total organic carbon concentrations were low, ranging from 2 to 3 μg/l, and ammonia concentrations were below detection limits, indicatng little or no contributions from either septic tanks or domestic animals within the study area. Cyanide and chlorine were also below detection limits. Spring Creek ion concentrations were moderate, with a conductivity of 421 μmhos/cm, 2.7 times greater than at control station 011. Conductivity at station 013 was 226 μmhos/cm, but increased to 269 μmhos/cm at station 018 as a result of additional secondary inflow sources high in ion concentrations downstream from Spring Creek. This downstream increase was also reflected in alkalinity and hardness, which showed similar downstream trends. Turbidity in Spring Creek was higher than in Prickly Pear Creek; however, water clarity or suspended solids were not a water quality problem during this investigation. Temperature, dissolved oxygen and pH levels were typical of fall conditions for temperate streams and were indicative of good water quality.

Hydrology

Stream flow at the U.S. Geological Survey gaging station, located 2 km downstream from station 018, ranged from 35 to 42 cubic feet per second (cfs) during the toxicity testing period and was typical of seasonal low flows over the last 3 years. Peak flow on October 2 was due to a small storm event that occurred on September 30 and to snow melt from a storm that occurred on September 18.

Stream flow in Prickly Pear Creek increased from 11 cfs at station 011 to 37 cfs at station 018. Measured tributary inflows accounted for 62 percent of the increase in flow. Estimated unmeasured inflows between stations 013, 014, and 018 were approximately 5 and 8 cfs, respectively. There were no other major surface inflows and the majority of the unmeasured increase in flow was due to groundwater inputs.

The percent of Spring Creek water volume to the total water volume at downstream Prickly Pear Creek stations 013, 014, and 018 was 17.3, 7.2, and 2.4 percent, respectively, based on concentrations of Rhodamine WT injected into

Spring Creek on September 23. Dye retention time from the Spring Creek confluence to station 018 was just over 11 hours.

Biota

Salmonid fishes were abundant at all Prickly Pear Creek stations. However, there was a downstream shift in species abundance. Brook trout (*Salvelinus fontinalis*) was the only salmonid found at station 011. Both brook trout and rainbow trout (*Salmo gairdneri*) were abundant at stations 013 and 014, and the brown trout (*Salmo trutta*) also occurred with the other salmonid species at station 018. The species shift in salmonids was probably not due to metal toxicity from Spring Creek, but rather to the increased frequency of pool habitats downstream.

Previous investigations have shown major reductions in both macroinvertebrate and periphyton numbers and diversity in the Prickly Pear Creek impact zone (station 013) and a gradual downstream reoccurrence of these species between stations 014 and 018, the recovery zone. These studies were conducted in the summer. Quantitative analyses of periphyton and macroinvertebrate samples were not part of this investigation. A superficial examination of the macroinvertebrate samples at the time of collection indicated no evident reduction in either species types or species number in the impact zone. This lack of reduction may have been a physiological response to temperature and needs to be validated quantitatively. Water temperatures during this investigation were approximately 7°C compared to summer temperatures of 16 to 20°C.

Metals in Sediment and Tissue

Sediment metal concentrations at station 013 were approximately an order of magnitude higher than those found at the upstream station 011. Sediment metal concentrations at Spring Creek and station 013 were similar, indicating high sediment deposition from Spring Creek in the area of station 013. Sediment concentrations decreased downstream from station 013 and were four to five times lower at station 018. However, concentrations at station 018 were substantially higher than concentrations found at the control station 011, further demonstrating the extent of downstream impacts from Spring Creek. Sediment metals were a potential source of downstream toxicity. However, hydrological conditions during the testing period were very stable, and increased downstream water metal concentrations resulting from sediment resuspension probably did not occur or were very minimal. Sediment-water interactions were not determined in this study and should be investigated to determine the extent sediments act as a source of or sink for metals under various hydrological conditions in Prickly Pear Creek.

Tissue metal concentrations were highest in periphyton followed by macroinvertebrates and fish. Periphyton and macroinvertebrate tissue concentrations were substantially higher at station 013 and decreased downstream relative to ambient water and sediment concentrations. Metal uptake by periphyton and macroinvertebrates represented a potential metal sink; however, these organisms are also a source of metals when ingested by other organisms. Fish tissue metal concentrations were not exceptionally high and there was no substantial difference in tissue concentrations between stations. Previous investigations have found significantly higher tissue metal concentrations in most organs (kidneys, gills, brains, heart, and gonads) from fish collected in the impact areas of Prickly Pear Creek. However, muscle tissue did not have elevated metal concentrations. In this investigation, whole fish were used for tissue analyses and the inclusion of muscle tissue probably masked metal concentrations in the organs.

General Discussion

The results of the Prickly Pear Creek instream toxicity measurements (after mixing of the effluent), indicate such methods along with assessments of stream biota communities can provide a great deal of information about the nature and extent of effluent impacts on resident biota. These analyses aid in identifying needs for limiting effluent toxicity.

An important and often overlooked aspect of effluent and instream toxicity is the persistence of toxicity within a receiving system and the potential spatial extent and severity of impact to the biota. Obviously, pollutants that are rapidly degraded to nontoxic forms, lost to the atmosphere, or rendered unavailable through other processes pose far less threat to biota than the more persistent forms.

Conditions in Prickly Pear Creek were such that conservative behavior in toxicity was favored. Hydrological retention time from the Spring Creek confluence to the downstream Prickly Pear Creek stations was relatively short (11 hours) and would limit oxidation and reduction processes, especially at stream temperatures (7°C) found during this investigation. Suspended solids and organic compounds were also very low and toxicity was not highly influenced by particle adsorption or by complexing with organic compounds. Prickly Pear Creek was not acutely toxic to larval fathead minnows, and bioassay results were not applicable to testing the fate of Spring Creek toxicity. *C. reticulata* was more sensitive than fathead minnows and bioassay results did demonstrate conservative behavior in toxicity.

Toxicity found in both test organisms clearly paralleled past changes in native fish and macroinvertebrate communities attributed to toxicity. Distribution and abundance of fish and macroinvertebrates in Prickly Pear Creek have been well documented for summer conditions in previous investigations. These studies have shown that toxicity from Spring Creek has little or no effect on native fish, but a definite impact zone and recovery zone were found in Prickly Pear Creek relative to macroinvertebrate community diversity and species abundance. If fathead minnows and *C. reticulata* bioassays from this study were used to predict downstream biotic conditions in Prickly Pear Creek, identical impacts and/or zones would be designated. Therefore, it does appear that these bioassays reflect summer levels of toxicity affecting native fish and macroinvertebrate communities in this system.

The work reported here, addressing toxicity persistence in a receiving stream, represents an initial step toward field validation of procedures for establishing water quality based effluent limits using biological data. Although the primary source of metals to Prickly Pear Creek was a tributary stream rather than an effluent pipe, Spring Creek was treated as an effluent for which load limits and required reductions could be established and a permit issued. Data from this and other projects will provide information on the conservative (or nonconservative) nature of various types of pollutants in a range of receiving systems. Such case-history information will enable EPA's Office of Water Regulations and Standards to assess the validity of the mass balance modeling approach to predicting instream toxicity persistence. An eventual goal is to include in this testing all steps leading to, and including, the issuance of permits using biological data. Analyses to be included in these tests would consist of:

1. identification of water quality limited systems;
2. water body survey and assessment;
3. review and, if necessary, revision of designated uses;
4. establishment of appropriate criteria;
5. performance of waste load allocation; and
6. identification of control technology requirements.

These analyses, when completed, would result in assurance of a water quality based permit that would allow the water quality standard to be met. Issuance of the permit would be followed by monitoring to ensure water quality improvements are being achieved.

Conclusions

Metal concentrations in Prickly Pear Creek were significantly increased downstream from its tributary Spring Creek, which produced elevated levels due to gold mining tailing and settling ponds in the drainage basin. Concentrations of cadmium, zinc, and copper measured over a 10-day period exceeded EPA acute criteria for aquatic life at one or more of the downstream sampling stations in Prickly Pear Creek. Sediments were a potential downstream source of metals, but probably did not contribute to ambient water metal concentrations due to stable hydrological conditions. Elevated metal concentrations were the only water quality problems observed in Prickly Pear Creek during this investigation.

Spring Creek toxicity to test organisms (*C. reticulata* and *P. promelas*) was primarily due to zinc and copper. Other unidentified toxicants were present and Spring Creek was ot the only tributary serving as a source of toxicity for Prickly Pear Creek waters. Although there were additional sources, changes in toxicity (persistence) in Prickly Pear Creek were primarily due to downstream dilution of Spring Creek water. Therefore, Spring Creek toxicity did exhibit a conservative behavior in its downstream distribution in Prickly Pear Creek and complied with toxicity model assumptions.

Sensitivity of the two test organisms to toxicity in Spring Creek and Prickly Pear Creek was very different. *C. reticulata* was highly sensitive, and bioassay results were applicable in assessing toxicity persistence in Prickly Pear Creek. *P. promelas* had a higher tolerance and could not be used in assessing toxicity persistence. Although sensitivity of larval fathead minnows and the cladoceran was different, both appeared to be highly representative of toxic effects in Prickly Pear Creek native fish and macroinvertebrate communities found in previous studies.

Problems were encountered in the field bioassay procedures used for both organisms. These problems were related to the food regime used in each of the bioassays. Cerophyl proved to be a better food source than yeast in *C. reticulata* tests. Chronic toxicity was not measured in *P. promelas*, apparently because of underfeeding, and either a quantitative food regime should be developed for this test or a nonfeeding test should be used in future field testing.

John R. Baker and Barry P. Baldigo are with Lockheed Engineering & Management Services Co., Inc., Las Vegas, NV 89114.
***Wesley L. Kinney** is the EPA Project Officer (see below).*

The complete report, entitled "Toxicity Persistence in Prickly Pear Creek, Montana," (Order No. PB 85-137 149; Cost: $11.50, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

The EPA Project Officer can be contacted at:

Environmental Monitoring Systems Laboratory
U.S. Environmental Protection Agency
P.O. Box 15027
Las Vegas, NV 89114

☆U. S. GOVERNMENT PRINTING OFFICE:1985/559-111/10768