PROGRESS REPORT
(Quarters I and II)

BIOAVAILABILITY AND TOXICITY OF SILVER TO BENTHIC ORGANISMS IN FRESHWATER SYSTEMS CONTAINING SEDIMENTS OF DIFFERENT CHARACTERISTICS

to

National Association of Photographic Manufacturers
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by

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March 31, 1997

INTRODUCTION

Since the proposal for this project was submitted in early August, 1996, and approved by the National Association of Photographic Manufacturers (NAPM) for study starting on October 1, 1997, several changes have been discussed with members of the scientific panel within NAPM and with other national experts on silver and other heavy metals. Daniel Call had an opportunity to present the planned research at a meeting of renowned environmental chemists and toxicologists in early September, 1996, who had convened for a meeting on the development of national criteria for chemicals in sediment at the EPA Laboratory in Duluth, MN. This meeting was attended by several EPA-Duluth staff, including Gary Ankley, David Mount, and Russell Erickson; Mary Reilly of EPA-Washington, D.C.; Richard Swartz of EPA-Newport, OR; David Hansen and Walter Berry of EPA-Narragansett, RI; Dominic DiToro and Paul Paquin of HydroQual; and Herbert Allen of the University of Delaware. Based upon discussion following this presentation and a subsequent teleconference call that included Joe Gorsuch of Eastman Kodak Co., Dominic DiToro of HydroQual, Tom Purcell of NAPM, Dave Mount of EPA-Duluth, and David Hansen and Walter Berry of EPA-Narragansett, it was concluded that the most useful information to both the silver industry and the regulatory community would be achieved by modifying the Year I plan from that of the approved proposal. The major suggested changes were to focus on a single sediment, and to perform a definitive toxicity test with a single freshwater sediment, rather than to use the four-sediment matrix as originally proposed; and to increase the chemical analyses and resultant documentation of silver chemistry in this definitive test.

This progress report documents our efforts from October 1, 1996, through March 31, 1997. We presented our results to Joe Gorsuch and Ken Robillard in a half-day session on silver on March 26, 1997. Methods and results are presented by task.

I. Sediment Collection and Characterization

A. Preliminary Studies. Sediment was collected in the fall of 1996 from three lakes - West Bearskin Lake and Airport Pond, both from St. Louis County, MN, and Bond Lake from Douglas County, WI. The sediments were analyzed for percent dry weight and for AVS and TOC content. Bond Lake has a very sandy substrate, and had the lowest AVS and TOC levels (Table 1). West Bearskin Lake was intermediate in AVS and TOC, and Airport Pond was the highest for both parameters. Based upon the previously mentioned teleconference call, West Bearskin Lake sediment was selected as having the most desirable characteristics for use in the project, and this sediment was used in subsequent studies.

Table 1. Characteristics of sediment collected from three lakes in northern Wisconsin and Minnesota.

Lake	Mean Percent Dry Wt. (± s.d.)	Mean AVS (μmol/g) ± s.d.	Mean TOC (%) (± s.d.)
Airport Pond	45.8 (2.2)	52.7 (1.5)	1.9 (0.3)
Bond Lake	81.1 (0.9)	<0.3	0.2
West Bearskin Lake	57.4 (0.5)	2.4 (1.2)	1.1 (0.1)

II. Comparison of AVS and SEM Methodologies

West Bearskin Lake sediment was further characterized for its SEM metals by a conventional procedure which uses a "train" of glassware and extraction with 1M HCl. Two samples were run in duplicate, and the results are presented in Table 2. Zn was most abundant, followed in decreasing order by Ni, Cu, Pb and Cd for a ΣSEM of about 0.8 μmol/g. The "train" method was compared to a "jar" method at both 1M HCl and 1M HNO_3, as well as at 0.3 M HCl and 0.3 M HNO_3. The 0.3 M comparisons were made based upon the use of this concentration of HNO_3 with silver by John Mahony and colleagues at Manhattan College. The results of these comparisons are shown in Table 2. Higher AVS levels were obtained when using 1M HCl and the jar method as compared to 1M HCl and the train method. Concentrations of individual metals and the ΣSEM values were similar with use of either the train or jar procedure.

The use of 0.3M HNO_3 resulted in AVS values that were considerably lower than the corresponding train or jar procedure with 1M HCl. Values of ΣSEM were also reduced when extracted with 0.3M HNO_3 compared to 1M HCl, particularly Cu.

AVS and SEM analyses were performed following acid extraction at the 0.3M HNO_3 again, and at increased molar concentrations of HNO_3 (i.e., 0.6 and 1.0M). These extractions were performed using both the train and jar methods. SEM Cu and Zn were analyzed in addition to AVS. Extraction by 1M HCl was again performed for an additional comparison of results between acids. AVS measurements were higher using the jar procedure at an acid concentration of 1M, an observation that applied to both HCl and HNO_3 (Table 3). For concentrations of Cu, Zn and ΣCu + Zn SEM, the values were more comparable between the jar and train procedures using 1M HCl than with 1M HNO_3. The concentrations of Cu decreased when extracted with 0.6M HNO_3 in the train as compared to 1M HNO_3, and decreased further when extracted with

Table 2. Comparison of AVS and SEM concentrations determined by gas train and jar methods using 1M HCl and 0.3M HNO_3.

Sample ID	Method	SEM Values							AVS
		Ag (μmol/g)	Cd (μmol/g)	Cu (μmol/g)	Ni (μmol/g)	Pb (μmol/g)	Zn (μmol/g)	Σ SEM	Concs. (μmol/g)
WB 1	Train 1M HCl	<0.008	0.004	0.102	0.116	0.061	0.485	0.767	1.1
WB 1 Dup	Train 1M HCl	<0.007	0.002	0.108	0.117	0.059	0.494	0.780	1.1
WB 2	Train 1M HCl	<0.008	0.004	0.094	0.113	0.068	0.457	0.737	1.1
WB 2 Dup	Train 1M HCl	<0.008	0.004	0.103	0.124	0.079	0.514	0.825	1.1
WB 1	Jar 1M HCl	<0.006	0.004	0.109	0.117	0.072	0.482	0.784	2.9
WB 1 Dup	Jar 1M HCl	<0.007	0.004	0.116	0.130	0.077	0.534	0.861	2.2
WB 2	Jar 1M HCl	<0.007	0.003	0.105	0.122	0.074	0.496	0.801	2.5
WB 2 Dup	Jar 1M HCl	<0.007	0.003	0.127	0.149	0.080	0.543	0.901	2.3
WB 1	Train 0.3M HNO_3	<0.009	0.003	0.033	0.126	0.058	0.410	0.631	0.5
WB 1 Dup	Train 0.3M HNO_3	<0.009	0.002	0.032	0.116	0.048	0.403	0.602	0.6
WB 2	Train 0.3M HNO_3	<0.010	0.003	0.036	0.120	0.051	0.424	0.634	0.5
WB 2 Dup	Train 0.3M HNO_3	<0.011	0.003	0.042	0.124	0.055	0.461	0.685	0.8
WB 1	Jar 0.3M HNO_3	<0.006	0.003	0.044	0.115	0.048	0.391	0.601	0.6
WB 1 Dup	Jar 0.3M HNO_3	<0.006	0.002	0.040	0.119	0.048	0.404	0.613	1.4
WB 2	Jar 0.3M HNO_3	<0.006	0.002	0.049	0.118	0.053	0.448	0.670	0.8
WB 2 Rep	Jar 0.3M HNO_3	<0.006	0.002	0.050	0.116	0.053	0.433	0.655	1.0
WB 2 Dup	Jar 0.3M HNO_3	<0.006	0.002	0.049	0.116	0.054	0.445	0.665	0.4

Table 3. Comparison of AVS and SEM concentrations determined by gas train and jar methods using 1M HCl and 0.3M, 0.6M and 1.0M HNO_3.

Sample ID	Method	Cu (μmol/g)	Zn (μmol/g)	Σ SEM	AVS Concs. (μmol/g)
WB 1A	Jar 1M HCl	0.127	0.558	0.685	1.9
WB 2A	Jar 1M HCl	0.114	0.493	0.607	0.8
WB 1A	Train 1M HCl	0.116	0.629	0.745	0.8
WB 2A	Train 1M HCl	0.087	0.478	0.565	1.0
WB 1A	Jar 1M HNO_3	0.047	0.443	0.490	2.4
WB 2A	Jar 1M HNO_3	0.044	0.377	0.421	1.9
WB 1A	Train 1M HNO_3	0.131	0.579	0.710	<0.4
WB 2A	Train 1M HNO_3	0.113	0.506	0.619	0.3
WB 1A	Jar 0.6M HNO_3	0.055	0.544	0.599	0.9
WB 2A	Jar 0.6M HNO_3	0.042	0.465	0.507	1.2
WB 1A	Train 0.6M HNO_3	0.037	0.515	0.552	1.0
WB 2A	Train 0.6M HNO_3	0.037	0.454	0.491	1.1
WB 1A	Jar 0.3M HNO_3	0.028	0.471	0.499	0.7
WB 2A	Jar 0.3M HNO_3	0.027	0.414	0.441	0.9
WB 1A	Train 0.3M HNO_3	0.032	0.550	0.582	0.4
WB 2A	Train 0.3M HNO_3	0.029	0.436	0.465	0.5

0.3M HNO_3.

Upon reviewing the results from these experiments, we have decided to use the conventional train methodology with 1M HCl, as this procedure has yielded consistent AVS measurements and it extracts considerably more metal, particularly Cu and Pb, from the sediment. This method of measuring AVS and SEM also has been most widely used, and will provide data that can be readily compared to literature data from other studies using the same procedure.

III. Peeper Development and Silver Equilibration Studies

Studies have been conducted to date with peepers of two different designs, referred to as the "vial" and "acrylic" peepers in this report. Vial peepers consisted of 6 mL polyethylene vials with snap caps. The centers of the snap caps had been removed and a peeper membrane placed over the mouth of the vial prior to placing the cap on the vial. Acrylic peepers were rectangular blocks of acrylic plastic with 6 mL volume chambers machined out of the plastic. A thin acrylic cover with openings corresponding in dimensions to the openings of the peeper chambers was placed over the acrylic block containing the 6 mL chambers, and fastened tightly with screws. Acrylic peepers containing chambers of two different sizes were experimented with. Initial experiments were with acrylic peepers containing chambers of 1x1x6 cm dimensions. However, these peepers are too wide for deployment in 300-mL beakers. A second type contains chambers of 1x2x3 cm, and does fit into the 300-mL beakers. This newly designed peeper will be used in subsequent studies of this project.

In the first study using vial peepers, the peepers were assembled with deionized water in the chambers and they were placed into beakers containing dechlorinated laboratory water spiked with silver nitrate solution in deionized water. The exposure surface area of the peeper membrane was about 1.8 cm^2. The results of the equilibration studies are provided in Tables 4, 5 and 6. Each table indicates the length of time the peeper had been in the silver solution before the samples were collected, the silver concentration of the bulk solution and within the peeper chambers, and the percentage of silver in the peeper chamber compared to the concentration in the bulk solution.

Table 4. Silver equilibration with 6 mL vials and a polyether sulfone membrane.

Sampling Time (Hr)	Bulk Ag Conc. (mg/L)	Peeper Ag Conc. (mg/L)	% in Peeper
25	0.163	0.049	30.1
48	0.166	0.073	44.0
72	0.153	0.074	48.4
144	0.145	0.092	63.4
240	0.134	0.095	70.9

At measured concentrations of Ag ranging from 0.134 to 0.166 mg/L, a steady increase in concentration was measured within the peeper chamber over the 10-day period (Table 4).

Similar equilibration time studies were next conducted with acrylic plastic peepers having peeper chambers of two different sizes, but with identical surface areas of about 6 cm^2 (Tables 5 and 6). Equilibration was more rapid than for the vial peeper design, and quite comparable between the acrylic peepers.

Table 5. Silver equilibration with acrylic peepers (1 x 1 x 6 chambers) and a polyether sulfone membrane.

Sampling Time (Hr)	Bulk Ag Conc. (mg/L)	Peeper Ag Conc. (mg/L)	% in Peeper
24	0.162	0.127	78.4
48	0.150	0.125	83.3
72	0.135	0.113	83.7
96	0.132	0.111	84.1
168	0.116	0.099	85.3

Table 6. Silver equilibration with acrylic peepers (1 x 2 x 3 chambers) and a polyether sulfone membrane.

Sampling Time (Hr)	Bulk Ag Conc. (mg/L)	Peeper Ag Conc. (mg/L)	% in Peeper
26	0.200	0.151	75.5
52	0.195	0.149	76.4
122	0.178	0.140	78.7

An additional study with the polyether sulfone peeper membrane was conducted to determine if equilibration time was related to the concentration of $AgNO_3$. Four concentrations of silver (0.05, 0.10, 0.25 and 0.50 mg/L) in dechlorinated lab water were used. Samples collected at both 26 and 96 hr showed that the two highest concentrations had not equilibrated as completely as the lower two concentrations, and that the extent of equilibration appeared to vary inversely with concentration for the nominal concentrations of 100, 250 and 500 μg/L (Table 7). These results have led us to conclude that additional studies are necessary prior to the selection of the type of peeper membrane to be used in subsequent testing. We are currently exploring the use of other types of membranes. A polycarbonate membrane containing pores of 0.4 μm diameter is currently being studied.

Table 7. Silver equilibration with acrylic peepers (1 x 2 x 3 chambers) and a polyether sulfone membrane.

Sampling Time (Hr)	Bulk Ag Conc. (mg/L)	Peeper Ag Conc. (mg/L)	% in Peeper
26	0.047	0.043	91.5
26	0.094	0.088	93.6
26	0.228	0.151	66.2
26	0.463	0.178	38.4
96	0.042	0.037	88.1
96	0.089	0.083	93.3
96	0.216	0.157	72.7
96	0.420	0.204	48.6

IV. Toxicity Tests with Potassium Nitrate and Sodium Nitrate.

Since fairly high concentrations of $AgNO_3$ will be added to the sediment in the sediment toxicity test, we are interested in knowing the sensitivity of *Chironomus tentans* to NO_3^-, as well as to Ag^+. Static toxicity tests have been conducted with *Chironomus tentans* larvae using both KNO_3 and $NaNO_3$ salts. LC50s for NO_3^- after 48 hr of exposure ranged from 2,153 to 3,072 mg/L (Table 8). The overall mean 48 hr LC50 for NO_3^- regardless of starting salt was 2,659 $\pm$ 355 mg/L. Toxicity may increase with increased exposure, although more tests are needed to distinguish between chemical toxicity and artifactual toxicity associated with the tests themselves. It is anticipated that most of the NO_3^- in the $AgNO_3$-spiked sediment will be eliminated from the exposure system during the period of 7-10 days that the sediment is placed into the test system preceding the addition of test animals.

Table 8. Toxicity tests with *Chironomus tentans* larvae and potassium or sodium nitrate.

LC50 (mg/L)				
Chemical	Date	48 Hr	96 Hr	240 Hr
KNO_3	10/14/96	4332	2122	n.d.
as K^+ (38.7%)	10/14/96	1676	821	n.d.
as NO_3^- (61.3%)	10/14/96	2655	1301	n.d.
$NaNO_3$	10/14/96	3426	n.d.[1/]	n.d.
as Na^+ (27.0%)	10/14/96	925	n.d.	n.d.
as NO_3^- (73.0%)	10/14/96	2501	n.d.	n.d.
KNO_3	10/29/96	4989	n.d.	n.d.
as K^+	10/29/96	1931	n.d.	n.d.
as NO_3^-	10/29/96	3058	n.d.	n.d.
$NaNO_3$	10/29/96	2949	n.d.	n.d.
as Na^+	10/29/96	796	n.d.	n.d.
as NO_3^-	10/29/96	2153	n.d.	n.d.
KNO_3	11/5/96	4102	2197	1087
as K^+	11/5/96	1587	850	421
as NO_3^-	11/5/96	2515	1347	666
$NaNO_3$	11/5/96	4211	4019	2100
as Na^+	11/5/96	1139	1087	567
as NO_3^-	11/5/96	3072	2932	1533
KCl	7/29/96	6357	n.d.	n.d.

[1/] No data.

V. Project Plans and Schedule.

The overall plan for Year I of this project is to conclude with a definitive toxicity test of Ag-spiked sediment for *Chironomus tentans* larvae. To accomplish this, it will be necessary to determine the total binding capacity of West Bearskin Lake sediment for Ag, and to spike Ag into the sediment in a graded series of concentrations to provide both complete survival of the test animals as well as complete mortality. Ideally, the intermediate concentrations would exhibit partial mortalities in a dose-dependent series.

We have determined the following tentative schedule for performing the necessary tasks to accomplish these objectives:

Date		Research Task
April 30, 1997		Completion of peeper membrane equilibration studies in water containing $AgNO_3$
May 30, 1997	(1)	Completion of peeper membrane equilibration studies in silver-spiked sediment
	(2) ✓	Completion of 10-day water-only toxicity test with $AgNO_3$ and *Chironomus tentans*
June 30, 1997	(1)	Completion of sediment-spiking experiments to determine the binding capacity of West Bearskin Lake sediment for silver
	(2)	Completion of range-finding sediment toxicity test with *Chironomus tentans*
August 15, 1997		Completion of definitive 10-day acute toxicity test in silver-spiked sediments