The Comparative Toxicity of Silver to Aquatic Biota

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Silver studies were initiated as a toxicological component in evaluating the leachability and potential environmental effects of coal and coal wastes. The initial effort was devoted to developing a flow-through model system for analyzing leachates. Thirty-three inorganic elements traceable to coal toxicology were detectable in different waters perfused through fly ash and other coal products (Birge, 1978; Birge *et al.*, 1978; 1979). The leaching chamber, developed for use with solid wastes, was adjustable for water to solid ratio, general water quality parameters, flow rate, and the collection of settleable solids. Continuous leachability of toxic metals from coal fly ash was evident for more than 80 days.

Chemical analytical data were used in initial assessments of the impact potential of complex ash effluents but comparisons with U.S. EPA aquatic criteria and other data were complicated by lack of pertinent information. Consequently, laboratory studies were initiated 1) to characterize the toxicity of inorganic elements detected in coal leachates and 2) to apply direct toxicological testing to untreated and treated coal effluents. Independent laboratory toxicity data were used 1) to prioritize coal elements as to impact potential and 2) to categorize animal test species according to sensitivity, including the selection of surrogate species. Each inorganic element was evaluated with at least three aquatic species and with up to 14 animal species for metals considered to be more problematic.

The application and modeling of independent laboratory toxicity data, together with the chemical effluent monitoring results, facilitated identification of the most problematic elements. However, this traditional approach was not fully effective in quantifying the biological activity resulting from the chemical milieu contained in complex coal effluents, or in evaluating effluent treatability and/or sediment sorption of water column metals. Therefore, direct toxicological testing (*i.e.* biomonitoring) with continuous coal effluent was performed with early life stages of fish and amphibian species. Good dose response data were obtained for effluent dilutions using embryo-larval stages, and biomonitoring consistently provided more reliable results than aquatic life criteria for assessing the biological impact of coal effluents. The results of this early investigation formed the basis of our long-term effort in effluent biomonitoring, and led to the conclusion that leachability, bioavailability and/or transport of priority pollutants cannot necessarily be predicted accurately with the use of U.S. EPA aquatic criteria or conventional laboratory toxicity data.

Laboratory toxicity tests with silver were conducted with six species of amphibians and four species of fish. Test organisms were maintained in reconstituted water of 100 to 200 hardness (mg $CaCO_3$/L) using twelve-hour static renewal procedures (Birge *et al.*, 1985a; Weber *et al.*, 1989). Organisms and test parameters were monitored once to twice daily (*e.g.* pH, dissolved oxygen, conductivity, hardness, alkalinity). Sample size varied from 100 to 150 organisms, except for *A. opacum* (n = 35). Exposure was maintained from fertilization through four days posthatching, and overall treatment ranged from six to eight days for amphibians up to twenty-eight days for rainbow trout. Results were based on mortality and gross terata of embryos and larvae. These responses were combined and threshold values (LC_1, LC_{10}) and median lethal concentrations (LC_{50}) were determined by probit analyses (Table 1). The leopard frog (*Rana pipiens*) and rainbow trout (*Oncorhynchus mykiss*) were the most sensitive species, with LC_{10} values of 0.7 to 0.8 µg/L, and an LC_{50} value of 10 µg/L. The LC_{10} values for the other species ranged from 1 to 30 µg/L and the LC_{50} values varied from 10 to 240 µg/L. The probit LC_{50} and threshold (LC_1, LC_{10}) values were comparable to MATC and chronic values determined in various chronic tests (*e.g.* life cycle studies) by other investigators (Davies *et al.*, 1978; U. S. EPA, 1980; Nebeker *et al.*, 1983; Eisler, 1995).

Toxic effects of silver also were investigated using *Ceriodaphnia dubia* and the three-brood procedure. The NOEC values were 5 and 4 µg/L and the LOEC values were 10 and 8 µg/L in two independent experiments conducted using U.S. EPA methods (Weber *et al.*, 1989). The chronic values were 7.1 and 5.7 µg/L and the IC_{10}, IC_{25} and IC_{50} values were 5.8 and 4.5 µg/L, 7.3 and 5.2 µg/L, and 9.8 and 6.4 µg/L, respectively. The above studies revealed the LC_{10} to be a more reliable indicator of threshold effects than the IC_{25} suggested by U.S. EPA. By comparison, Nebeker *et al.* (1983) reported similar values obtained in three 21 day life cycle tests with *Daphnia magna*. They determined a mean LC_{50} of 3.5 µg/L based on survival, and LOECs of 4.1 µg/L and 10.1 µg/L based on survival and reproduction, respectively. Thus, there was remarkably little variation among results for both cladoceran species.

In fish and amphibian tests, silver was among the more toxic metals evaluated (*i.e.* LC_{50}). In an attempt to further characterize biological effects of silver, data were combined for different combinations of sensitive and tolerant species to give mean toxicity indices. Results for amphibian species are given in Table 2. The leopard frog (*Rana pipiens*), pickerel frog (*Rana palustris*) and narrow-mouthed toad (*Gastrophryne carolinensis*) were the most sensitive amphibians, with LC_{10} values of 0.7 to 2 µg/L and an LC_{50} value of 10 µg/L. Combining responses for these three species, the LC_{10} was 1.0 µg/L and the LC_{50} remained 10 µg/L. For the more tolerant species, the individual LC_{10} values ranged from 3 to 34 µg/L and the LC_{50} values varied from 20 to 240 µg/L. The combined LC_{10} and LC_{50} values were 3 and 90 µg/L, respectively.

Silver nitrate, one of the more soluble forms of silver, was used in the above investigations. Based on calculations with the aquatic equilibrium program MINEQL$^+$ (Schecher and McAvoy, 1991), it was concluded that the silver nitrate used in these studies was highly dissociated and that the observed effects were attributable to the "free" silver ion. Predictably, other forms of silver (eg. silver chloride, silver sulfide) would be less soluble and results based on total recoverable metal concentrations would reflect proportionally less toxicity (Le Blanc *et al.*, 1984; Wood *et al.*, 1995). Considering either single-species or combined data (Tables 1, 2), silver was remarkable in providing less diversity of response among test organisms than did mercury or most other metals. However, in further studies of coal toxicity silver was deprioritized because of its more limited leachability and/or bioavailability.

Numerous investigations have demonstrated that the embryo-larval procedure used in these studies is sufficiently sensitive to give reliable predictions of the chronic toxic effects of single compounds or complex effluents, and that test results correlate well with life cycle studies used to develop aquatic criterion values (Birge *et al.*, 1981; 1985b; Birge and Black, 1990; Weber *et al.*, 1989). It should be noted, however, that more soluble forms of silver have been used in most laboratory investigations, and that test procedures and/or the use of reconstituted water likely exacerbate stresses to test organisms and optimize bioavailability of the free silver ion. Consequently, laboratory test results may overestimate silver toxicity in the field. In general, caution should be used in applying laboratory toxicity data to assessments of ecological impact.

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Table 1. Silver Toxicity Values for Early Life Stages of Fish and Amphibians

	mg/L[b]			
Species[a]	**LC_{50}**	**LC_{25}**	**LC_{10}**	**LC_{1}**
R. pipiens	0.01	0.004	0.0007	0.0001
O. mykiss	0.01	0.005	0.0008	0.0001
R. palustris	0.01	0.007	0.001	0.0001
I. punctatus	0.01	0.007	0.002	0.0003
G. carolinensis	0.01	0.007	0.002	0.0006
R. catesbeiana	0.02	0.01	0.003	0.0005
C. auratus	0.02	0.01	0.004	0.001
M. salmoides	0.11	0.07	0.018	0.004
B. fowleri	0.23	0.07	0.004	0.0001
A. opacum	0.24	0.13	0.03	0.007
Geometric Mean	0.03	0.02	0.003	0.0004
Arithmetic Mean	0.07	0.03	0.007	0.001

[a] Organisms were maintained through four days posthatching.
[b] Probit values were calculated using the EPASTATS program.

Table 2. Combined Silver Toxicity Values for Amphibians

	mg/L[a]			
Species	**LC_{50}**	**LC_{25}**	**LC_{10}**	**LC_{1}**
R. pipiens	0.01 (0.007-0.015)	0.004	0.0007 (0.0003-0.0013)	0.0001 (0.0000-0.0002)
R. palustris	0.01 (0.003-0.050)	0.007	0.001 (0.0000-0.0040)	0.0001 (0.0000-0.0009)
G. carolinensis	0.01 (0.004-0.030)	0.007	0.002 (0.0001-0.0060)	0.0006 (0.0000-0.0023)
R. catesbeiana	0.02 (0.017-0.032)	0.012	0.003 (0.0010-0.0040)	0.0005 (0.0002-0.0009)
B. fowleri	0.23 (0.150-0.350)	0.073	0.004 (0.0020-0.0080)	0.0001 (0.0000-0.0004)
A. opacum	0.24 (0.150-0.360)	0.130	0.034 (0.0120-0.0640)	0.007 (0.0010-0.0180)
All Species	0.03 (0.019-0.052)	0.012	0.001 (0.0004-0.0028)	0.0001 (0.0000-0.0003)
Geometric Mean	0.03	0.017	0.003	0.0004
Arithmetic Mean	0.09	0.039	0.007	0.001
More Sensitive Species				
R. pipiens *R. palustris* *G. carolinensis*	0.01 (0.009-0.015)	0.007	0.001 (0.0008-0.0020)	0.0002 (0.0001-0.0003)
Geometric Mean	0.001	0.006	0.001	0.0002
Arithmetic Mean	0.01	0.006	0.001	0.0003
More Tolerant Species				
R. catesbeiana *B. fowleri* *A. opacum*	0.09 (0.020-0.340)	0.033	0.003 (0.000-0.0070)	0.0002 (0.000-0.0019)
Geometric Mean	0.10	0.048	0.007	0.001
Arithmetic Mean	0.16	0.072	0.013	0.003

[a] Probit values were calculated using the EPASTATS program.

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Questions & Answers: The Comparative Toxicity of Silver to Aquatic Biota

Q. ARUN MUKHERJEE (Univ. of Helsinki): I understand that you worked with coal and analyzed the ashes that yield when you burn the coal at high temperature. For example, if you take one pound of coal and know what is the amount of silver within the coal and you know the efficiency of your precipitator, how much silver would go into ashes and how much would go into the atmosphere?

A. You're talking about the precipitator for the ash?

Q. Yes. Could you tell me how much would go into the atmosphere?

A. The only thing I can tell you is what we analyze in the air before we start the leachate. Normally, we're in the range of 40 ppb and, after some time, as much as 600 ppb, rarely maybe even 700 ppb. Normally, it's in the 40-60 ppb range if the precipitator works with chloride.

Q. Ppb. That means, 2 ppb is 0.002 ppm. Am I correct?

A. Yes. So we have 0.04 mg/kg.

Q. 0.04 mg/kg, good. And how much will go out into the atmosphere?

A. How much goes into the atmosphere? Actually, that's a difficult question, really, to answer. What happens there, at the lead-high temperature, is that some of the elements are not sorbed to particulates. But, then again, in the gas phase they do sorb, and then, if you look at the leachate part for sorption effects, you find that some do and some don't sorb. So what's going up secondarily, a lot of it is probably collected on particulates also and sent out into the atmosphere and then deposited.

Q. Do you have any experience with domestic waste?

A. We don't look only at sewage waste. We have several other studies around where we're looking at, or examining what comes out from power plants. But there, about the only elements that turn up in soils around the site of the plants would be in much higher concentration than in coal. So we're normally looking at aluminum and iron, copper, zinc. I don't recall actually seeing major silver amounts.

Q. ANDERS ANDREN (Univ. of Wisconsin): You presented an incredible amount of data, so there are lots of questions, but I'll probably just limit myself to two. The first one, when you characterize your leachate; did you filter your material or is that total concentration of metals, or what?

A. We were doing total metal, full recovery metal.

Q. I defy anyone to make any sense out those data, then, in terms of ascribing any effects to any particular metal.

A. That's an interesting point to debate that we're heading at here, the dissolved versus the solid argument, depending on the filter. I hope we have the time to do it. But remember, a great amount of subtoxic and almost all the toxicity data on metals were done for total metal concentrations, and with these data were given the bioavailability and response level. I think that this may give problems for some other scientists, but I'd be happy to discuss this.

Q. The other question deals with your LC_{50}s, etc., how can you, — as I don't have much biology background — how can you do an LC_1?

A. Well, with the photo systems we were using, you can't really do this with all the available photo systems out there. We were using a special process and, basically, you have the opportunity to get a read-out anywhere from 1 to 99.

Q. But you have to determine the concentrations at that level?

A. It would depend on the inhomogeneity of your animal response data and water flea data. We realized that they really don't vary so much. The variability within these tests isn't great. A lot of these animals are adapted to toxicity tests; that reduces mortality and the ability of a chemical to get a critical life support mechanism knocked down. The opportunity for more individual variation is less here. When we're looking at embryonic development; that is, the period when the DNA is doing its thing. That's when there is more regulation, more control and more reproduction in the lab, at least for the organisms we looked at. Many of you have studied embryonic development, you know what kind of reproducibility is involved. An enormously complex major event would be needed to take influence and they all would change in the same way. That can be taken advantage of in various toxicity tests.

And we can show that when you do a normal batch test you often get less variability in your data than you expect, but it leads to a better opportunity for further analysis. With a lot of chemicals we can't do an LC_1 with reasonable confidence, but that's when we back off and we do the LC_{10} and we think it works out pretty well. We do all kind of other kinds, not just the LC_1.

Q. What concentrations of DOC, chloride, and particulate matter do you have in your bioassays?

A. In these particular assays? I have information on that but I didn't bring it with me. I have a list over there. We don't have a lot of the usual things as effect level variability and normal water quality and a lot of these parameters. It's more homogeneous with effect to a lot of the nonmetal constituents that you can find in different forms. I have some information on that I can show you later. Anything else? The question that I want to leave you with is, why don't we see a more varied response of silver during the early life stages? A lot is depending on the life stage, for example, the LC_{50} we studied with adult fish, they tend to be different, and the main thing for this is inhomogeneous response. There's got to be a reason for this. You can either be looking at some universal receptors that are out there, or it is a matter of membrane composition and individual toxic patterns, but with the same level of sensitivity as for the first (that is, the universal receptors). Thank you.