

Collaborative Research and Development Program
Project (CRD 227776/99). Final Report

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Collaborative Research and Development Program Project (CRD 227776/99) Final Report

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2. Title of Project

Incidence and toxicological implications of <accidental> transport of silver across biological membranes.

3. Background Information

In aquatic and terrestrial toxicology, it is generally accepted that complexation of a metal leads to a decrease in its bio-availability – in effect, most dissolved ligands that bind metals form hydrophilic complexes, ML_n^\pm , and in such systems metal uptake, nutrition and toxicity normally vary as a function of the concentration of the free-metal cation in solution. Exceptions to this simple model of metal toxicity generally involve ligands that form lipophilic complexes, ML_n^0 , which can bypass normal metal transport mechanisms and cross biological membranes by simple diffusion. A number of intriguing metal toxicity experiments have however been reported in the literature where the metal's "residual" bio-availability in the presence of hydrophilic ML_n^\pm complexes has been found to exceed that which would have been predicted on the basis of the free-metal ion concentration at equilibrium. Most of these apparent exceptions to the FIM / BLM¹ of metal toxicity involve assimilable organic ligands, and this has led to the suggestion that "accidental" metal transport may occur in their presence (i.e., the ligand is assimilated as a metal-ligand complex and the metal "comes along for the ride").

In principle, uptake of intact hydrophilic metal-ligand complexes could also occur with inorganic ligands such as chloride or sulfate / thiosulfate. Uptake systems for such essential nutrient anions exist at biological interfaces; if these transport systems could be "fooled" into binding and transporting the intact metal-anion complex, then the metal would find its way into the cell "accidentally".

How does this information pertain to silver in the environment? In the absence of dissolved organic ligands and reduced sulfur(II) species, the speciation of silver is strongly influenced by two simple inorganic ligands - chloride and thiosulfate. Chloride is ubiquitous in natural fresh waters (typical concentrations 10^{-5} to 10^{-3} M), and may also be present at higher levels in industrial effluents and urban runoff. Thiosulfate is less common in natural waters, but is present in effluents from photo-finishing plants and is also found in the natural waters at interfaces between oxic and anoxic environments (e.g., in the interstitial waters of suboxic sediments) and in waste waters from sulfide ore flotation processes. Given the importance of these two inorganic ligands, we asked the question "Does the complexation of silver with chloride or thiosulfate reduce its bioavailability, its toxicity ... or do the silver-anion complexes contribute to the metal's bioavailability?" An alternative formulation might be "Does the BLM apply to silver in the presence of thiosulfate and chloride ions?" If the response to this latter question were

¹ This model of metal-organism interactions was originally known as the <Free-Ion Model> or FIM, but in recent years this (misleading) designation has been largely supplanted by the term <Biotic Ligand Model> or BLM. For algae both terms can be used interchangeably.

positive, then the current environmental regulations for silver, which are expressed in terms of total recoverable silver (not the free Ag^+ ion), would be needlessly conservative.

4. Objectives

In this project we sought to determine how the complexation of silver by assimilable inorganic ligands such as chloride or thiosulfate affects its bioavailability. The hypothesis to be tested was that silver could traverse biological barriers by accidental metal transport, via an unselective anion transporter, thus leading to enhanced silver uptake and toxicity. To explore new questions arising from the study, in Year 3 we extended our research on silver bioavailability to investigate the influence of organic ligands such as natural organic matter (NOM; i.e., humic and fulvic acids).

5. Summary of results

5.1 Overall experimental approach.

Silver uptake and toxicity experiments were designed to be carried out with unicellular algae. Such eukaryotic microorganisms are known to possess membrane-bound transport systems for the assimilation of chloride and sulfate / thiosulfate, and thus were appropriate models for testing the hypothesis that silver can cross biological membranes and enter cells via anion transport systems. To follow the influence of the test ligands (thiosulfate; chloride) on metal bioavailability we monitored several biological end-points concurrently: metal uptake rates, ligand uptake rates and algal growth rates. For these latter determinations, we used continuous cultures grown in a turbidostat to determine the effect of silver and its anion-complexes on algal growth rates and intracellular metal physiology. In such systems, cultures are kept at a constant density by regulating addition (and removal) of culture medium as a function of cellular growth, as monitored by *in situ* turbidity measurements. Turbidostat cultures can thus be kept in exponential growth at low cell densities. Fresh solution is pumped into the culture vessel periodically, to dilute the culture and maintain the cell population at the desired level; this continual influx of contaminant affords steady exposure conditions over time. In addition, turbidostats allow the algal cells to grow at high rates by supplying nutrient-replete medium (as opposed to chemostats which are designed for nutrient-limited cultures). This design thus facilitates the detection of any decreases in growth rate caused by silver toxicity.

5.2 Summary of work carried out.

Our research plan involved three sequential steps: (1) determine if silver uptake in the presence of chloride or thiosulfate is coherent with the Biotic Ligand Model; (2) if silver uptake is enhanced in the presence of chloride or thiosulfate, elucidate the mechanism(s) responsible for this behaviour; (3) if silver uptake is enhanced in the presence of chloride or thiosulfate, determine if this enhanced uptake also leads to increased toxicity. The presentation of our results follows this same sequence.

Inorganic ligands

In Years 1 and 2, investigations were conducted on a euryhaline unicellular green alga, *Chlamydomonas reinhardtii*. The key results from silver uptake tests with this alga are summarised below in bullet form:

- Short-term silver uptake (e.g., ≤ 30 min) by *C. reinhardtii* was greater than predicted in the presence of chloride – e.g., for a fixed free Ag^+ concentration (10 nM), silver uptake

increased markedly when the external chloride concentration was increased from 5 μM to 4 mM.

- However, no evidence could be found for the passive diffusion of the neutral AgCl^0 complex or for the facilitated uptake of the anionic AgCl_2^- complex. Instead, the rate enhancement proved to be related to the very high silver uptake rates demonstrated by the test alga, which led to diffusion limitation in the boundary layer surrounding the algal cell [Fortin and Campbell 2000]².
- Short-term silver uptake by *C. reinhardtii* was also enhanced in the presence of thiosulfate, to a much greater extent than in the earlier experiments with chloride. In this case we demonstrated the enhancement was indeed the result of "accidental transport", i.e. silver-thiosulfate complexes are transported across the plasma membrane via sulfate / thiosulfate transport systems; as expected, this uptake route is inhibited by high external sulfate concentrations [Fortin and Campbell 2001].
- In the absence of thiosulfate, free silver inhibited the growth of *C. reinhardtii* in continuous cultures ($\text{EC}_{50} \sim 21 \text{ nM}$ or 23 amol/cell).
- Based on this result we fixed the free Ag^+ concentration at 10 nM ($\sim 10\%$ growth inhibition in the absence of thiosulfate) and then progressively added both silver and thiosulfate in proportions such that the free Ag^+ concentration remained constant at 10 nM while the $\text{Ag}(\text{S}_2\text{O}_3)^-$ concentration increased. Under such conditions, the degree of growth inhibition due to silver should have remained constant at 10% if only Ag^+ were contributing to toxicity (i.e., if the Biotic Ligand Model applied). Instead, the growth rate diminished as $[\text{Ag}(\text{S}_2\text{O}_3)^-]$ increased. Silver toxicity is clearly enhanced in the presence of thiosulfate.

In summary, we showed that silver can enter an algal cell via an anion transporter in the presence of thiosulfate but not in the presence of chloride. Using the turbidostat cultures, we then demonstrated that this enhanced uptake of silver by *C. reinhardtii* leads to increased toxicity (i.e., reduced algal growth compared to a thiosulfate-free culture containing the same free Ag^+ concentration).

For Year 3, we set out to determine whether earlier results from *C. reinhardtii* were specific to the alga, or whether they applied generally to freshwater algae. To accomplish this goal, we chose a second test alga, *Pseudokirchneriella subcapitata* (aka *Selenastrum capricornutum*). The experimental approach developed for *C. reinhardtii*, i.e. steps 1, 2 and 3, was applied to *P. subcapitata*. We also adopted a third test alga, *Chlorella pyrenoidosa*, for the study of silver bioavailability (i.e., steps 1 and 2). Key results are summarised below in bullet form:

- Unlike the case with *C. reinhardtii*, short-term silver uptake (e.g., $\leq 30 \text{ min}$) by *P. subcapitata* was not influenced by the presence of chloride – for a fixed free Ag^+ concentration (10 nM), silver uptake rates remained constant despite dramatic changes in the total concentrations of Ag (from 10 to 104 nM) and Cl (from 5 μM to 4 mM), reflecting the constant free Ag^+ concentration. Similarly, short-term silver uptake by *C. pyrenoidosa* was not influenced by the presence of chloride under identical experimental conditions.

² Cited references can be found in Section 5.4 or Section 9.

- When total ambient [Ag] was held constant (10 nM or 115 nM) but [Cl] was varied between 5 μ M and 50 mM to explore the whole range of silver chloro-complexes, cellular silver content varied markedly along the chloride concentration gradient and exhibited a clear positive correlation with the ambient free Ag^+ ion concentrations for both algae, but not with the concentrations of AgCl^0 or AgCl_2^- .

The BLM was thus found to be a reliable tool for describing silver bioavailability in the presence of chloride for these two algae. These results differ from those obtained earlier with *C. reinhardtii*, where the BLM failed to predict silver uptake in the presence of chloride. This difference in behaviour can be explained by the much lower silver uptake rates observed for *P. subcapitata* and *C. pyrenoidosa*. For these two algal species, silver internalization appears to be the rate-limiting step and the first assumption³ of the BLM is satisfied. For *C. reinhardtii*, however, silver internalization is much more rapid and diffusion of silver from the bulk solution to the algal surface becomes rate-limiting. *We conclude that the BLM will apply to silver uptake by algae in the presence of chloride, provided that the rate of silver internalization does not exceed the rate of diffusive supply of silver from the bulk solution.* As was the case for *C. reinhardtii*, no evidence was found for the internalization of silver via anion transport (AgCl_2^-) or by passive diffusion (neutral AgCl^0) in the presence of chloride [Lee et al. *submitted*].

To compare silver toxicity (step 3) for *C. reinhardtii* and *P. subcapitata*, a follow-up test was performed with *P. subcapitata* under experimental conditions comparable to those used previously for *C. reinhardtii*. The results are summarised below in bullet form:

- In the absence of thiosulfate, free silver inhibited the growth of *P. subcapitata* in continuous cultures ($\text{EC}_{50} \sim 64$ nM or 109 amol/cell); these values are higher than those determined for *C. reinhardtii* ($\text{EC}_{50} \sim 21$ nM or 23 amol/cell), indicating that *P. subcapitata* is the more tolerant alga.
- When both silver and thiosulfate were added in proportions such that the free Ag^+ concentration remained constant at 10 nM ($\sim 10\%$ growth inhibition in the absence of thiosulfate), the result showed an increase of silver toxicity in the presence of thiosulfate, i.e. a trend similar to that observed with *C. reinhardtii*.
- However, the behaviour of the two algae in the presence of thiosulfate is not identical: based on the silver cell quotas corresponding to 50% growth inhibition, overall silver toxicity was not significantly changed in the presence of thiosulfate for *P. subcapitata* ($\text{EC}_{50} = 109 \pm 31$ amol/cell in the absence of thiosulfate vs. 95 ± 2 amol/cell in the presence of thiosulfate), whereas for *C. reinhardtii* the silver cell quota corresponding to 50% growth inhibition increased markedly in the presence of thiosulfate ($\text{EC}_{50} 23 \pm 0.3$ amol/cell in the absence of thiosulfate $\rightarrow 100 \pm 3$ amol/cell in the presence of thiosulfate).

In conclusion, thiosulfate not only enhanced silver uptake but also increased silver toxicity for both *C. reinhardtii* and *P. subcapitata*. However, cell quota-based results suggest that the two algae respond differently to the internalized Ag-thiosulfate complexes; for *C. reinhardtii* it

³ First assumption of BLM: metal internalization, or transport of metal across the plasma membrane, is *slower* than the advection or diffusion of the metal towards the plasma membrane [Campbell et al. 2002].

appears that the silver thiosulfate complex remains at least partially intact once it has entered the algal cell, thus being less toxic than free silver ions (i.e., in the absence of thiosulfate), whereas in the case of *P. subcapitata* the complex appears to dissociate in the cytosol, thus exerting an internal toxicity comparable to that attributable to an influx of free Ag^+ ions.

We hypothesised that *C. reinhardtii* and *P. subcapitata* may respond differently to the Ag-thiosulfate complex because of differences either in the intracellular target of silver toxicity, or in their capacity to detoxify intracellular silver using metal chelating cellular ligands. To test this hypothesis we exposed the two algae to silver and monitored the fate of internalized silver by determining the subcellular partitioning of silver and the intracellular content of metal chelating molecules (e.g., phytochelatins and other thiols). *P. subcapitata* showed far higher thiol contents than *C. reinhardtii*, which is consistent with our observation of a higher EC_{50} for *P. subcapitata* than for *C. reinhardtii* in the absence of thiosulfate.

Organic ligands

In Year 3, after consultation with our industrial partner, we extended our research on silver uptake and toxicity to systems containing natural organic matter (NOM; i.e., humic and fulvic acids). Silver speciation is known to be influenced by the presence of NOM, but unlike the case with simple inorganic ligands, the extent of silver complexation by NOM cannot simply be calculated with equilibrium speciation models (due to a lack of reliable thermodynamic data for Ag-NOM interactions). Therefore, to carry out our experiments we had to develop a method for measuring free Ag^+ concentrations in the presence of natural organic matter using an equilibrium ion-exchange technique (IET – Fortin and Campbell 1998). Using this technique we were able to titrate NOM solutions with silver and determine the ratios of free:bound silver for different quantities of added total Ag. Based on these titration results, we set up experimental media to test the hypothesis that Ag-NOM complexes are biologically unavailable to two test algae, *C. reinhardtii* and *P. subcapitata*, as would be predicted by the BLM.

Free Ag^+ was measured in the presence of NOM using the IET. The technique was first validated for Ag^+ in the presence of variable concentrations of Cl, since the complexation constants for the reactions of Ag with chloride are well established. The calibrated resin was then used to measure free silver in the presence of Suwannee River humic acid (SRHA); results for solutions containing 5 → 100 nM Ag and realistic organic carbon concentrations (5 mg C/L) indicated that the natural SRHA has a very high affinity for silver. The results of these titration experiments allowed us to define appropriate exposure conditions for the algal uptake experiments.

- Short-term silver uptake (e.g., ≤ 60 min) by *C. reinhardtii* was enhanced in the presence of natural organic matter (SRHA) – e.g., for a fixed free silver concentration (10, 50 or 90 nM Ag^+), silver uptake over time was greater in the presence of NOM (5 mg C/L) than in the inorganic control medium. In a second series of experiments (fixed exposure time but different free Ag^+ concentrations), silver uptake was again consistently higher in the presence of NOM than in the control media (Porcher and Campbell 2002).
- Since total silver concentrations are higher in the NOM-containing media than in the organic-free control media, it is possible that the greater than expected uptake in the presence of NOM is related to the very high silver uptake rates demonstrated by *C. reinhardtii*, leading to diffusion limitation in the boundary layer surrounding the algal cell (i.e., a situation similar to that described above for the chloride system). To test this explanation, silver uptake

experiments were also carried out on *P. subcapitata*, an alga that takes up silver much more slowly than *C. reinhardtii*, such that diffusion in the boundary layer is not rate-limiting [Lee et al. *submitted*]. Contrary to our expectation, short-term silver uptake by *P. subcapitata* was also affected by the presence of SRHA, the trends being similar to those observed with *C. reinhardtii*. We conclude that the greater than expected silver uptake in the presence of SRHA cannot be explained entirely by the diffusion rate argument, and that the organic matter must be acting directly on the algal cells.

5.3 Research training.

The initial work on silver uptake by *C. reinhardtii* in the presence of chloride or thiosulfate was performed by Claude Fortin, a PhD student who graduated in November 2000 and who subsequently spent two years with the *Institut de Radioprotection et Sûreté Nucléaire* in France. Following a brief stint with Environment Canada, Dr. Fortin recently returned to the INRS as an assistant professor. Most of the toxicity work with the turbidostat cultures was carried out by two post-doctoral fellows, Dr. Véronique Hiriart-Baer (PhD 2000, University of Waterloo) and Dr. Dae-Young Lee (PhD 1998, State University of New York, Albany). Dr. Hiriart-Baer is currently at the Wilfrid Laurier University in Waterloo, ON, whereas Dr. Lee is still with us. Julie Deault (BSc 1999, University of Ottawa) and Céline Porcher (BSc 2000, McGill University) worked on silver-algae interactions for their MSc research; both Ms Deault and Ms Porcher are writing up their MSc theses.

5.4 Dissemination of research results.

Journal publications

Fortin, C. and P.G.C. Campbell (2000). Silver uptake by the green alga, *Chlamydomonas reinhardtii*, in relation to chemical speciation: influence of chloride. *Environ. Toxicol. Chem.* **19**: 2769-2778.

Fortin, C. and P.G.C. Campbell (2001). Thiosulphate enhances silver uptake by a green alga: role of anion transporters in metal uptake. *Environ. Sci. Technol.* **35**: 2214-2218.

Campbell, P.G.C., O. Errécalde, C. Fortin, V.P. Hiriart-Baer and B. Vigneault (2002). Metal bioavailability to phytoplankton - applicability of the Biotic Ligand Model. *Comp. Biochem. Physiol.* **133C**: 185-202.

Lee, D.-Y., C. Fortin, and P. G. C. Campbell (2003). Influence of chloride on silver uptake by two green algae, *Pseudokirchneriella subcapitata* and *Chlorella pyrenoidosa*. *Environ. Toxicol. Chem.* Submitted February 2003.

Hiriart-Baer, V.P., C. Fortin and P.G.C. Campbell (2003). Toxicity of silver to the freshwater algae, *Chlamydomonas reinhardtii* and *Pseudokirchneriella subcapitata*, grown under continuous culture conditions. In preparation.

Conference presentations

Fortin, C. and P.G.C. Campbell (1998). Interactions of silver with a unicellular alga: transport mechanisms through the cell wall and the plasma membrane in relation to chemical speciation. 19th Annual meeting, Society of Environmental Toxicology and Chemistry (SETAC), Charlotte, NC, USA, November 1998.

- Fortin, C. and P.G.C. Campbell (1998). Interactions of silver with a unicellular alga: transport mechanisms through the cell wall and the plasma membrane in relation to chemical speciation. 25th Aquatic Toxicity Workshop, Quebec, QC, Canada, October 1998.
- Fortin, C. and P.G.C. Campbell (1999). Prise en charge de l'argent par une algue verte en fonction de la spéciation chimique en présence de chlorures : un cas d'exception au modèle de l'ion libre. 67^{ème} Congrès de l'ACFAS, Ottawa, ON, Canada, May 1999.
- Fortin, C. and P.G.C. Campbell (1999). Uptake of silver by a unicellular alga: exceptions to the free-ion model. 20th Annual meeting, Society of Environmental Toxicology and Chemistry (SETAC), Philadelphia, PA, USA, November 1999.
- Fortin, C. and P.G.C. Campbell (2000). Uptake of silver by a unicellular alga: exceptions to the free-ion model. 4th International Symposium on Speciation of Elements in Biological, Environmental, and Toxicological Sciences, Whistler, BC, Canada, June 2000.
- Fortin, C. and P.G.C. Campbell (2000). Uptake of silver by a unicellular alga: exceptions to the free-ion model. International Conference on Heavy Metals in the Environment, Ann Arbor, MI, USA, August 2000.
- Campbell, P.G.C. (2001). Uptake of silver by a green alga: the effect of chloride and thiosulfate. Colloquium on the Biotic Ligand Model (BLM): current status and future directions. Washington, DC, USA, January 2001.
- Fortin, C. and P.G.C. Campbell (2001). Thiosulfate enhances silver uptake by a green alga: role of anion transporters in metal uptake. 11th Annual Meeting of SETAC-Europe, Society of Environmental Toxicology and Chemistry, Madrid, Spain, May 2001.
- Hiriart, V.P., P.G.C. Campbell, C. Fortin and J. Deault (2001). The effects of thiosulfate on the uptake and toxicity of silver on freshwater algae. 22nd Annual Meeting, Society of Environmental Toxicology and Chemistry, Baltimore, MD, USA, November 2001.
- Porcher, C. and P.G.C. Campbell (2002). Interactions de l'argent (Ag) avec les algues d'eau douces en présence de matière organique dissoute naturelle. Congrès de l'ACFAS, Ste-Foy, QC, Canada, May 2002.
- Campbell, P.G.C., C. Fortin and B. Vigneault (2002). Metal speciation and bioavailability, a continuum: external solution → cell surface → intracellular environment. 85th Annual Conference of the Canadian Chemical Society, Symposium on Metal Speciation, Vancouver, BC, Canada, June 2002.
- Porcher, C. and P.G.C. Campbell (2002). Interactions de l'argent (Ag) avec les algues d'eau douces en présence de matière organique dissoute naturelle. 6^e Colloque annuel du Chapitre St-Laurent, Ste-Foy, QC, Canada, June 2002.
- Porcher, C. and P.G.C. Campbell (2002). Effects of natural dissolved organic matter on silver speciation and silver uptake by a green alga. 23rd Annual meeting, Society of Environmental Toxicology and Chemistry (SETAC), Salt Lake City, UT, USA, November 2002.

Meetings with Industry representatives

Philadelphia, November 1999: P.G.C. Campbell and C. Fortin (INRS) met with J. Gorsuch and K. Robillard (Kodak) at the SETAC meeting.

Quebec, May 2000: T. Smith and J. Gorsuch (Kodak) met with P.G.C. Campbell, V. Hiriart, C. Fortin and J. Deault.

Nashville, November 2000: P.G.C. Campbell met with J. Gorsuch and K. Robillard (Kodak) at the SETAC meeting.

Quebec, August 2001: T. Smith and J. Gorsuch (Kodak) met with P.G.C. Campbell, V. Hiriart, J. Deault and C. Porcher.

Quebec, August 2002: T. Smith and J. Gorsuch (Kodak) met with P.G.C. Campbell, D.-Y. Lee, C. Fortin and C. Porcher.

6. Follow-up studies.

We initially planned to test the influence of two inorganic ligands, chloride and thiosulfate on silver bioavailability to a freshwater alga, *C. reinhardtii*, and later expanded our test organisms to include two other green algae, *P. subcapitata* and *C. pyrenoidosa*. Clear conclusions have emerged from this work. Silver uptake in the presence of chloride conforms to the predictions of the Biotic Ligand Model (BLM), except when the silver internalization rate exceeds the rate of diffusive supply of silver from the bulk solution to the algal surface. No evidence was found for the internalization of silver via anion transport (AgCl_2^-) or by passive diffusion (neutral AgCl^0) in the presence of chloride. In contrast, silver uptake and toxicity in the presence of thiosulfate do not follow the predictions of the BLM. Clear evidence was presented for the internalization of silver by anion transport (AgS_2O_3^-), via the membrane transport system involved in algal uptake of sulfate. Since sulfate transport is a ubiquitous (and necessary) process for freshwater algae, we would predict that this exception to the BLM will prove widespread. We are currently following up these observations with studies on other metals (e.g., Cd and Hg) known to form strong complexes with thiosulfate.

In the second phase of our project (Year 3), we extended our experiments to include natural organic matter (NOM) as a silver-binding ligand. Conclusions from this part of the study remain preliminary, but our results suggest that NOM affects Ag uptake not only by complexing the silver ion and reducing the free Ag^+ concentration (as anticipated by the BLM), but also by interacting directly with the algal cells. Several avenues of research are being considered, including the extension of the study to saline media (predominance of the neutral AgCl^0 complex among the inorganic forms of silver), to different sources of NOM, and to different algal types (cyanophytes, diatoms). Partial funding has been obtained from the Canadian Network of Toxicology Centres to pursue this work.

7. Discussion and Comments

The problems encountered in this project were relatively minor. The postdoctoral fellow initially hired to work on the project (Dr. Hiriart-Baer) left after only one year, for personal reasons (marriage!), and the recruitment of her replacement proved difficult – there was a lapse of 10 months before Dr. Lee took up the position in March 2002. The other problem was of a technical nature. The turbidostats that we used for the silver toxicity studies proved to be hypersensitive to external electronic "noise" and susceptible to electronic drift. Since we were purposely using low

cell densities, these periodic instabilities (if not detected and corrected manually) could lead to either a build-up of cells in the turbidostat or a washout of the algal culture. A second-generation turbidostat is currently undergoing testing in our laboratory.

As reported in the Progress Reports for this project, the collaboration with our industrial partner (Kodak) was uniformly positive. We met regularly with Kodak representatives, both in Quebec City and at the annual North America SETAC meetings. In addition to the constructive feedback on our own project, we also benefited from advance knowledge of the results obtained by other researchers working on the environmental chemistry and toxicology of silver. Kodak supported an extensive network of university researchers working in this area (*cf.* Synergy award in 2001), and their representatives have generously shared their knowledge with us.

8. Benefits to the industrial partner

To predict metal bioaccumulation and toxicity in the aquatic environment one must consider the speciation of the metal (in the exposure medium, at the biological surface, and within the target organism). Environmental regulators are increasingly aware of the links between «speciation» and «bioavailability / toxicity» and are actively considering how water quality criteria for metals might be refined to take into account the various factors that influence metal bioavailability. The Biotic Ligand Model (Gorsuch et al. 2002) has been suggested as a logical basis for the development of the next generation of water quality criteria.

The current research project has contributed to a better understanding of the strengths and weaknesses of the Biotic Ligand Model. Benefits from the research have already accrued to our industrial partner and to environmental regulators. Industry has gained a better appreciation of the bioavailability of silver in the aquatic environment and is benefiting from increased credibility vis-à-vis environmental regulators when discussing the rationale for site-specific water quality criteria for silver. An improved balance is slowly being established between industry and regulators, avoiding both over-regulation and under-regulation.

9. References cited in the report (other than those listed in Section 5.4)

Fortin, C. and P.G.C. Campbell (1998). An ion-exchange technique for free-metal ion measurements (Cd^{2+} , Zn^{2+}): applications to complex aqueous media. *Int. J. Environ. Anal. Chem.* **72**:173-194.

Gorsuch, J.W., C.R. Janssen, C.M. Lee and M.C. Reiley [Eds.] (2002). Special Issue: The Biotic Ligand Model for Metals - Current Research, Future Directions, Regulatory Implications. *Comp. Biochem. Physiol.* **133C**: 1-343