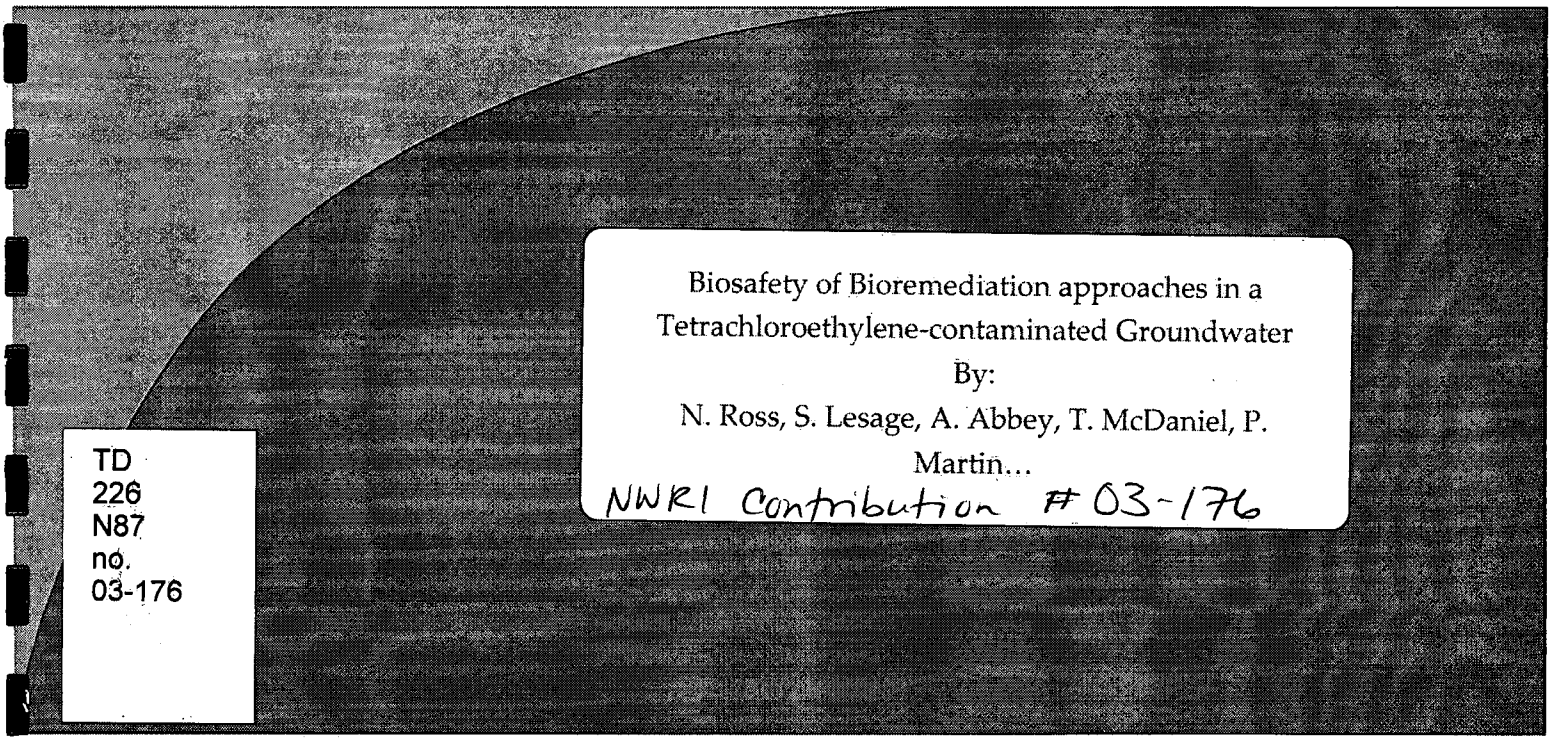
Environment Canada

Water Science and Technology Directorate

Direction générale des sciences et de la technologie, eau

Environnement Canada



Biosafety of Bioremediation approaches in a Tetrachloroethylene-contaminated Groundwater

By:
N. Ross, S. Lesage, A. Abbey, T. McDaniel, P. Martin...

NWRI Contribution # 03-176

03-176

BIOSAFETY OF BIOREMEDIATION APPROACHES IN A TETRACHLOROETHYLENE-CONTAMINATED GROUNDWATER

Nathalie Ross, Suzanne Lesage, Ann-Marie Irwin Abbey, Tana V. Mc Daniel, Pamela A. Martin, David W. Major, and Elizabeth A. Edwards

ABSTRACT

To assess whether bioaugmentation (AU) is as biosafe as biostimulation (ST) or natural attenuation (NA), a large-scale aquifer was divided in three lanes for a comparative study in a tetrachloroethylene(PCE)-contaminated groundwater. Results from the first 250 days confirmed that AU was effective for reductive dechlorination to ethene, whereas *cis*-DCE remained in the effluent from the ST lane and no degradation products were measured in the NA lane. A model amphibian was chronically exposed to effluents from the three lanes. Although the froglets had a significantly higher weight compared to the controls, the survivorship and metamorphic transformation were not significantly affected by the effluents. This information will be used to help define regulatory requirements for *in situ* bioremediation approaches.

BIOSÉCURITÉ DES MÉTHODES DE BIORESTAURATION DES EAUX SOUTERRAINES CONTAMINÉES PAR LE TÉTRACHLOROÉTHYLÈNE

Nathalie Ross, Suzanne Lesage, Ann-Marie Irwin Abbey, Tana V. Mc Daniel, Pamela A. Martin, David W. Major et Elizabeth A. Edwards

RÉSUMÉ

Dans le but d'évaluer si la bioaugmentation (AU) est aussi biosécuritaire que la biostimulation (ST) ou l'atténuation naturelle (AN), on a divisé un modèle d'aquifère à grande échelle en trois sections pour y mener une étude comparative sur les eaux souterraines contaminées par le tétrachloroéthylène (ou perchloroéthylène, PCE). Les résultats des 250 premiers jours confirment que l'AU est efficace pour la déchloration réductive en éthène, que le *cis*-DCE persiste dans l'effluent de la section ST et qu'aucun produit de dégradation ne se trouve dans la section AN. Un amphibien témoin a été soumis à une exposition chronique aux effluents des trois sections. Même si le poids des jeunes grenouilles est beaucoup plus grand que celui des témoins, leur survie et leur métamorphose ne sont pas touchées de manière significative par les effluents. Ces résultats serviront à définir les exigences réglementaires relatives aux méthodes de biorestauration *in situ*.

NWRI RESEARCH SUMMARY

Plain language title
Biosafety of bioremediation approaches in a tetrachloroethylene-contaminated groundwater

What is the problem and what do sicentists already know about it?
The application of bioremediation techniques is subject to environmental regulations requiring the provision of information on the environmental fate and ecological effects of injected microorganisms.

Why did NWRI do this study?
NWRI has the expertise on microbiology and ecotoxicology assessment. The large-scale model aquifer, in AQUEREF at NWRI, was an ideal facility to conduct such a comparative biotechnological study.

What were the results?
Bioaugmentation, the addition of adpated bacteria to degrade a targeted contaminant, was effective to transform a fraction of PCE into ethene whereas intermediate compounds were found where biostimulation, the addition of nutrients to stimulate the indigenous microbial population, was applied in the model aquifer. Enumeration of bacteria in groundwater and on the soil particles suggested that the active population is attached to the soil. As a potential receptor of groundwater, a model amphibian was chronically exposed to effluents; although the froglets had a significantly higher weight compared to the controls, the survivorship and metamorphic transformation were not significantly affected by the effluents. Bioaugmentation is effective to biodegrade PCE to harmless compounds, and no significant toxicity was measured from the effluent.

How will these results be used?
This information will be used to help define regulatory requirements for in situ bioremediation approaches.

Who were our main partners in the study?
Canadian Wildlife Service, University of Toronto, and GeoSyntec Consultants

Sommaire des recherches de l'INRE

Titre en langage clair
Biosécurité des méthodes de biorestauration des eaux souterraines contaminées par le tétrachloroéthylène.

Quel est le problème et que savent les chercheurs à ce sujet?
L'application de techniques de biorestauration est assujettie aux règlements environnementaux en vertu desquels des renseignements sont requis sur le devenir dans l'environnement et les effets écologiques des microorganismes injectés.

Pourquoi l'INRE a-t-il effectué cette étude?
Le personnel de l'INRE possède des compétences dans les domaines de la microbiologie et de l'évaluation écotoxicologique. Le modèle d'aquifère à grande échelle du laboratoire AQUEREF (Aquatic Ecosystem Restoration Evaluation Facility) de l'INRE était idéal pour mener une étude comparative sur des biotechnologies.

Quels sont les résultats?
La bioaugmentation, c'est-à-dire l'ajout de bactéries adaptées pour dégrader un contaminant visé, est efficace pour transformer une partie du PCE en éthène; des composés intermédiaires sont produits lorsqu'on applique la biostimulation, qui consiste à ajouter des substances nutritives pour stimuler la population microbienne indigène, au modèle d'aquifère. Le dénombrement des bactéries dans les eaux souterrraines et sur les particules de sol laisse croire que la population active est dans le sol. En tant que récepteur potentiel des eaux souterraines, un amphibien témoin a été soumis à une exposition chronique aux effluents; même si le poids des jeunes grenouilles est beaucoup plus grand que celui des témoins, leur survie et leur métamorphose ne sont pas touchées de manière significative par les effluents. La bioaugmentation est efficace pour biodégrader le PCE en composés inoffensifs, et aucune toxicité importante n'a été mesurée dans l'effluent.

Comment ces résultats seront-ils utilisés?
Ces renseignements serviront à définir les exigences réglementaires relatives aux méthodes de biorestauration *in situ*.

Quels étaient nos principaux partenaires dans cette étude?
Service canadien de la faune, Université de Toronto et GeoSyntec Consultants

BIOSAFETY OF BIOREMEDIATION APPROACHES IN A TETRACHLOROETHYLENE-CONTAMINATED GROUNDWATER

Nathalie Ross[1], Suzanne Lesage[1], Ann-Marie Irwin Abbey[1], Tana V. Mc Daniel[2], Pamela A. Martin[2], David W. Major[3], and Elizabeth A. Edwards[4]

[1]National Water Research Institute, Environment Canada, 867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario, L7R 4A6 Canada

[2]Canadian Wildlife Service, Environment Canada, 867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario, L7R 4A6 Canada

[3]GeoSyntec Consultants, 130 Research Lane, Suite 2, Guelph, Ontario, N1G 5G3 Canada

[4]Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street Toronto, Ontario, M5S 3E5 Canada

Fax: (905) 336-6430; E-mail: nathalie.ross@ec.gc.ca

ABSTRACT

To assess whether bioaugmentation (AU) is as biosafe as biostimulation (ST) or natural attenuation (NA), a large-scale aquifer was divided in three lanes for a comparative study in a tetrachloroethylene(PCE)-contaminated groundwater. Results from the first 250 days confirmed that AU was effective for reductive dechlorination to ethene, whereas *cis*-DCE remained in the effluent from the ST lane and no degradation products were measured in the NA lane. A model amphibian was chronically exposed to effluents from the three lanes. Although the froglets had a significant higher weight compared to the controls, the survivorship and metamorphic transformation were not significantly affected by the effluents. This information will be used to help define regulatory requirements for *in situ* bioremediation approaches.

1. INTRODUCTION

The application of bioremediation techniques is subjected to environmental regulations requiring the provision of information to governmental agencies with information on the environmental fate and ecological effects of injected micro-organisms [1, 2]. Biomolecular techniques are successfully used [3] to assess the fate of injected bacteria, but further scientific evidence has to be provided for the application of these regulations regarding bioremediation activities.

As information requested with respect to the ecological effects of the injected micro-organisms, receptor species likely to be exposed should be included in a battery of biotests. As such, amphibians are an important component of wetland ecosystems and may be receptors through groundwater recharge. Limited information is available on the toxicity of chloroethylenes to amphibians [4]; however, early embryonic exposures with TCE have shown teratogenic damage to developing amphibian larvae [5].

Bioaugmentation has been shown to be effective in remediating groundwater contaminated with chlorinated products [6], but the biosafety of this approach, as required by recent environmental regulations, needs to be demonstrated. The present study—comparing three bioremediation approaches: natural attenuation (NA), biostimulation (ST), and bioaugmentation (AU)—combines the monitoring of volatile organic carbons (VOCs), the assessment of the fate of injected bacteria, and the measurement of ecological effects. The selected results reported herein summarizes the first 250 days of this ongoing multidisciplinary study.

2. MATERIALS AND METHODS

2.1 The model aquifer

The model aquifer consisted of a 6-m long, 2.4-m wide, and 1.8-m deep stainless-steel tank, divided in three 0.8-m lanes, and filled with clean, medium-to-fine grain sand (Figure 1). Groundwater, pumped on-site, was introduced into three head tanks, and the flow was maintained gravimetrically at 80 mL min^{-1}. Each lane was equipped with 66 sampling ports distributed along three depths and nine longitudinal transects, a PCE-source well, three injection wells, and a withdrawal well. As a control treatment, NA was compared to ST (injection of methanol and lactic acid twice weekly) and AU (injection of nutrients plus a single injection of the KB-1 culture).

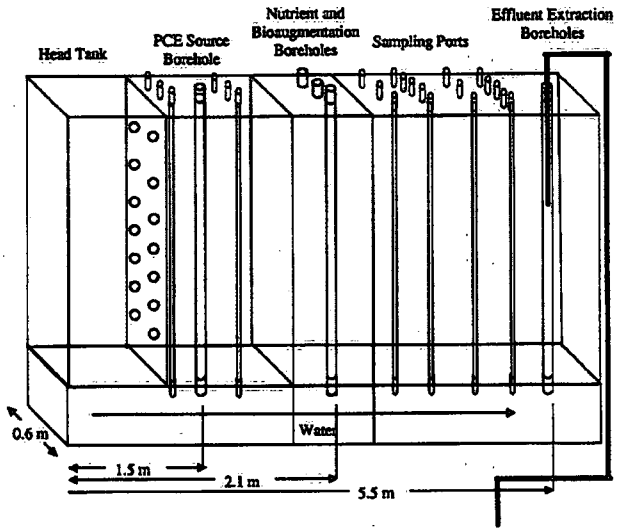


FIGURE 1. Cross-sectional view of one lane of the model aquifer

The PCE source consisted of PCE in silicone oil (10 % w/w) mixed with coarse sand introduced in a 30-cm, 200-μm meshed sock inserted at 1 m deep. The nomenclature used for the sampling ports was as follows: lane (NA, ST, or AU) , length from the head tank (ft), width from the center of the lane (A and E = 20 cm, B and D = 10 cm, and C = 0 cm), depth (ft); (ex. AU11D3).

2.2 VOCs and microbiological analyses

Groundwater samples were analyzed for VOCs by purge-and-trap GC/MSD (HP 5890 gas chromatograph/5973 mass spectrometer equipped with a DB-624 column) following USEPA methods 5030B/8260B. Because of the high concentrations of the contaminants, they were diluted by as much as 4300 fold by using 100 μL of sample in a 43-mL VOC vial.

Groundwater was sampled through ports located downgradient of the wells in each of the three lanes in June and September 2002. Samples were refrigerated at 4°C until analyzed, within 24-h time frame. A *Bac*Light™ viability test was performed as outlined by Boulos *et al.* (1999) [7] to assess the total bacterial density. A soil core was removed from each of the three lanes in July 2002. Soil collected was mixed to form a composite sample and analysis for cell density using *Bac*Light™ enumeration. One gram of the soil was added to a mixture of sterile 9.5 ml sterile 0.1 % sodium pyrophosphate buffer (pH 7.0) and 3 g glass beads [8] in a 50 ml Erlenmeyer flask. The slurry was shaken at 150 rpm at room temperature for 45 min to separate bacterial cells from the soil particles [9]. Serial dilutions of the slurry were prepared by adding 1 ml of the slurry to 9 ml of the sterile sodium pyrophosphate. A BacLight™ viability test was then performed on the dilutions.

2.3 Chronic exposures of the aquifer effluents to amphibian embryos

Xenopus tadpoles and embryos were exposed to 25 % effluent from each of the three lanes diluted in filtered dechlorinated tap water. Controls were exposed to groundwater from the head tank diluted in filtered tap water. Three replicates of 30 individuals were studied. Exposures were initiated on embryos less than 24-h old and continued for 100 days or until individuals reached metamorphosis. When *Xenopus* reached metamorphic transformation, they were euthanized, weighed, and snout vent length (SVL) was measured.

3. RESULTS AND DISCUSSION

3.1 Fate of VOCs in the model aquifer

The evolution of the VOC concentrations in the three treatment lanes and the effluents are summarized in Figure 2. The data at monitoring points 4C4 demonstrate the dissolution of the PCE from the source, prior to stimulating or augmenting. The fluctuation in the data reflects the source heterogeneity and possible escapes of DNAPL blobs from the source; nevertheless, the three different sources were relatively similar and provided an average input concentration of 200 μmoles/L for the first 50 days, after which they started to decline exponentially. However, the source in the AU lane did not last as

long as the other two lanes. The effluent concentrations peaked simultaneously in the three lanes at about 100 days before declining as well. Shortly after that point, degradation products began to appear in the effluents.

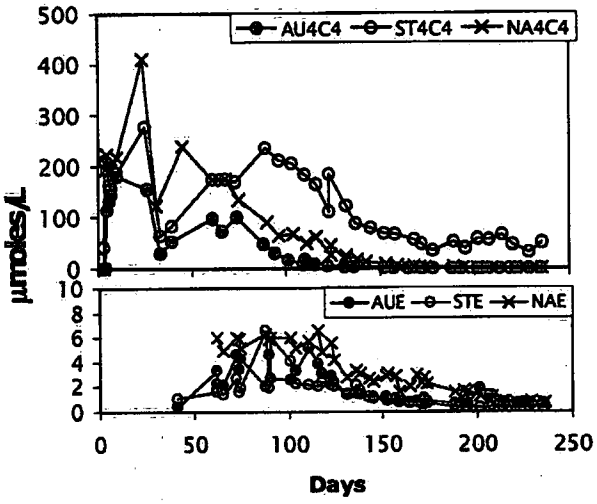


FIGURE 2. Source dissolution in the three treatments and the effluents concentrations. The source contained 10% tetrachloroethylene and 90% silicone oil.

3.2 Microbiological monitoring

The bacterial density in groundwater was consistent over time and space (Table 1). The bacterial density attached to the soil was 7 orders of magnitude higher than in suspension in the groundwater, indicating that the majority of active bacteria were attached [10]. Therefore, the partitioning of the bioaugmentation culture, KB-1 isolated from a TCE-contaminated site, might be preferentially attached to the soil particles. Additional microbiological analyses, such as DGGE and monitoring KB-1 with biomolecular probes, will give insights into the fate of the bioaugmented bacterial population in the model aquifer.

TABLE 1. Enumeration of total bacteria using BacLight™

Sample	Groundwater (log total bacteria / ml)		Soil (log total bacteria / g)
	June 2002	September 2002	July 2002
Holding tank		5.25	
NA6C4	5.53		
NA8C4	5.65		
NA15C5	5.37	5.23	
NA-soil composite			12.01
ST15C5		5.43	
ST-soil composite			12.06
AU15C5		5.01	
AU-soil composite			11.71

3.3 Assessment of the impacts of bioremediation using amphibian larvae

There was no significant difference in the survivorship between the control group and those exposed to any of the three treatments ($p > 0.05$, Table 2). Of the survivors, there was no significant difference in the proportion of individuals to reach metamorphic transformation within the 100-d period. There was a significant difference in weight and SVL of *Xenopus* froglets with controls being smaller than animals exposed to the three effluents. However, no obvious bacterially induced lesions were observed in tadpoles or transformed froglets.

TABLE 2. Endpoints measured on *Xenopus* tadpoles exposed to effluents from the model aquifer

Treatment	Survivorship t = 68 d (%)	Survivorship t = 100 d (%)	Transformed (%)	Weight (g)	Snout Length (mm)	Vent
NA	66.3 ±17.4	70.0 ±20.3	38.4 ±14.8	0.44 ±0.20	16.12 ±0.27	
ST	66.7 ±27.8	65.4 ±25.0	48.1 ±14.6	0.39 ±0.20	15.84 ±0.23	
AU	60.6 ±5.8	58.4 ±4.3	52.3 ±11.5	0.44 ±0.20	13.26 ±0.21	
Control	88.3 ±1.4	70.2 ±11.4	32.0 ±14.0	0.36 ±0.20	15.23 ±0.20	

4. CONCLUSIONS

Bioaugmentation generated complete dechlorination product, whereas biostimulation enhanced existing bacterial population, which very often lack the ability to degrade PCE beyond *cis*-DCE. In the absence of intervention, no biological degradation was observed after 250 days. Unfortunately, even in the AU lane, large amounts of parent product were still reaching the effluent, and this in a very small narrow plume intersected with three injection wells. In this experiment, there was only one AU event, and it is possible that the culture did not establish itself over the whole plume particularly given its apparent sessile nature.

The results suggest that the bioremediation approaches had no negative impact on amphibian embryos. Bacteriological screening of tissues from *Xenopus* exposed to the effluents will be direct indicators of any adverse effects not detected by the reported end-points. Additional information on the partitioning/identifying of the bacterial population in the three lanes will help in assessing the biosafety of AU compared to the ST and the NA approaches.

5. ACKNOWLEDGEMENTS

The authors acknowledge the participation of Susan Brown and Helena Steer in performing laboratory analyses. This study was funded by the Environmental Management of Biotechnology Regulation and Research (EMBRR).

6. REFERENCES

[1] Environment Canada (1997) *Canadian Environmental Protection Act - New Substances Notification Regulations.*

[2] United States Environmental Protection Agency (1997) *Point to consider in the preparation of TSCA biotechnology submissions for microorganisms*, Office of Pollution Prevention and Toxics: Washington, D.C., 71.

[3] van Elsas, J.D., G.F. Duarte, A.S. Rosado, and K. Smalla (1998) "Microbiological and molecular biological methods for monitoring microbial inoculants and their effects in the soil environment", *J. Microbiol. Methods*, 32:133-154.

[4] McDaniel, T.V., Martin, P. A., Ross, N., Brown, S., Lesage, S., and Pauli, B. D (2003) "Effects of chlorinated solvents on four species of North American amphibians", *Arch. Environ. Contamination Toxicol.*, Submitted.

[5] Fort, D.J., E.L. Stover, J.R. Rayburn, M. Hull, and J.A. Bantle (1993) "Evaluation of the developmental toxicity of trichloroethylene and detoxification metabolites using *Xenopus*", *Terat. Carcinogen. Mutagen.*, 13:35-45.

[6] Major, D.W., M.L. McMaster, E.E. Cox, E.A. Edwards, S.M. Dworatzek, E.R. Hendrickson, M.G. Starr, J.A. Payne, and L.W. Buonamici (2002) "Field demonstration of succesful bioaugmentation to acheive dechlorination of tetrachloroethene to ethene", *Environ. Sci. Technol*, 36:5106-5116.

[7] Boulos, L., M. Prévost, B. Barbeau, J. Coallier, and R. Desjardins (1999) "LIVE/DEAD BacLight: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water", *Microbiol. Methods*, 37:77-86.

[8] Leung, K.T., A. Watt, H. Lee, and J.T. Trevors (1997) "Quantitative detection of pentachlorophenol-degrading *Sphingomonas* sp. UG30 in soil by a most-probable-number / polymerase chain reaction protocol", *J. Microbiol. Methods*, 31:59-66.

[9] Van Dyke, M.I., H. Lee, and J.T. Trevors (1996) "Survival of luxAB-marked *Alcaligenes eutrophus* H850 in PCB-contaminated soil and sediment", *J. Chem. Tech. Biotechnol.*, 65:115-122.

[10] van Schie, P.M. and M. Fletcher (1999) "Adhesion of biodegradative anaerobic bacteria to solid surfaces", *Appl. Environ. Microbiol.*, 65:5082-5088.

PRINTED IN CANADA
IMPRIMÉ AU CANADA
ON RECYCLED PAPER
SUR DU PAPIER RECYCLÉ

National Water Research Institute
Environment Canada
Canada Centre for Inland Waters
P.O. Box 5050
867 Lakeshore Road
Burlington, Ontario
L7R 4A6 Canada

National Hydrology Research Centre
11 Innovation Boulevard
Saskatoon, Saskatchewan
S7N 3H5 Canada

NATIONAL WATER RESEARCH INSTITUTE
INSTITUT NATIONAL DE RECHERCHE SUR LES EAUX

Institut national de recherche sur les eaux
Environnement Canada
Centre canadien des eaux intérieures
Case postale 5050
867, chemin Lakeshore
Burlington, Ontario
L7R 4A6 Canada

Centre national de recherche en hydrologie
11, boul. Innovation
Saskatoon, Saskatchewan
S7N 3H5 Canada

Environment Canada Environnement Canada

Canada