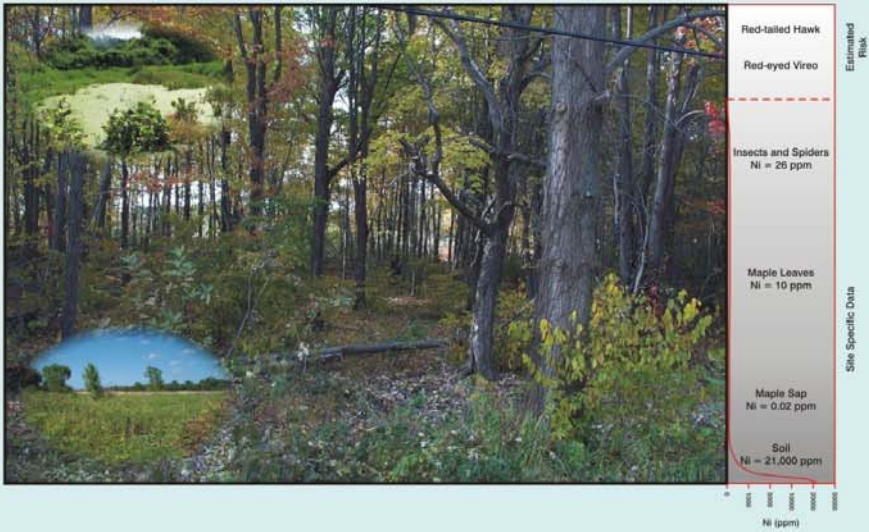
COMMUNITY BASED RISK ASSESSMENT
PORT COLBORNE, ONTARIO

ECOLOGICAL RISK ASSESSMENT
NATURAL ENVIRONMENT



Volumes II-V

September 2004

Jacques Whitford

INCO

PORT COLBORNE CBRA – ECOLOGICAL RISK ASSESSMENT

NATURAL ENVIRONMENT

VOLUME II

FIELD DATA COLLECTION AND ANALYSIS PROTOCOLS

Project No. ONT33828

Prepared for

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September, 2004

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INTRODUCTION

Volume II of the ERA-Natural Environment Report presents the protocols that were developed for field data collection, laboratory analysis of field samples and protocol for data interpretation for Risk Characterization. For the Community Based Risk Assessment (CBRA), to insure an open public process, prior to conducting field data collection for the Ecological Risk Assessment (ERA) draft protocols where developed by Jacques Whitford Environment Limited for review by the Technical Subcommittee (TSC) and the Public Liaison Committee (PLC).

Draft protocols were developed and reviewed through the field seasons of 2001 and 2002. Initial draft protocols detailed the rational for the collection of site-specific data, the collection methods, general sample collection locations, number of samples and the handing and laboratory analysis of samples. These initial draft protocols were reviewed by the PLC's consultant, and where required, changes to the protocols where made prior to undertaking the field collection of data.

During field collection, for some of the draft protocols, the locations for sample collection and/or the number of samples collected had to be modified from the draft protocols due to a number of factors, including:

- Habitat types (woodlot/field) were not found to occur in appropriate soil type (Clay/organic) and/or soil concentration of Nickel;
- Target species (i.e. voles, frogs, tadpoles) were either scarce or were not found to occur in predetermined sample locations; or
- Access to private lands could not be obtained

During the field programs, where deviation from a protocol was required, changes where agreed to in the field by a representative of the PLC's consultant who accompanied Jacques Whitford biologists during all field programs. For the purpose of the ERA report, the protocols presented in this Volume II represent the final approved protocols that detail and reflect what was undertaken for the study. Maps 1 and 2, located at the back of this Volume, presents the locations of sample sites for the study that are referenced in the individual protocols presented here. The following page presents a list of the protocols and their tab identifier.

ERA-NATURAL ENVIROMENT
Port Colborne Community Based Risk Assessment
Field Data Collection and Analysis Protocols

Quality Assurance and Quality Control for Field Sampling and Laboratory Procedures
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

As part of the Port Colborne Community Based Risk Assessment (CBRA), Jacques Whitford has carried out sampling and chemical analyses on various types of sample medium including soil, water, air and garden/orchard produce. Details of sampling methodology and analytical procedures for each sample medium is provided in separate written protocols.

Jacques Whitford has developed a protocol for determination of analytical parameters, sample standards, and laboratory quality assurance and quality control (QA/QC). This protocol has been designed for PSC of Mississauga, Ontario, the designated laboratory carrying out the chemical analyses on the Port Colborne samples. This protocol delineates Jacques Whitford policies for PSC with respect to field QA/QC, laboratory standardization, data management and laboratory QA/QC.

As outlined in the MOE Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario (MOE, 1996), laboratories accredited by the Canadian Association of Environmental Analytical Laboratories (CAEAL) are recommended for use for analytical purposes. PSC is such a CAEAL accredited analytical laboratory for most of the required analytes.

2. SAMPLING METHODS QA/QC

As outlined in Section five of the MOE (1996) guidance document, all samples were collected by Jacques Whitford in MOE-recommended containers. PSC provided the Jacques Whitford team with the required sample containers.

The following field sampling QA/QC procedures were followed:

- Clean latex gloves were worn by the Jacques Whitford technicians during sampling and were changed before each new sample was collected.

- The sampling equipment was cleaned after collection of each sample set. Cleaning involved a detergent solution wash, followed by rinses with distilled water and then allowed to air-dry before the next sampling.

- Samples were stored in a cooler (provided by PSC) at 4°C in the field and will be delivered to PSC after collection as soon as possible.

The field procedures for collecting soil and water samples are similar, except that different types of containers and preservatives are used for water sampling.

3. REQUIRED CONTAINERS

As outlined in Section five of the MOE (1996) guidance document all samples, including field samples, field blanks and travelling blanks were collected by Jacques Whitford in MOE recommended containers. PSC provided Jacques Whitford with the required sample containers. The following procedures in Table 1 describe container and preservative requirements during sampling and transportation of samples from the field to the analytical laboratory.

Table 1: Appropriate Containers, Preservatives and Storage for Soil and Water Samples, Inco, Port Colborne, Ontario

Parameter	Container	Preservative	Maximum Holding Time
Soil Samples			
Total Metals	Plastic or glass	None	180 days
Water Samples			
Total Metals (excluding mercury and hexavalent chromium)	Polyethyleneterephthalate (PET) or glass with plastic-lined cap	HNO_3 (containing <1mg/L of total metals) to pH between 1.5 and 2.0	60 days

From: *Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario*, Sections 5.1 and 5.2, (MOE, 1996).

Prior to undertaking field data collection programs the following was undertaken:

- Prior to the onset of field program, Jacques Whitford technicians informed PSC of the sample medium, analytes of interest and required sample containers and coolers.

- PSC provided Jacques Whitford with the required number of containers with appropriate preservatives, when required.

4. FIELD QA/QC

As outlined in Section 7 of the MOE (1996) guidance document, all sampling and sample handling was conducted with utmost care, to prevent cross-contamination of samples. The following procedures describe field QA/QC requirements during sampling and transportation of samples from the field to the analytical laboratory.

4.1 Blanks

Blanks are analytical quality control samples analysed in the same manner as site samples. They were used to determine whether contamination has been introduced into a sample either in the field while the samples were being collected, or in the laboratory during sample preparation or analysis.

4.1.1 Travelling Blank

A travelling blank is a sample of uncontaminated water free of the analytes of interest that is prepared by the laboratory performing the chemical analysis. Travelling blanks are used to determine whether sample contamination occurred in the sample containers and/or as a result of sample cross-contamination during sample transport and storage. For the field programs, where travelling blanks were required the following was undertaken:

- PSC provided adequate travelling blank(s) to Jacques Whitford prior to the onset of field investigation.
- The travelling blank accompanied the sample containers to the sampling location. Jacques Whitford carried the travelling blank to the field and returns it, unopened, to the PSC laboratory for chemical analysis.

4.1.2 Travelling Spiked Blank

A travelling spiked blank is a sample of uncontaminated matrix (i.e. water, soil, sediment, air absorbent) free of any interfering substances to which a known amount of standard solution containing known amounts of the analytes of interest and appropriate preservatives have been added by the laboratory performing the chemical analysis. For the field programs, where travelling spiked blanks were required the following was undertaken:

- PSC prepared and provide samples of travelling spiked blanks to Jacques Whitford prior to the onset of any field investigation.

- PSC spiked the travelling spiked blank with solutions containing all the target parameters required to be analysed at a level of five-to-ten times the concentrations of each analyte of interest at the specific site.

- The travelling spiked blank was prepared within 24 hours of accompanying the containers required for sampling at the site. Jacques Whitford carried the travelling spiked blank to the field and returned it, unopened, to the PSC laboratory for chemical analysis.

4.1.3 Field Blank

Field blanks were used during the collection of surface water samples. A field blank is a sample of uncontaminated water free of the analytes of interest that is prepared by the laboratory performing the chemical analysis. Field blanks were used to determine whether sample contamination occurred in the sample containers and/or as a result of sample cross contamination during sample collection procedures in the field. For the surface water collection program, the following was undertaken:

- PSC provided samples of a field blank to Jacques Whitford prior to the onset of field collection of samples.

- The field blank accompanied the sample containers to the sampling location. At the sampling location Jacques Whitford opened the field blank container, at least as long as the filling of other sample bottles was required, closed the container, and returned the container to the laboratory with the samples for analysis.

4.2 Duplicate Samples

Duplicate samples are any number of additional samples collected in the same place and at the same time as the original sample. Duplicates samples were collected and analysed to provide an estimate of sample variability. For the various field data collection programs the following was undertaken:

- Jacques Whitford collected duplicate samples for all test groups as outlined in Section 7.2 of the MOE (1996) guidance document.

- Jacques Whitford calculated the percentage differences between analysed values for the original and duplicate samples as part of an overall QA/QC program to determine the reproducibility or variability related to sampling procedures and sample homogeneity.

5. DOCUMENTATION

5.1 Documentation and Shipping

Proper documentation by Jacques Whitford staff in the field is an important for ensuring the integrity of samples shipped from the field to the laboratory. Proper documentation includes: field observations, station sampling summaries, chain of custody forms, correct shipping conditions for samples and Transportation of Dangerous Goods (TDG) compliance, when required.

5.2 Chain of Custody Records

Chain of Custody Records were completed and accompanied all field samples collected during the ERA field programs. For field programs the following was undertaken:

- PSC provided the Chain of Custody Forms. A sample copy is provided in Appendix A.
- A Jacques Whitford field technician completed all relevant sections of the Chain of Custody Form during sampling and Jacques Whitford's Project Manager (PM), or a person designated by the PM, was responsible for clearly indicating the required analytical testing on the Chain of Custody form.

5.3 Shipping

To in insure quality control of samples during shipping the following was undertaken for samples collected during the field programs:

- PSC provided the required sample coolers with ice or cold packs. Jacques Whitford will ensure proper packaging to prevent spillage and breaking of glass bottles.
- Jacques Whitford was responsible for preserving the samples at optimum temperature at 4°C until the laboratory receives the samples.

If possible, the samples were delivered by a Jacques Whitford field technician in person. However, when this was not possible, PSC's courier was used. Once the samples were delivered to the laboratory, the Chain of Custody form was signed by both parties to ensure the tracking of sample movement. Both Jacques Whitford and PSC retained copies of these signed forms.

All analytical methods used by PSC included details of sample pretreatment/preparation, clean-up (if required), instrumental measurement method, and data reporting procedures. All documentation is accompanied by references.

6. LABORATORY TESTING GUIDELINES

PSC undertook the chemical analysis of the samples after they were submitted by a Jacques Whitford representative. A properly completed and signed Chain of Custody form was included with all sample batches submitted to PSC. Instructions for analyses of specific chemical parameters with previously agreed upon (Jacques Whitford and PSC) method detection limits (EQL) appropriate for the regulatory criteria to which the results were compared, was included with each sample submitted to PSC.

6.1 Sample Preparation and Digestion

As outlined in Section 5.1 of the MOE (1996) guidance document, PSC prepared all samples prior to analysis conducted by instrument. Sample preparation and digestion procedures that were followed by PSC for inorganic analyses are outlined below.

6.1.1 Soils

- Soil samples were spread out on drying trays in a dust free environment and dried at 30-35°C to constant weight (overnight). A drying blank was prepared and analysed. PSC retained this moisture data and reported this information as part of the Certificate of Analysis.

- The sample was then be disaggregated with a mortar and pestle and screened through a 2 mm sieve. The fraction greater than 2 mm was discarded. The fraction less than 2 mm was ground to pass through a 355 µm sieve.

- The samples were then digested using concentrated nitric acid and hydrochloric acids. The digestion involves the following procedure.
  - One (1) g of soil sample was weighed into a beaker.
  - 2mls of concentrated HNO_3 was added and the mixture is allowed to sit at room temperature for 1 hour.
  - 6mls of concentrated HCl was added and the mixture is sample is left at room temperature for 30 minutes.
  - The sample was refluxed at 90^{o}C for 1 hour and then evaporated to incipient dryness.
  - 1.5mls of concentrated HNO_3 was added and the volume is diluted to 25mls with de-ionized water.

- After adding de-ionized water to the sample, the sample was allowed to settle before analysis was conducted. If the sample contained floatable particles it was centrifuged.

6.1.2 Water

- No digestion is required for surface water samples.
- Groundwater samples were digested using a concentrated nitric acid and hydrochloric acid similar to the soil samples.
- Groundwater samples were field filtered and preserved. A filter blank was analysed for each sample set to determine whether the sample contamination occurred during the sample collection procedures in the field or not.

6.1.3 Vegetation/Garden/Orchard Produce

- Once received, PSC washed the samples with distilled water. Samples were digested using hot nitric acid at 90oC until the biomass is dissolved. Time required to dissolve biomass varies depending on the type of sample.
- Once the biomass was dissolved completely, the sample was allowed to settle before analysis, if the sample still contained floatable particles it was centrifuged.

6.1.4 Maple Sap

- Maple sap samples were collected by Jacques Whitford in accordance with the University of Toronto, Faculty of Forestry collection protocol (U of T 1992).
- Prior to chemical analysis, the maple sap samples were digested using hot nitric acid.

6.1.5 Air

- Once collected, the air filters were submitted to PSC for chemical analysis. Prior to chemical analysis, the samples (air filters) were digested using hot nitric acid. Digest is centrifuged and the supernatant is analysed.
- A filter blank was analysed with each sample set.

6.2 Sample Analysis

Analytical procedures and instruments were pre-selected by Jacques Whitford and PSC in accordance with the MOE (1996) guidance document. This selection was based on a sample matrix, detection limits to be reached, comparability to guidelines, parameters analysed, availability and suitability of techniques and instrumentation.

Analytical methods and QA/QC protocols have been referenced by PSC to recognized standard setting organizations such as US EPA, CSA, and ASTM. If alternate equivalent methods were used, then these are demonstrated in the specific protocol to meet acceptable precision, accuracy and sensitivity requirements. Table 2 shows the analytical guidelines for metal parameters.

Table 2: Analytical Methods and Instrumentation, Inco, Port Colborne, Ontario

Parameter	Analytical Method	Instrument
Soil, Water, Air (Particulate)* Samples		
Metals (17 including, Al, Ba, Be, Ca, Cd, Cu, Co, Cr, Fe, Mg, Mn, Mo, Ni, Pb, Sr, V and Zn)	US EPA Method 6010, Rev. 0	Inductively Coupled Plasma-Atomic Emission Spectrometer
Metals (As, Sb and Se)	US EPA Method 7061 and 7741 (Modified)	Hydride Generation Atomic Absorption Spectrophotometer
Garden Produce and Maple Sap Samples		
Metals (17including, Al, Ba, Be, Ca, Cd, Cu, Co, Cr, Fe, Mg, Mn, Mo, Ni, Pb, Sr, V and Zn)	U.S.EPA Method 200.8 (Modified)	Inductively Coupled Plasma-Mass Spectrometer
Metals (As, Sb and Se)	U.S. EPA Method 7061 and 7741 (Modified)	Hydride Generation Atomic Absorption Spectrophotometer

Note: *For Air Particulate Sampling (Filter): US EPA, 40 CFR, Part 53- Ambient Air Monitoring Reference and Equivalent Methods

Table 3 shows the sample matrix and pre-selected EQL's for all seventeen (17) ICP metal parameters as well as for arsenic, selenium and antimony for the Port Colborne samples.

Table 3: Method Detection Limit (EQL) Criteria and Sample Matrix, Inco, Port Colborne, Ontario

Parameter	Soil[a] (µg/g)	Vegetation (µg/g)	Groundwater[a] (µg/L) (2.5 m)*	Surface Water[b] (µg/L) bh102-1 (0.5 m)*	Air[c] ((µg/Filter)
Aluminum	20	0.6	5.0	5.0	5.0
Antimony	0.2	0.05	0.5	0.5	0.2
Arsenic	0.2	0.2	2.0	2.0	1.0
Barium	5.0	0.5	5.0	5.0	2.0
Calcium	50	50	500	500	200
Cadmium	0.3	0.01	0.1	0.1	0.05
Cobalt	2.0	0.01	0.1	0.1	0.05
Copper	1.0	0.05	0.5	0.5	0.5
Chromium	1.0	0.5	5.0	5.0	2.0
Iron	50	5.0	30	30	20
Magnesium	20	20	50	50	20
Manganese	1.0	0.5	5.0	5.0	2.0
Molybdenum	3.0	0.1	5.0	5.0	0.5
Nickel	2.0	0.1	1.0	1.0	0.5
Lead	5.0	0.05	0.5	0.5	0.2
Selenium	0.2	0.2	2.0	2.0	1.0
Strontium	0.3	0.1	1.0	1.0	0.5
Vanadium	1.0	0.05	0.5	0.5	0.5
Zinc	5.0	0.5	5.0	5.0	5.0

Notes: a Meet MOE Table A Residential/Parkland Land Use Criteria
B Meet MOE's Provincial Water Quality Objective (PWQO) Criteria
C Meet MOE's Ambient Air Quality Criteria (AAQC)

7. STANDARD REFERENCE MATERIALS (SRM)

PSC and Jacques Whitford purchased commercial standard reference materials (SRMs) for QA/QC for the laboratory analysis of soil and vegetation samples as an added check on the variability related to an analytical procedure. SRM's, Spinach leaves and San Joaquin soil, were purchased from the National Institute of Standards & Technology. The Certificate of Analysis for the SRM's are attached to this protocol (Appendix B). Jacques Whitford compared data from PSC's results on the SRM samples to those referenced by the originating authority. For the assessment of the quality of PSC's analysis of field samples, Jacques Whitford calculated the percentage difference of the PSC's results on SRM samples to determine the accuracy of each analytical determination.

8. LABORATORY QA/QC

As outlined in the MOE (1996) guidance document, PSC observed the following QA/QC procedures to perform the chemical analyses.

- Pre-run* QC:
  - labware and reagent blanks;
  - instrument setup standard;
  - reference standard to validate in-house standards; and
  - instrument detection limits (IDLs) and detector linearity curves (minimum of 5-point calibration).

- In-run* QC:
  - baseline drift blanks;
  - standards; and
  - instrument checks.

- Run* QC:
  - method recovery blanks;
  - mthod blanks;
  - in-house matrix check material;
  - duplicates (minimum of one set per run* of 30 samples). As mentioned in the MOE (1996) guidance document, a duplicate sample is defined as a second aliquot from the same sample container;
  - surrogates (added prior to organic extraction). The surrogates should be selected to cover the whole range of the particular scan. It is recommended to use a minimum of three surrogates per organic type scan, except PCBs, where one surrogate can be used. Surrogates are not used in inorganic analyses and thus will not be used in these analyses of the Port Colborne samples;
  - spiked samples, if applicable;
  - certified standard reference materials (SRMs) to validate method recovery; and
  - Estimated Quantitation Limit (EQL) for each parameter.

** "run" refers to a group of samples submitted as one group, and consisting of 30 or fewer samples.*

9. DATA MANAGEMENT: QA/QC

The quality of data depends upon planning, sampling, analysis and reporting. As a means of determining the reproducibility or variability related to analytical procedures of the sample homogeneity, Jacques Whitford calculated the percentage differences between analysed values for the original and duplicate samples.

Further, as a means of determining sample accuracy, Jacques Whitford calculated the percentage differences between the analytical results of the SRM samples and the referenced SRM correlation data.

For sample reproducibility calculations, percentage differences were calculated by Jacques Whitford for those chemical parameters with analytical values greater than 3 X LOQ (LOQ is the limit of quantification, i.e., the lowest level of a parameter that can be identified with confidence by an analytical laboratory).

Percentage differences were determined using the following formula:

$$Percentage\ difference\ of\ \ Analyte\ A = \frac{(Analyte\ A\ in\ test\ 1 - Analyte\ A\ in\ test\ 2)\ x\ 100}{(Analyte\ A\ in\ test\ 1 + Analyte\ A\ in\ test\ 2) / 2}$$

10. REPORT OF ANALYSIS

The Laboratory Report of Analysis as provided by PSC includes the sample results as well as all run quality QC, recovery data and EQL data. The acceptability of the laboratory data will include the following considerations:

- The analytical method performance should meet the requirement criteria as outlined in Section 8.3, 8.4 and 8.5 of the MOE (1996) guidance document.
- The results of laboratory QC samples that are applicable to the matrix and contaminant groups of interest (method blank, spiked blank, spiked sample) will be within the statistically determined control limits of 30%. PSC was responsible for any QC results that exceed the control limits.
- A table of the precision and accuracy estimates associated with the reported results was provided based on duplicate/replicate analyses of Port Colborne samples, and through periodic analysis of standard or certified reference materials as available for each analyte selected at appropriate concentrations.
- Analytical data was reported without correction, unless correction is clearly identified and described.

11. REFERENCES

Ontario Ministry of the Environment, June 1996, *Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario.*

University of Toronto, Faculty of Forestry, November 1992, *Relationship of Sugar Maple Acer Saccharum Decline and Corresponding Chemical Changes in the Sap Composition (Carbohydrates and Trace Elements).*

APPENDIX A

SAMPLE CHAIN OF CUSTODY RECORD

APPENDIX B

STANDARD REFERENCE MATERIAL

Certificate of Analysis

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1570a

Trace Elements in Spinach Leaves

This Standard Reference Material (SRM) is intended primarily for use in evaluating the reliability of analytical methods for the determination of major, minor, and trace elements; proximates; calories; and total dietary fiber in botanical materials, agricultural food products, and materials of similar matrix. A unit of SRM 1570a consists of 60 g of finely powdered dried spinach leaves.

Certified Concentration Values: The certified concentration values of the constituent elements are given in Table 1. These concentrations are based on the agreement of results from at least two independent analytical methods or from a method of known accuracy. Analytical methods are provided in Appendix A.

Reference Concentration Values: Reference concentration values of constituent elements are provided in Table 2; analytical methods are provided in Appendix A. Reference concentration values for selected proximates and total dietary fiber are provided in Table 3; analytical methods are provided in Appendix B. Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Information Concentration Values: Information concentration values for additional constituent elements are provided in Table 4. Information values for carbohydrate, caloric content, fat, and individual fatty acids are provided in Table 5. These are noncertified values with no reported uncertainties as there is insufficient information to assess uncertainties. The information values are given to provide additional characterization of the material. Use of this SRM to quantitatively monitor method performance for analytes other than those with certified or reference concentration values in Tables 1 through 3 is not warranted.

Expiration of Certification: The certification of this SRM lot is valid until **31 August 2008**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. Value assignment is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certified values before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

The original technical and support aspects involved in the certification and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by R.A. Alvarez and T.E. Gills. Revision of this certificate was coordinated through the NIST Standard Reference Materials Program by J.C. Colbert.

Willie E. May, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Revised Certificate Issue Date: 31 August 2001
See Certificate Revision History on Last Page

John Rumble, Jr., Acting Chief
Standard Reference Materials Program

Coordination of analytical measurements for the characterization of this SRM was performed by D.A. Becker and K.E. Sharpless of the NIST Analytical Chemistry Division.

Analytical measurements at NIST were performed by E.S. Beary, D.A. Becker, C.M. Beck II, M.S. Epstein, J.D. Fassett, K.M. Garrity, R.R. Greenberg, R.M. Lindstrom, E.A. Mackey, P. Morales, K.E. Murphy, P.J. Paulsen, B.J. Porter, T.A. Rush, R. Saraswati, J.M. Smeller, G.C. Turk, R.D. Vocke, R.L. Watters, Jr., and L.J. Wood. Additional elemental analyses were performed by D.L. Anderson (Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Washington, DC), A.R. Byrne (Nuclear Chemistry Department, Jozef Stefan Institute, Ljubljana, Slovenia), and J. Kucera (Nuclear Physics Institute, Academy of Sciences of the Czech Republic, Rez, Czech Republic). Several elements were also measured in an International Atomic Energy Agency (IAEA) interlaboratory comparison exercise. Proximates, calories, fatty acids, and total dietary fiber were determined by Covance Laboratories (Madison, WI), Lancaster Laboratories (Lancaster, PA), Medallion Laboratories (Minneapolis, MN), and Southern Testing and Research Laboratories (Wilson, NC).

Statistical analysis of the experimental data was performed by W. Guthrie, S.B. Schiller, and L.M. Gill of the NIST Statistical Engineering Division.

NOTICE AND WARNINGS TO USERS

Stability: This material was radiation sterilized at an estimated minimum dose of 27.8 kGy for microbiological control; however, its stability has not been rigorously assessed. Spinach leaves have a tendency to rapidly bleach and to turn a tan or light brown color in the presence of visible light. Based on 15 years experience with the original SRM 1570, there is no evidence documenting any change in elemental concentrations as a result of that color change. However, NIST will monitor this material and will report any substantive changes in certified values to the purchaser.

Storage: The material should be kept tightly closed in its original bottle and stored in the dark at a temperature between 10 °C and 30 °C. It should not be exposed to intense sources of radiation. Ideally, the bottle should be kept in a desiccator under the conditions indicated above.

Instructions for Use: The contents of a bottle should be thoroughly mixed by rotating and/or rolling before each use. Allow the contents to settle for 1 minute prior to opening to minimize the loss of fine dust particles. A minimum sample mass of 150 mg of the material, dried as described in the section on "Instructions for Drying", should be used to relate analytical determinations to the certified values on this certificate. In some cases, especially for volatile elements such as mercury, it is preferable to analyze samples from the bottle without drying, determine the moisture content on a separate sample from the same bottle taken at the same time, and convert the analytical results to a dry-mass basis.

Digestion procedures should be designed to avoid loss of volatile elements, such as arsenic and mercury. Digestion of the SRM in nitric and perchloric acids was found to be incomplete, with a small residue of siliceous material remaining. This residue must be considered an integral part of this SRM and should be dissolved with a small amount of hydrofluoric acid to obtain total dissolution. All certified values are based on the total dissolution.

Instructions for Drying: Samples of this SRM must be dried by one of the following two procedures in order for certified values to be valid:

1. Drying in a desiccator at room temperature (approximately 22 °C) for 120 h over fresh anhydrous magnesium perchlorate. The sample depth should not exceed 1 cm.

2. Freeze-drying for 24 h at a pressure of 13.3 Pa or lower and a shelf temperature of –5 °C or lower after having frozen the sample (not to exceed 1 cm in depth) at –40 °C or lower for at least 1 h. At the end of the 24 h period, samples should be placed immediately in a desiccator with fresh anhydrous magnesium perchlorate. Samples should be weighed after allowing a minimum of 4 h to establish temperature equilibrium.

Note: Vacuum drying at room temperature and oven drying at elevated temperatures have resulted in excessive mass losses and therefore are **NOT** recommended.

Source and Preparation of Material: The material (approximately 2270 kg) for this SRM was obtained from commercial supplier Oregon Freeze-Drying Corp., Albany, OR. It consists of U.S. Grade A chopped frozen spinach. The material was thawed, placed in a ribbon mixer, thoroughly mixed, and blended. After mixing, the spinach was freeze-dried. The freeze-dried material was then ground in a stainless steel grinder and shipped to NIST. At NIST, the freeze-dried material was sieved through a polypropylene sieve having openings of 0.25 mm (equivalent to a U.S. Series 60 standard sieve). The sieved material was then jet milled and air classified to a particle size of approximately 75 µm (200 mesh). After mixing in a large blender, the spinach was irradiated with cobalt-60 radiation to a minimum absorbed dose of approximately 27.8 kGy for microbiological control and bottled.

Homogeneity Assessment: Samples from randomly selected bottles of SRM 1570a were tested for homogeneity by instrumental neutron activation analysis (INAA). No evidence of statistically significant inhomogeneity was observed.

Table 1. Certified Concentration Values of Constituent Elements[a,b]

Element	Mass Fraction (%)
Calcium	1.527 ± 0.041
Phosphorus	0.518 ± 0.011
Potassium	2.903 ± 0.052
Sodium	1.818 ± 0.043

Element	Mass Fraction (mg/kg)	Element	Mass Fraction (mg/kg)
Aluminum	310 ± 11	Mercury	0.030 ± 0.003
Arsenic	0.068 ± 0.012	Nickel	2.14 ± 0.10
Boron	37.6 ± 1.0	Selenium	0.117 ± 0.009
Cadmium	2.89 ± 0.07	Strontium	55.6 ± 0.8
Cobalt	0.39 ± 0.05	Thorium	0.048 ± 0.003
Copper	12.2 ± 0.6	Vanadium	0.57 ± 0.03
Manganese	75.9 ± 1.9	Zinc	82 ± 3

[a] The certified concentrations are equally weighted means of results from two or more different analytical methods or the mean of results from a single method of known high accuracy. In the case of two or more methods, each uncertainty is the sum of a 95 % confidence limit and an allowance for systematic error between the methods used. In the case of a method of known accuracy, each uncertainty is the sum of a 95 % confidence limit and the known systematic error of the method.

[b] These certified values are reported on a dry-mass basis. For certified values to be valid, the material must be dried according to the instructions provided above.

Table 2. Reference Concentration Values of Constituent Elements[a,b,c]

Element	Mass Fraction (%)
Nitrogen (Total)[d]	6.06 ± 0.20
Nitrogen (Organic)[d]	6.20 ± 0.25
Nitrogen (Protein)[d]	5.68 ± 0.13

Element	Mass Fraction (mg/kg)	Element	Mass Fraction (mg/kg)
Europium	0.0055 ± 0.0010	Rubidium	12.7 ± 1.6
Scandium	0.0055 ± 0.0006	Uranium	0.155 ± 0.023

[a] NIST has replaced the previously used term "non-certified" with "reference value" or "information value," as appropriate.

[b] Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of results provided by NIST and/or collaborating laboratories. The uncertainty in the reference concentration values is calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty calculated according to the ISO Guide [1], which accounts for the combined effect of the within-laboratory variance for all participating laboratories at one standard deviation and bias between methods. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte.

[c] These reference values are reported on a dry-mass basis. In order for these reference values to be valid, the material must be dried according to the instructions provided above.

[d] Data from three methods for the determination of nitrogen have been treated separately. Total nitrogen was determined by prompt gamma activation analysis; "organic" nitrogen was determined by the Dumas method; and "protein" nitrogen was determined by the Kjeldahl method.

Table 3. Reference Concentration Values of Selected Proximates and Total Dietary Fiber[a]

Analyte	Mass Fraction, as received (%)	Mass Fraction, dry-mass basis (%)[b]
Moisture[c]	3.45 ± 0.25	0 (by definition)
Solids[c]	96.55 ± 0.25	100 (by definition)
Ash	14.66 ± 0.38	15.18 ± 0.38
Protein[d]	35.8 ± 3.0	37.0 ± 3.1
Total dietary fiber	30.5 ± 4.3	31.6 ± 4.4

[a] Each reference concentration value, expressed as a mass fraction on an as-received or dry-mass basis, is an equally weighted mean of results from the laboratories shown in Appendix C. (NIST and one of these laboratories provided results used in value assignment of mass fractions of moisture and solids; see footnote c.) The uncertainty in the reference values is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO Guide [1]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Appendix B.

[b] Results have been converted to a dry-mass basis using the reference value for solids. Uncertainty in the solids determination has been included in the uncertainties provided for the mass fractions on a dry-mass basis.

[c] Moisture has been determined by NIST (using freeze-drying and desiccation) and one of the collaborating laboratories (using desiccation) as specified in this certificate. Drying in a forced-air or vacuum oven by three laboratories resulted in a moisture value of 6.3 % ± 1.5 %.

[d] The protein concentration was calculated from the nitrogen values reported by the laboratories (two laboratories using the Dumas method, two laboratories using Kjeldahl) using a conversion factor of 6.25. The value for protein is the mean of the individual protein calculations reported by the laboratories shown in Appendix C. If the mean nitrogen values above are used for calculation, the mean protein concentrations are 35.8 % and 37.1 % on an as-received and dry-mass basis, respectively.

Table 4. Information Concentration Values of Constituent Elements[a]

Element	Mass Fraction (%)
Magnesium	0.89
Sulfur	0.46
Element	**Mass Fraction (mg/kg)**
Lead	0.20

[a] NIST has replaced the previously used term "non-certified" with "reference value" or "information value," as appropriate.

Table 5. Information Concentration Values of Carbohydrate, Fat, Caloric Content, and Selected Fatty Acids (as Triglycerides)[a]

Analyte	Mass Fraction, as received (%)	Mass Fraction, dry-mass basis (%)
Carbohydrate[b]	45	46
Fat	2	2
Calories[b,c]	340 kcal/100g	350 kcal/100g
Pentadecanoic Acid (C15:0)	0.010	0.011
Hexadecanoic Acid (C16:0) (Palmitic Acid)	0.61	0.64
Heptadecanoic Acid (C17:0) (Margaric Acid)	0.006	0.006
Octadecanoic Acid (C18:0) (Stearic Acid)	0.031	0.032
(Z)-9-Octadecenoic Acid (C18:1) (Oleic Acid)	0.25	0.26
(Z,Z)-9,12-Octadecadienoic Acid (C18:2) (Linoleic Acid)	0.27	0.28
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3) (Linolenic Acid)	0.63	0.65
Linolenic Acid (C18:3)	0.048	0.050
Docosanoic Acid (C22:0) (Behenic Acid)	0.028	0.029
Tetracosanoic Acid (C24:0) (Lignoceric Acid)	0.044	0.046

[a] These information values, reported on an as-received and dry-mass basis, are the equally weighted means of results reported by the collaborating laboratories shown in Appendix C. These values are based on results from determinations by two to four of the laboratories and are included to provide additional characterization of the material; no uncertainties are provided. Analytical methodology information is provided in Appendix B.

[b] These information values are calculated from the results reported by one laboratory.

[c] If the mean proximate values in Tables 2 and 4 are used for calculation, with caloric equivalents of 9, 4, and 4 for fat, protein, and carbohydrate, respectively, the mean caloric content is 340 kcal/100 g and 350 kcal/100g on an as-received and dry-mass basis, respectively.

REFERENCE

[1] *Guide to the Expression of Uncertainty in Measurement*, ISBN 92-67-10188-9, 1st Ed. ISO, Geneva, Switzerland, (1993); see also Taylor, B.N. and Kuyatt, C.E., "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results," NIST Technical Note 1297, U.S. Government Printing Office, Washington, DC, (1994); available at http://physics.nist.gov/Pubs/.

Certificate Revision History: 31 August 2001 (This technical revision reports the addition of reference and information values for proximates, calories, total dietary fiber, and fatty acids and a change from non-certified to reference and information values for several inorganic constituents); 15 July 1996 (editorial change); 20 October 1994 (original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet http://www.nist.gov/srm.

Appendix A. Methods Used in Elemental Determinations

Element	Method Code[a]	Element	Method Code[a]
Aluminum	ICP INAA	Nitrogen	DUMAS KJEL PGAA
Arsenic	FI-HGAAS RNAA	Phosphorus	COLOR ICP
Boron	IDICPMS PGAA	Potassium	IDTIMS INAA
Cadmium	IDICPMS PGAA RNAA	Rubidium	IAEA INAA
Calcium	IDTIMS INAA	Scandium	IAEA INAA
Cobalt	INAA RNAA	Selenium	FI-HGAAS INAA RNAA
Copper	ICP RNAA	Sodium	PGAA INAA
Europium	IAEA INAA	Strontium	IDTIMS INAA
Lead	IAEA IDICPMS	Sulfur	PGAA IAEA
Magnesium	IDICPMS INAA	Thorium	INAA RNAA
Manganese	INAA LEAFS	Uranium	RNAA
Mercury	CVAAS RNAA	Vanadium	IDTIMS INAA
Nickel	IDICPMS RNAA	Zinc	ICP INAA

[a] Acronyms for analytical methods:

CVAAS = Cold-Vapor Atomic Absorption Spectrometry
FI-HGAAS = Flow Injection Hydride Generation Atomic Absorption Spectrometry
IAEA = International Atomic Energy Agency Interlaboratory Comparison Exercise
ICP = Inductively Coupled Plasma Optical Emission Spectrometry
IDICPMS = Isotope Dilution, Inductively Coupled Plasma Mass Spectrometry
IDTIMS = Isotope Dilution, Thermal Ionization Mass Spectrometry
INAA = Instrumental Neutron Activation Analysis
KJEL = Kjeldahl Nitrogen Determination
LEAFS = Laser-Excited Atomic Fluorescence Spectrometry
PGAA = Prompt Gamma Activation Analysis
RNAA = Radiochemical Neutron Activation Analysis

Appendix B. Methods Used in the Determination of Proximates, Caloric Content, Fatty Acids, and Total Dietary Fiber

Ash – mass loss after ignition in a muffle furnace
Calories – calculated; [(9 × fat) + (4 × protein) + (4 × carbohydrate)]
Carbohydrate – calculated; [solids – (protein + fat + ash)]
Fat – sum of individual fatty acids
Fatty acids – hydrolysis followed by gas chromatography
Moisture – mass loss after drying at room temperature in a desiccator (1 laboratory + NIST); freeze-drying (NIST)
Nitrogen – Dumas (1 laboratory); modified Dumas (1 laboratory); Kjeldahl (2 laboratories + NIST)
Protein – calculated from nitrogen reported by 4 laboratories using a factor of 6.25
Solids – calculated; (sample mass – moisture)
Total dietary fiber – enzymatic digestion followed by gravimetry

Appendix C. Collaborating Laboratories for Proximate, Fatty Acid, Total Dietary Fiber, and Caloric Determinations

Covance Laboratories, Madison, WI, USA
Lancaster Laboratories, Lancaster, PA, USA
Medallion Laboratories, Minneapolis, MN, USA
Southern Testing and Research Laboratories, Wilson, NC, USA

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 2709

San Joaquin Soil

Baseline Trace Element Concentrations

This Standard Reference Material (SRM) is intended primarily for use in the analysis of soils, sediments, or other materials of a similar matrix. SRM 2709 is an agricultural soil that was oven-dried, sieved, and blended to achieve a high degree of homogeneity. A unit of SRM 2709 consists of 50 g of the dried material.

The certified elements for SRM 2709 are given in Table 1. The values are based on measurements using one definitive method or two or more independent and reliable analytical methods. Noncertified values for a number of elements are given in Table 2 as additional information on the composition. The noncertified values should not be used for calibration or quality control. Analytical methods used for the characterization of this SRM are given in Table 3 along with analysts and cooperating laboratories. All values (except for carbon) are based on measurements using a sample weight of at least 250 mg. Carbon measurements are based on 100 mg samples.

NOTICE AND WARNINGS TO USERS

Expiration of Certification: This certification is valid for 5 years from the date of shipment from NIST. Should any of the certified values change before the expiration of the certification, purchasers will be notified by NIST. Return of the attached registration card will facilitate notification.

Stability: This material is considered to be stable; however, its stability has not been rigorously assessed. NIST will monitor this material and will report any substantive changes in certification to the purchaser.

Use: A minimum sample weight of 250 mg (dry weight - see Instructions for Drying) should be used for analytical determinations to be related to the certified values on this Certificate of Analysis.

To obtain the certified values, sample preparation procedures should be designed to effect complete dissolution. If volatile elements (i.e., Hg, As, Se) are to be determined, precautions should be taken in the dissolution of SRM 2709 to avoid volatilization losses.

Statistical consultation was provided by S.B. Schiller of the NIST Statistical Engineering Division.

The overall direction and coordination of the analyses were under the chairmanship of M.S. Epstein and R.L. Watters, Jr., of the NIST Inorganic Analytical Research Division.

The technical and support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the Standard Reference Materials Program by T.E. Gills and J.S. Kane.

Gaithersburg, MD 20899
August 23, 1993
(Revision of certificate dated 10-30-92)

Thomas E. Gills, Acting Chief
Standard Reference Materials Program

(over)

Instructions for Drying: When nonvolatile elements are to be determined, samples should be dried for 2 h at 110 °C. Volatile elements (i.e., Hg, As, Se) should be determined on samples as received; separate samples should be dried as previously described to obtain a correction factor for moisture. Correction for moisture is to be made to the data for volatile elements before comparing to the certified values. This procedure ensures that these elements are not lost during drying. The weight loss on drying has been found to be in the range of 1.8 to 2.5%.

Source and Preparation of Material: The U.S. Geological Survey (USGS), under contract to the NIST, collected and processed the material for SRM 2709. The soil was collected from a plowed field, in the central California San Joaquin Valley, at Longitude 120° 15' and Latitude 36° 30'. The collection site is in the Panoche fan between the Panoche and Cantu creek beds. The top 7.5-13 cm (3-5 in) of soil containing sticks and plant debris was removed, and the soil was collected from the 13 cm level down to a depth of 46 cm (18 in) below the original surface. The material was shoveled into 0.114 m^3 (30 gal) plastic buckets and shipped to the USGS laboratory for processing.

Addendum to SRM Certificates
2709 San Joaquin Soil
2710 Montana Soil
2711 Montana Soil

Leachable Concentrations Using U.S. EPA Method 3050 for Flame Atomic Absorption Spectrometry and Inductively Coupled Plasma Atomic Emission Spectrometry

The certified concentrations of constituent elements in essentially all National Institute of Standards and Technology (NIST) chemical composition Standard Reference Materials (SRMs) are given as total concentrations. The certified concentrations are based on measurements obtained by two or more independent methods or techniques. The measurement methods require complete sample decomposition, or the sample may be analyzed nondestructively. Where complete sample decomposition is required, it can be accomplished by digestion with mixed acids, or by fusion. For mixed acid decomposition, hydrofluoric acid must be included in the acid mixture used to totally decompose siliceous materials such as soils and sediments.

For a number of environmental monitoring purposes, the concentrations of labile or extractable fractions of elements are more useful than total concentrations. Concentrations of labile or extractable fractions are generally determined using relatively mild leach conditions which are unlikely to totally decompose the sample. It should be noted that results obtained using the mild leach conditions are often erroneously depicted in reports as total concentrations. However, reported concentrations of labile or extractable fractions of elements are generally lower than total concentrations; recovery can be total if an element in a given sample is completely labile. Results are often presented as measured concentration in the leachate in comparison to the total or certified concentration. The recovery of an element as a percent of total concentration is a function of several factors such as the mode of occurrence in the sample, leach medium, leach time and temperature conditions, and pH of the sample-leach medium mixture. References 1-27 may be consulted for detailed discussions of these factors and their effect on leach results. Some of these references provide leach data for one or more reference materials.

In its monitoring programs the U.S. Environmental Protection Agency (EPA) has established a number of leach methods for the determination of labile or extractable elements. They include Methods 3015, 3050, and 3051. A number of cooperating laboratories using the variation to U.S. EPA Method 3050 for FAAS and ICP-AES measurements, have reported data for SRMs 2709, 2710, and 2711. This variation of the method uses hydrochloric acid in its final step which is different from Method 3050 for ICP-MS and HGA-AAS measurements. The data obtained are presented in Tables 1, 2, and 3 of this addendum. The names of the cooperating laboratories are listed in Table 4. Several laboratories provided replicate (3-6) analyses for each of the three soil SRMs. The number of results for a given element varied from only one to as many as nine, as indicated in the data presented in Tables 1-3. Because of the wide range of interlaboratory results for most elements, only the data range and median of the individual laboratory means are given. Ranges differ somewhat from those in Ref. 26, since this addendum is based on a larger data set than had been available previously.

For SRMs 2710 and 2711, seventeen laboratories provided data as part of contract work for the U.S. EPA. Each SRM was treated as a blind sample in one quarter of 1992. Since there was no within-laboratory replication of analysis in the design of the exercise, the 17-laboratory means of results were treated as single laboratory results from laboratories using replication, in establishing the median of the full data set. In a few cases, however, the contract laboratories mean was the only result available for a particular element (e.g., Sb in 2710). In others, the contract laboratories mean is also the median for the full leach data set (e.g., As in 2710). An asterisk identifies those cases where the contract laboratories means are given as the median value.

Please note none of the values in Tables 1-3 are certified, but are given as information on the performance of the three (3) soils when used to evaluate, or to provide quality control for Method 3050 followed by FAAS and ICP-AES measurements only. The data should not be used for any other purpose. The certified values, provided as total concentrations, are the best estimate of the true concentrations.

Gaithersburg, MD 20899
August 23, 1993

Thomas E. Gills, Acting Chief
Standard Reference Materials Program

Table 1

Leach Data from Cooperating Laboratories for Soil SRM 2709

Element	Range Wt %	Median	N	% Leach Recovery†
Aluminum	2.0 - 3.1	2.6	5	35
Calcium	1.4 - 1.7	1.5	5	79
Iron	2.5 - 3.3	3.0	8	86
Magnesium	1.2 - 1.5	1.4	5	93
Phosphorus	0.06 - 0.09	0.07	3	100

Carbon	(1.2)	Cerium	(42)
		Cesium	(5.3)
		Dysprosium	(3.5)
		Europium	(0.9)
		Gallium	(14)
		Gold	(0.3)
		Hafnium	(3.7)
		Holmium	(0.54)
		Iodine	(5)
		Lanthanum	(23)
		Molybdenum	(2.0)
		Neodymium	(19)
		Rubidium	(96)
		Samarium	(3.8)
		Scandium	(12)
		Thorium	(11)
		Tungsten	(2)
		Uranium	(3)
		Ytterbium	(1.6)
		Yttrium	(18)
		Zirconium	(160)

Table 3. Analytical Methods Used for the Analysis of SRM 2709

Element	Certification Methods *	Element	Certification Methods *
Ag	ID ICPMS; RNAA	Mo	**ID ICPMS**
Al	XRF1; XRF2; INAA; DCP; ICP	Na	INAA; FAES; ICP
As	RNAA; HYD AAS; INAA	Nd	ICP
Au	**INAA; FAAS**	Ni	ID ICPMS; ETAAS; INAA
Ba	XRF2; FAES	P	DCP; COLOR; XRF2
C	**COUL**	Pb	**ID TIMS**
Ca	XRF1; XRF2; DCP	Rb	**INAA**
Cd	ID ICPMS; RNAA	S	**ID TIMS**
Ce	**INAA; ICP**	Sb	INAA; ETAAS
Co	INAA; ETAAS; ICP	Sc	**INAA; ICP**
Cr	INAA; DCP; ICP	Se	RNAA; HYD AAS
Cs	**INAA**	Si	XRF1; XRF2; GRAV
Cu	RNAA; FAES; ICP	Sm	**INAA**
Dy	**INAA**	Sr	ID TIMS; INAA; ICP
Eu	**INAA**	Th	**ID TIMS; INAA; ICP**
Fe	XRF1; XRF2; INAA; DCP	Ti	INAA; XRF1; XRF2; DCP
Ge	**INAA; ICP**	Tl	ID TIMS; LEAFS
Hf	**INAA**	U	**ID TIMS; INAA**
Hg	CVAAS	V	INAA; ICP
Ho	**INAA**	W	**INAA**
I	**INAA**	Y	**ICP**
K	XRF1; XRF2; FAES; ICP; INAA	Yb	**INAA**
La	**INAA; ICP**	Zn	ID TIMS; ICP; INAA; POLAR
Mg	INAA; XRF1; ICP	Zr	**INAA**
Mn	INAA; ICP		

*Methods in **bold** were used to corroborate certification methods or to provide information values.

COLOR - Colorimetry; lithium metaborate fusion.
COUL - Combustion coulometry.
CVAAS - Cold vapor atomic absorption spectrometry.
DCP - Direct current plasma atomic emission spectrometry; lithium metaborate fusion.
ETAAS - Electrothermal atomic absorption spectrometry; mixed acid digestion.
FAAS - Flame atomic absorption spectrometry; mixed acid digestion except for Au, leached with HBr-Br$_2$.
FAES - Flame atomic emission spectrometry; mixed acid digestion.
GRAV - Gravimetry; sodium carbonate fusion.
HYD AAS - Hydride generation atomic absorption spectrometry.
ICP - Inductively coupled plasma atomic emission spectrometry; mixed acid digestion.
ID ICPMS - Isotope dilution inductively coupled plasma mass spectrometry; mixed acid digestion.
ID TIMS - Isotope dilution thermal ionization mass spectrometry; mixed acid digestion.
INAA - Instrumental neutron activation analysis.
LEAFS - Laser enhanced atomic fluorescence spectrometry; mixed acid digestion.
POLAR - Polarography.
RNAA - Radiochemical neutron activation analysis; mixed acid digestion.
XRF1 - Wavelength dispersive x-ray fluorescence on fused borate discs.
XRF2 - Wavelength dispersive x-ray fluorescence spectrometry on pressed powder.

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The material was spread on 30.5 cm x 61 cm (1 ft x 2 ft) polyethylene-lined drying trays in an air drying oven and dried for three days at room temperature. The material was then passed over a vibrating 2 mm screen to remove plant material, rocks, and large chunks of aggregated soil. Material remaining on the screen was deaggregated and rescreened. The combined material passing the screen was ground in a ball mill to pass a 74 µm screen and blended for 24 h. Twenty grab samples were taken and measured for the major oxides using x-ray fluorescence spectrometry and for several trace elements using inductively coupled plasma atomic emission analysis to provide preliminary assessment of the homogeneity of the material prior to bottling. The material was bottled into 50 g units and randomly selected bottles were taken for the final homogeneity testing.

Analysis: The homogeneity, using selected elements in the bottled material as indicators, was assessed using x-ray fluorescence spectrometry and neutron activation analysis. In a few cases, statistically significant differences were observed, and the variance due to material inhomogeneity is included in the overall uncertainties of the certified values. The estimated relative standard deviation for material inhomogeneity is less than 1% for those elements for which homogeneity was assessed.

Certified Values and Uncertainties: The certified values are weighted means of results from two or more independent analytical methods, or the mean of results from a single definitive method, except for mercury. Mercury certification is based on cold vapor atomic absorption spectrometry used by two different laboratories employing different methods of sample preparation prior to measurement. The weights for the weighted means were computed according to the iterative procedure of Paule and Mandel (NBS Journal of Research 87, 1982, pp. 377-385). The stated uncertainty includes allowances for measurement imprecision, material variability, and differences among analytical methods. Each uncertainty is the sum of the half-width of a 95% prediction interval and includes an allowance for systematic error among the methods used. In the absence of systematic error, a 95% prediction interval predicts where the true concentrations of 95% of the samples of this SRM lie. The certified values were corroborated by analyses from nine Polish laboratories cooperating on the certification under the direction of T. Plebanski and J. Lipinski, Polish Committee for Standardization Measures, and Quality Control. The Polish laboratory work was supported by the Maria Sklodowska-Curie Joint Fund.

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Table 1. Certified Values

Element	wt. %			Element	µg/g		
Aluminum	7.50	±	0.06	Antimony	7.9	±	0.6
Calcium	1.89	±	0.05	Arsenic	17.7	±	0.8
	Iron		3.50±	0.11	Barium	968 ±	40
Magnesium	1.51	±	0.05	Cadmium	0.38	±	0.01
Phosphorus	0.062	±	0.005	Chromium	130	±	4
Potassium	2.03	±	0.06	Cobalt	13.4	±	0.7
Silicon	29.66	±	0.23	Copper	34.6	±	0.7
Sodium	1.16	±	0.03	Lead	18.9	±	0.5
Sulfur	0.089	±	0.002	Manganese	538	±	17
Titanium	0.342	±	0.024	Mercury	1.40	±	0.08
				Nickel	88	±	5
				Selenium	1.57	±	0.08
				Silver	0.41	±	0.03
				Strontium	231	±	2
				Thallium	0.74	±	0.05
				Vanadium	112	±	5
				Zinc	106	±	3

Noncertified Values: Noncertified values, shown in parentheses, are provided for information only. An element concentration value may not be certified if a bias is suspected in one or more of the methods used for certification, or if two independent methods are not available. Certified values for some of these elements will eventually be provided in a revised certificate when more data is available.

Table 2. Noncertified Values

Element	wt %	Element	µg/g

Potassium	0.26 - 0.37	0.32	5	16
Silicon	— —	<0.01	1	<1
Sodium	0.053 - 0.11	0.068	6	6
Titanium	0.03 - 0.04	0.038	3	11
	mg/kg			
Antimony	--- ---	<10	1	---
Arsenic	— ---	<20	2	---
Barium	392 - 400	398	2	41
Cadmium	--- —	< 1	5	---
Chromium	60 - 115	79	5	61
Cobalt	10 - 15	12	5	90
Copper	26 - 40	32	7	92
Lead	12 - 18	13	5	69
Manganese	360 - 600	470	7	87
Molybdenum	--- ---	< 2	2	---
Nickel	65 - 90	78	7	89
Selenium	nr nr	0.014	1	<1
Strontium	100 - 112	101	3	44
Vanadium	51 - 70	62	3	55
Zinc	87 - 120	100	7	94

† % Leach Recovery = $100 \times \left[\frac{\text{Median Value}}{\text{Certified/Info. Value}}\right]$

-- at or below the detection limit
--- no % Leach Recovery calculated
nr no range reported by the laboratory

Table 2

Leach Data from Cooperating Laboratories for Soil SRM 2710

Element	Range	Median	N	% Leach Recovery†
	Wt %			
Aluminum	1.2 - 2.6	1.8	6	28
Calcium	0.38 - 0.48	0.41	7	33
Iron	2.2 - 3.2	2.7	9	80
Magnesium	0.43 - 0.60	0.57	6	67
Phosphorus	0.106 - 0.11	0.11	2	100
Potassium	0.37 - 0.50	0.45	6	21
Silicon	--- ---	<0.01	1	<1
Sodium	0.049 - 0.062	0.054	5	5
Titanium	0.092 - 0.11	0.10	3	35
	mg/kg			
Antimony	5.4 - 12	7.9*	1*	21
Arsenic	490 - 600	590	3	54
Barium	300 - 400	360	3	51
Cadmium	13 - 26	20	8	92
Chromium	15 - 25	19	6	(49)
Cobalt	6.3 - 12	8.2	7	(82)
Copper	2400 - 3400	2780	8	92
Lead	4300 - 7000	5100	*	**

Manganese	6200	- 9000	7700	8	76
Mercury	27	37	32*	1*	98
Molybdenum	13	- 27	20	2	(100)
Nickel	8.8	- 15	10.1	8	71
Silver	24	- 30	28	3	79
Selenium	nr	- nr	0.002	1	...
Strontium	94	- 110	100	3	(42)
Thallium	0.50	- 0.76	0.62*	1*	(48)
Vanadium	37	- 50	43	4	56
Zinc	5200	- 6900	5900	9	83

† % Leach Recovery = $100 \times \left\{ \frac{\text{Median Value}}{\text{Certified/Info. Value}} \right\}$

( ) indicates that information value was used
--- at or below the detection limit
** no % Leach Recovery could be calculated
nr no range reported by laboratory
* U.S. EPA contract laboratories mean; treated as one laboratory since no within-laboratory replication; see text

Table 3

Leach Data from Cooperating Laboratories for Soil SRM 2711

Element	Range		Median	N	% Leach Recovery†
	Wt %				
Aluminum	1.2	- 2.3	1.8	5	28
Calcium	2.0	- 2.5	2.1	5	73
Iron	1.7	- 2.6	2.2	7	76
Magnesium	0.72	- 0.89	0.81	5	77
Phosphorus	0.06	- 0.09	0.088	3	100
Potassium	0.26	- 0.53	0.38	5	16
Silicon	---	---	<0.01	1	< 1
Sodium	0.020	- 0.029	0.026	4	2.3
Titanium	0.039	- 0.048	0.042	2	14
	mg/kg				
Antimony	---	---	<10	1	...
Arsenic	88	- 110	90	3	86
Barium	170	- 260	200	2	28
Cadmium	32	- 46	40	6	96
Chromium	15	- 25	20	4	(43)
Cobalt	7	- 12	8.2	5	(82)
Copper	91	- 110	100	6	98
Lead	930	- 1500	1100	7	95
Manganese	400	- 620	490*	7	77
Molybdenum	---	---	<2	2	...
Nickel	14	- 20	16	7	78
Silver	2.5	- 5.5	4.0	1	86
Selenium	nr	nr	0.009	1	< 1
Strontium	48	- 55	50	3	20
Vanadium	34	- 50	42	3	51
Zinc	290	- 340	310	7	89

† % Leach Recovery = $100 \times \left\{ \frac{\text{Median Value}}{\text{Certified/Info. Value}} \right\}$

( ) indicates information value was used
--- at or below detection limit
** no Leach Recovery could be calculated
nr no range reported by laboratory

* U.S. EPA contract laboratories mean; treated as one laboratory since no within-laboratory replication; see text

Table 4

Leach Study for Cooperating Laboratories

SRMs 2709, 2710, and 2711

S.A. Wilson, U.S. Geological Survey; Lakewood, CO.

J. Lipanski and T. Piekanski, Polish Committee for Standardization, Measures and Quality Control; Warsaw, Poland.

E. Gorecka, Polish Geological Institute; Warsaw, Poland.

M. Paul, Research Institute of Vegetable Crops; Skierniewice, Poland.

I. Matuszczyk, Forest Research Institute; Warsaw, Poland.

Z. Jonca, Institute of Environmental Protection; Warsaw, Poland.

B. Kaluzek, Geological Enterprise; Warsaw, Poland.

I. Twardowska, Polish Academy of Sciences, Institute of Environmental Engineering; Zabrze, Poland.

SRMs 2710 and 2711

L. Butler and D. Hillman, U.S. Environmental Protection Agency; Las Vegas, NV and 17 contract laboratories.

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Laboratory Protocol for Analysis of Biological Tissues
Ecological Risk Assessment & Human Health Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft - November 2002

1. INTRODUCTION

Jacques Whitford Environment Limited (Jacques Whitford) has been retained by Inco Limited (Inco) to undertake work for an Ecological Risk Assessment (ERA) as part of the overall Community Based Risk Assessment (CBRA) process in the Port Colborne area. In order to evaluate the potential risk Inco's industrial activities have had on the natural environment, certain biological samples have been collected from inland ponds, fields, and woodlots. Chemical analysis of these samples provides data that will assist in determining the uptake of chemicals of concern (COCs) by various animals and plants from three media in the environment of Port Colborne (soils, sediment and water).

Samples, including Green and Leopard Frogs, frog livers and gastrointestinal (GI) tracts, tadpoles and tadpole GI tracts, Meadow Voles and vole livers, earthworms, caterpillars, deer meat, rabbit meat, perch meat, spiders, insects, maple leaves, maple cotyledons, wood cores, and corn kernels were sent to Philip Analytical Services Inc. (PSC) for chemical analysis. Tissues were analyzed for 24 elements at the lowest estimated quantitation limit (EQL) attainable for each sample. This report outlines the laboratory procedures for the preparation and chemical analysis of each type of tissue sample mentioned above.

2. OBJECTIVES

- To accurately determine the levels of 24 elements including the chemicals of concern (CoCs) nickel, cobalt, copper and arsenic in the tissues of Green and Leopard Frogs, frog livers and GI tracts, tadpoles and tadpole GI tracts, Meadow Voles and vole livers, spiders, insects, caterpillars, earthworms, perch, deer, rabbits, maple leaves, maple cotyledons, wood cores, and corn kernels.

- To accomplish this objective, this protocol provides guidance as to the analysis of these tissues, including the use of three sub-samples of some specimens.

3. APPARATUS AND MATERIALS

This section presents a list of standard equipment, materials, and reagents that were used at PSC during the analysis of biomaterials identified in Section 1.0.

3.1 Equipment and Materials

- Hotplate capable of maintaining a temperature between 90-95^{o} C.
- Analytical Balance
- Pyrex glass beakers
- Blenders with various sized cups used according to the size of sample being blended.
- Pyrex weighing dishes
- Glass rods

3.2 Reagents

Reagent grade chemicals were used in all cases. The purity of reagents was confirmed prior to their use, by analysis of method blanks. Only those reagents with impurity levels less than the method detection limit were acceptable.

- Reagent water- reverse osmosis deionized (RODI).
- Nitric Acid (concentrated), Environmental Grade.
- Nitric Acid (dilute, 5%).
- Hydrogen Peroxide (30%).

4. PROCEDURES

In this section, the procedures for sample preparation, digestion and analysis are provided for each of the following: tadpole bodies, tadpole GI tracts, frog bodies, frog liver and GI tracts, Meadow Vole bodies, Meadow Vole livers, spider and insect bodies, earthworms, caterpillars, perch, rabbit, deer meat, maple leaves, maple cotyledons, wood cores, and corn kernels. For each sample type, a summary of the method is provided as an overview, followed by a list of apparatus and reagents to be used. Sample handling and storage is addressed under the relevant sections, followed by outlines for sample preparation, moisture determination and sample digestion (U.S. EPA SW – 846 3010 A). Methods used for elemental analyses follow standard references (see Section 5.). Method detection limit criteria are covered in Section 5.1.

4.1 Composite Tadpole Samples

All tadpoles were dissected to remove their gastrointestinal (GI) tracts. In most cases, the mass of an individual tadpole carcass or its GI tract was too small for accurate analysis. Therefore all individual tadpole carcasses and all GI tracts from a given site were combined into two separate composite samples comprising a composite tadpole and composite GI tract sample for each site.

At sites where large tadpoles (approximately 5 grams each) existed, a minimum of five individuals were collected, dissected to remove the GI tract, and then combined to provide composite carcass and GI tract samples of that site. In ponds where only small tadpoles (>0.25 grams) existed, a minimum of 20 individuals were collected and carcass and GI tract samples were combined for that site. This procedure provided PSC with adequate sample size for meaningful chemical analysis.

4.1.1 Summary of Method

1. Composite tadpole samples, excluding GI tracts, were pulverized in the glass beaker to be used for acid digestion using a glass rod.

2. A portion of each composite sample was removed for moisture determination.

3. The glass rod was cleaned with dilute nitric acid into the beaker, to minimize loss of tissue.

4. Digestion of each composite sample was accomplished using nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) at 95°C.

5. The results were reported on a dry weight basis following correction for moisture content.

4.1.2 Sample Handling and Storage

Composite samples were submitted to the lab in pre-weighed numbered plastic containers. Samples were kept frozen and thawed immediately prior to preparation.

4.1.3 Sample Preparation

1. Composite tadpole samples were submitted to PSC in pre-weighed numbered containers.

2. Upon receipt by PSC the samples were weighed. The sample weight was determined by calculating the difference between the pre-weighed container weight and the total weight (container and sample).

3. The sample was removed from the container and placed in a glass beaker, and was subsequently homogenized using a glass rod.

4. A sub-sample of the mixture was set aside for moisture determination and, if enough sample was available, a separate sub-sample for replicate analysis.

5. The weight of the sample digested was equal to the sample weight minus the weight of the removed sub-samples.

6. The glass rod was thoroughly rinsed with a small volume of dilute nitric acid into the pyrex glass beaker to minimize sample loss.

4.1.4 Moisture Determination

The moisture content was determined on the sub-sample set aside for moisture calculations.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt = B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105°C to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.1.5 Sample Digestion

All pyrex glass beakers used for the digestion process were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid and then subjected to a hot concentrated nitric acid reflux for 10 min. This was conducted in the following manner:

1. The sample was weighed (approximately 1gram of biomaterial) and placed into a pre-cleaned 50 or 100ml glass beaker.

2. Ten ml of concentrated nitric acid were added to the glass beaker.

3. The sample was digested at 90 – 95°C until complete dissolution of solid material was achieved; this typically took 3 to 4 hours to complete.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion. If further dissolution of any remaining solid material was necessary additional drops of concentrated nitric acid were added.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube and made to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

Note: If the total sample weight was less than 0.5 grams, the final digestate volume was made up to 25 ml rather than 50 ml so as to maintain consistent estimated quantitation limit (EQL) reporting.

4.2 Composite Tadpole GI Tract Samples

GI tracts of tadpoles from a given site were composited and submitted to PSC for analysis.

4.2.1 Summary of Method

1. Using a glass rod, each entire composite tadpole GI tract sample was pulverized in the glass beaker to be used for acid digestion. The glass rod was cleaned into the glass beaker with dilute nitric acid into the beaker, to minimize loss of tissue.

2. A sub-sample of each composite sample was removed for moisture determination.

3. Digestion was accomplished using nitric acid and hydrogen peroxide at 95°C.

4. The results were reported on a dry weight basis following correction for moisture content.

4.2.2 Sample Handling and Storage

Composite samples were submitted in pre-weighed numbered plastic containers. Samples were kept frozen and thawed immediately prior to preparation.

4.2.3 Sample Preparation

1. Composite tadpole GI tract samples were submitted in pre-weighed numbered plastic containers.

2. Upon receipt by PSC the samples were weighed. The sample weight was determined by calculating the difference between the pre-weighed plastic container and the total weight (container with sample).

3. The sample was removed from the container and placed in a pyrex glass beaker, and was subsequently homogenized by using a glass rod until a visually homogeneous mixture was obtained.

4. A sub-sample of the homogenous sample was set aside for moisture determination and, if possible, an additional sub-sample for replicate analysis.

5. The weight of the sample digested was equal to the sample weight minus the weight of the removed sub-samples.

6. The glass rod was thoroughly rinsed with a small volume of dilute nitric acid.

4.2.4 Moisture determination

The moisture content was determined on the sub-sample set aside for moisture calculations.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt = B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.2.5 Sample Digestion

All pyrex glass beakers used for the digestion process were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid and then subjected to a hot concentrated nitric acid reflux for 10 min. This was conducted in the following manner:

1. A four gram sample was accurately weighed and placed into a pre-cleaned 50 or 100ml pyrex glass beaker.

2. Ten ml of concentrated nitric acid were added to the glass beaker.

3. The sample was digested at 90 – 95°C until complete dissolution of solid material was achieved; this typically took 3 to 4 hours to complete.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion. If further dissolution of any remaining solid material was necessary additional drops of concentrated nitric acid were added.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube and made up to a final volume of 50 ml using RODI water.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

Note: If the total sample weight was less than 0.5 grams, the final digestate volume was made up to 25 ml rather than 50 ml to maintain consistent EQL reporting.

4.3 Individual Frog Carcass Samples

Frog carcasses excluding their GI tracts and livers were submitted to PSC and analyzed individually.

4.3.1 Summary of Method

1. Individual frog carcasses excluding liver and GI tract were thoroughly homogenized in a blender using RODI water to facilitate the homogenization process at an approximate ratio of 1 frog:2 water.

2. Homogenate was separated into five sub-samples (three replicate samples to provide a measure of variability due to the homogenization and analytical process, one sub-sample for moisture determination, one archive sample).

3. The sub-samples were then digested using nitric acid and hydrogen peroxide at 95°C.

4. The results were reported on a dry weight basis following correction for moisture content.

4.3.2 Homogenization

Due to the physical nature of certain components of the sample such as bones, variability between sub-samples occurred due to particulate matter in the homogenate. For this reason, triplicate analysis was conducted on 25% of the frog samples to determine the degree of variability. Since percent differences between triplicates were generally below 30%, the remaining frog samples were not analyzed in triplicate as this additional work was not necessary.

4.3.3 Sample Handling and Storage

Individual samples were submitted in labeled plastic bags. Samples were kept frozen and thawed immediately prior to preparation.

4.3.4 Sample Preparation

1. Blending equipment was thoroughly cleaned using detergent and hot water, followed by a RODI water rinse.

2. Individual samples were weighed and transferred to a blender cup with a volume of RODI water equivalent to 3 times the sample's weight.

3. The sample was homogenized using a blender at high speed until a visually homogeneous mixture was obtained.

4. The homogenate was then transferred to a polypropylene container labeled with the sample ID and initial weight.

4.3.5 Moisture Determination

Since homogenate included water added for the purpose of homogenization, moisture correction was required to determine the percent moisture of the original biological sample. The following calculation was used based on the amount of water that was added to the sample to facilitate homogenization.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt = B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.3.6 Sample Digestion

All glass beakers used for the digestion process were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid and then refluxed with hot concentrated nitric acid for 10 min. Sample preparation and analysis was conducted in the following manner.

Homogenate Sub-Sample

This procedure was done in triplicate on 25% of homogenate frog samples, to provide a measure of homogenate and analytical variability.

1. A four gram sub-sample was accurately weighed (equivalent to approximately 1 gram of biological sample) and placed into a pre-cleaned 50 or 100 ml pyrex glass beaker

2. Ten ml of concentrated nitric acid were added into the glass beaker.

3. The sub-sample was digested at 90 – 95°C until complete dissolution of solid material was achieved; this typically took 3 to 4 hours to complete.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion. If further dissolution of any remaining solid material was necessary additional drops of concentrated nitric acid were added.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. The sub-sample was submitted for analysis (see Section 5.).

4.4 Individual Frog Liver and GI Tract Samples

Livers and GI tracts were analyzed separately, but each had similar procedures for sample preparation and analysis, as follows.

4.4.1 Summary of Method

1. Using a glass rod, individual samples were pulverized in a pyrex glass beaker to be used for acid digestion. The glass rod was cleaned into the glass beaker with a small volume of dilute HNO_3 to minimize sample loss.

2. Digestion was accomplished using nitric acid and hydrogen peroxide at 95°C.

3. The results were reported on a dry weight basis following correction for moisture content. Moisture values used were a mean of livers or GI tracts large enough to obtain moisture contents from. In addition, a trial analysis and moisture determination was conducted on six livers and GI tracts prior to all other analysis of bio-materials. These six moisture values were also incorporated into the mean moisture value for livers and GI tracts. See Volume V for reported moisture contents used in moisture corrections.

4. Due to the small sample sizes of livers and GI tracts, replicate analysis was not preformed.

4.4.2 Sample Handling and Storage

Samples were submitted in pre-weighed plastic containers. Samples were kept frozen and thawed immediately prior to preparation.

4.4.3 Sample Preparation

1. Individual liver and GI tract samples were submitted in pre-weighed numbered snap cap vials.

2. Upon receipt by PSC the samples were weighed. The sample weight was determined by calculating the weight difference between the pre-weighed container and the sample plus the container.

3. The sample was placed in a glass beaker used for digestion, and homogenized using a glass rod until a visually homogeneous mixture was obtained. The rod was rinsed with a small volume of dilute HNO_3 to minimize sample loss.

4.4.4 Moisture Determination

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt = B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105^{o}C to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.4.5 Sample Digestion

All pyrex glass beakers used for the digestion process were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid and then refluxed with hot concentrated nitric acid for 10 min. This was conducted in the following manner:

1. The weighed sample was placed into a pre-cleaned 50 or 100 ml pyrex glass beaker.

2. Ten ml of concentrated nitric acid were added.

3. The sample was digested at 90 – 95°C until complete dissolution of solid material was achieved; this typically took 3 to 4 hours to complete.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube and made up to a final volume of 50 ml using RODI water.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

Note: If the total sample weight was less than 0.5 grams the final digestate volume was made up to 25 ml rather than 50 ml to maintain more consistent reporting limits.

4.5 Individual Meadow Vole Carcass Samples

Voles excluding their livers were submitted to PSC and analyzed individually.

4.5.1 Summary of Method

1. Individual vole carcasses excluding liver were thoroughly homogenized in a blender using water to facilitate the homogenization process.

2. Homogenate was separated into a maximum of five sub-samples (three replicate samples for 25% of the voles to provide a measure of variability due to the homogenization and analytical process, one sub-sample for moisture determination, one archive sample).

3. Samples were then digested using nitric acid and hydrogen peroxide at 95^{o}C.

4. Results were reported on a dry weight basis following correction for moisture content.

4.5.2 Homogenization

Due to the physical nature of certain components of the sample such as bones, fur and pelt, variability between sub-samples occurred due to the difficulty in obtaining a truly homogenous sample. For this reason, triplicate analysis was conducted on 25% of the vole samples to determine the degree of variability. Since percent differences between triplicates were generally below 30%, the remaining vole samples were not analyzed in triplicate since variability did not warrant this additional work.

4.5.3 Apparatus and Materials

- Hotplate capable of maintaining a temperature between 90 – 95°C.
- 50 ml polypropylene capped centrifuge tubes.
- Pyrex glass beakers 50 & 100 ml.
- Blenders with various sized cups were used according to the size of sample being blended.

4.5.4 Reagents

Reagent grade chemicals were used in all cases. The purity of reagents was confirmed prior to their use, by analysis of method blanks. Only reagents with impurity levels less than the method detection limit are acceptable. The following reagents were used.

- Reagent water- reverse osmosis deionized (RODI).
- Nitric Acid (concentrated) Environmental Grade.
- Nitric Acid (dilute, 5%).
- Hydrogen Peroxide (30%).

4.5.5 Sample Handling and Storage

Individual samples were submitted in labeled plastic bags. Samples were kept frozen and thawed immediately prior to preparation.

4.5.6 Sample Preparation

1. Blending equipment was thoroughly cleaned using detergent and hot water, followed by a RODI water rinse.

2. Individual samples were weighed and transferred to a blender cup with a volume of RODI water equivalent to three times the sample's weight.

3. The sample was homogenized in the blender cup at high speed until a visually homogeneous mixture was obtained.

4. The homogenate was then transferred to a polypropylene container and labeled with the sample ID and initial weight.

4.5.7 Moisture Determination

Since homogenate included water added for the purpose of homogenization, moisture correction is required to determine the percent moisture of the original biological sample. The following calculation was used based on the amount of water that was added to the sample to facilitate homogenization.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt = B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.5.8 Sample Digestion

All pyrex glass beakers used for digestion were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid and then refluxed with hot concentrated nitric acid for 10 minutes.

Homogenate Sub-Sample

This procedure was done in triplicate on 25% of homogenate vole samples, to provide a measure of homogenate and analytical variability.

1. A four gram sub-sample was accurately weighed and placed into a pre-cleaned 50 or 100 ml beaker, (equivalent to approximately 1 gram of biological sample).

2. Ten ml of concentrated nitric acid were added.

3. The sample was digested at 90 – 95°C until complete dissolution of solid material were achieved; this required 3 to 4 hours.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion. If further dissolution of any remaining solid material was necessary additional drops of concentrated nitric acid were added.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

4.6 Individual Meadow Vole Liver Samples

Vole livers were submitted and analyzed individually.

4.6.1 Summary of Method

1. Using a glass rod, each vole liver was pulverized in the pyrex glass beaker to be used for acid digestion. The glass rod was cleaned in the glass beaker with dilute nitric acid to minimize loss of tissue.

2. Digestion was accomplished using nitric acid and hydrogen peroxide at 95°C.

3. The results were reported on a dry weight basis following correction for moisture content.

4.6.2 Sample Handling and Storage

Samples were submitted in pre-weighed plastic snap cap vials. Samples were kept frozen and thawed immediately prior to preparation.

4.6.3 Sample Preparation

1. Individual liver samples were submitted in pre-weighed numbered snap cap vials.

2. Upon receipt the samples were weighed. The sample weight was determined by calculating the difference between the pre-weighed vial weight and the sample.

3. After the sample was placed in a pyrex glass beaker, it was homogenized by using a glass rod until a visually homogeneous mixture was obtained.

4. A sub-sample of the homogenized sample was set aside for moisture determination and weight permitting, an additional sub-sample for replicate analysis.

5. The weight of the sample digested was equal to the sample weight minus the weight of the removed sub-samples.

6. The glass rod was rinsed into the pyrex glass beaker with a small volume of dilute HNO_3 to minimize sample loss. A Jacques Whitford representative was present to determine the length of time required for this homogenization procedure.

4.6.4 Moisture Determination

Moisture content was determined on the sub-sample set aside for moisture calculations in the following manner.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt=B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.6.5 Sample Digestion

All pyrex glass beakers used for digestion were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid and then subjected to a hot concentrated nitric acid reflux for 10 min. This was conducted in the following manner for the total sample.

1. A sample was weighed and placed into a pre-cleaned 50 or 100 ml pyrex glass beaker.

2. Ten ml of concentrated nitric acid was added to the beaker.

3. The sample was digested at 90 to 95°C until complete dissolution of solid material was achieved. This typically took 3 to 4 hours to complete.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion.

5. The final volume of the digestate was reduced to approximately. 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

Note: If the total sample weight was less than 0.5 grams, the final digestate volume was made up to 25 ml rather than 50 ml to maintain more consistent reporting limits.

4.7 Composite Insect and Spider Samples

For each field sampled for insects, two composite samples were submitted to PSC for chemical analysis. First, a portion of grasshoppers from each site was submitted to constitute one grasshopper composite sample. A second composite sample was submitted which included all other insects and spiders collected from the same field.

For each woodlot sampled for insects, two composite samples were submitted to PSC for chemical analysis. First, all spiders from each woodlot were combined into one vial and submitted to PSC as one composite sample. A second composite sample was submitted which included all other insects captured in that woodlot.

4.7.1 Summary of Method

Composite insect, grasshopper, and spider samples were pulverized in the pyrex glass beaker to be used for acid digestion using a glass rod. The glass rod was cleaned with dilute nitric acid into the beaker, to minimize loss of tissue.

1. Digestion was accomplished using nitric acid and hydrogen peroxide at 95°C.

2. The results were reported on a dry weight basis following correction for moisture content.

4.7.2 Sample Handling and Storage

Samples were submitted in pre-weighed plastic snap cap vials. Samples were kept frozen and thawed immediately prior to preparation.

4.7.3 Sample Preparation

1. Composite samples were submitted in pre-weighed numbered snap cap vials.

2. Upon receipt the samples were weighed. The sample weight was determined by calculating the difference between the pre-vial weight and the sample.

3. The sample was crushed using the blunt end of a glass rod until only small fragments (i.e., carapace) were visible. The rod was thoroughly rinsed with a small volume of dilute nitric acid to prevent sample loss.

4. When possible, separate sub-samples were taken for determination of moisture content, replicate analysis and archival purposes.

4.7.4 Moisture Determination

Moisture content was determined on the sub-samples set aside for moisture calculations.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt=B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105^{o}C to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.7.5 Sample Digestion

All pyrex glass beakers used for the digestion process were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid and then subjected to a hot concentrated nitric acid reflux for 10 min. This was conducted in the following manner:

1. A sample was weighed (approximately 1 gram) and placed into a pre-cleaned 50 or 100 ml pyrex glass beaker.

2. The sample was crushed using the blunt end of a glass rod until only small fragments (i.e., carapace) were visible. The rod was thoroughly rinsed with a small volume of dilute nitric acid to prevent sample loss.

3. Ten ml of concentrated nitric acid were added to the beaker.

4. The sample was digested at 90 to 95°C until complete dissolution of solid material was achieved; this required 3 to 4 hours.

5. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion. If dissolution of any remaining solid material was necessary, further additions of concentrated nitric acid were added dropwise.

6. The final volume of the digestate was reduced to approximately. 1 ml. The sample was not allowed to dry.

7. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

8. The polypropylene centrifuge tube was capped and shaken.

9. Sample was submitted for analysis (see Section 5.).

Note: If the total sample weight was less than 0.5 grams, the final digestate volume was made up to 25 ml rather than 50 ml to maintain more consistent reporting limits.

4.8 Deer Samples

Meat samples of deer were submitted to PSC and analyzed as individual samples.

4.8.1 Summary of Method

1. Individual deer meat samples were thoroughly homogenized in a blender using a 1:4 ratio of deer meat to water in order to facilitate the homogenization process.

2. Homogenate was separated into sub-samples (one for analysis, one archive sample, and one sample for replicate analysis, when required for 10% replication for standard QA/QC procedures by PSC).

3. Samples were then digested using nitric acid and hydrogen peroxide at 95°C.

4. The results for samples were reported on a dry weight basis following correction for moisture content.

4.8.2 Sample Handling and Storage

Individual samples were submitted in labeled plastic bags. Samples were kept frozen and thawed immediately prior to preparation.

4.8.3 Sample Preparation

1. Blending equipment was thoroughly cleaned using detergent and hot water, followed by RODI water rinse.

2. Individual samples were weighed and transferred to a blender cup with a volume of RODI water equivalent to 3 times the sample's weight.

3. The sample was homogenized using a blender at high speed until a visually homogeneous mixture was obtained.

4. The homogenate was then transferred to a polypropylene tube labeled with the sample ID and initial weight.

4.8.4 Moisture Determination

Since homogenate included added water for the purpose of homogenization, moisture correction was required to determine the percent moisture of the original biological sample. The following calculation was used based on the amount of water that was added to the sample to facilitate homogenization.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt=B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.8.5 Sample Digestion

All digestion beakers were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid and then refluxed with hot concentrated nitric acid for 10 min. Sample digestion was conducted in the following manner for the homogenate aliquot:

1. A 4-gram sample was weighed (equivalent to approximately 1 gram of biological sample) and placed into a pre-cleaned 50 or 100 ml pryex glass beaker.

2. Ten ml of concentrated nitric acid were added.

3. The sample was digested at 90 to 95°C until complete dissolution of solid material was achieved; this required 3 to 4 hours.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion. If dissolution of any remaining solid material was necessary, further drop wise additions of concentrated nitric acid were made.

5. The final volume of the digestate was reduced to approximately 1ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

4.9 Rabbit Meat Samples

Meat samples from rabbits were submitted to PSC and analyzed as individual samples.

4.9.1 Summary of Method

1. Individual rabbit meat samples were thoroughly homogenized in a blender using a 1:4 ratio of rabbit meat to water in order to facilitate the homogenization process.

2. Homogenate was separated into sub-samples (one for analysis, one archive sample, and one sample for replicate analysis, when required for 10% replication for standard QA/QC procedures by PSC).

3. Samples were then digested using nitric acid and hydrogen peroxide at 95°C.

4. The results for samples were reported on a dry weight basis following correction for moisture content.

4.9.2 Sample Handling and Storage

Individual samples were submitted in labeled plastic bags. Samples were kept frozen and thawed immediately prior to preparation.

4.9.3 Sample Preparation

1. Blending equipment was thoroughly cleaned using detergent and hot water, followed by RODI water rinse.

2. Individual samples were weighed and transferred to a blender cup with a volume of RODI water equivalent to three times the sample's weight.

3. The sample was homogenized using a blender at high speed until a visually homogeneous mixture was obtained.

4. The homogenate was then transferred to a polypropylene container labeled with the sample ID and initial weight.

4.9.4 Moisture Determination

Since homogenate included water that was added for the purpose of homogenization, moisture correction was required to determine the percent moisture of the original biological sample. The following calculation was used based on the amount of water that was added to the sample to facilitate homogenization.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt=B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.9.5 Sample Digestion

All pyrex glass beakers used for the digestion process were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid and then refluxed with hot concentrated nitric acid for 10 min. Digestion was conducted in the following manner for the homogenate aliquot:

1. A 4-gram prepared sample was weighed (equivalent to approx. 1 gram of biological sample) and placed into a pre-cleaned 50 or 100 ml pyrex glass beaker.

2. 10 ml of concentrated nitric acid were added.

3. The sample was digested at 90 – 95°C until complete dissolution of solid material was achieved; this required 3 to 4 hours.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion. If dissolution of any remaining solid material was necessary then further additions of concentrated nitric acid were added dropwise.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

4.10 Perch Meat Samples

Perch were caught from two locations. At each location, 12 perch were caught and filleted. 24 individual perch tissue samples were submitted to PSC for analysis.

4.10.1 Summary of Method

1. Individual perch tissue samples were thoroughly homogenized in a blender using a 1:4 ratio of perch tissue to water in order to facilitate the homogenization process.

2. Homogenate was separated into sub-samples (one for analysis, one archive sample, and one sample for replicate analysis, when required for 10% replication for standard QA/QC procedures by PSC).

3. Samples were then digested using nitric acid and hydrogen peroxide at 95°C.

4. The results for samples were reported on a dry weight basis following correction for moisture content.

4.10.2 Sample Handling and Storage

Individual samples were submitted in labeled plastic bags. Samples were kept frozen and thawed immediately prior to preparation.

4.10.3 Sample Preparation

1. Blending equipment was thoroughly cleaned using detergent and hot water, followed by RODI water rinse.

2. Individual samples were weighed and transferred to a blender cup with a volume of RODI water equivalent to three times the sample's weight.

3. The sample was homogenized with a blender at high speed until a visually homogeneous mixture was obtained.

4. The homogenate was then transferred to a polypropylene tubes labeled with the sample ID and initial weight.

4.10.4 Moisture Determination

Since homogenate included water added for the purpose of homogenization, moisture correction was required to determine the percent moisture of the original biological sample. The following calculation was used based on the amount of water that was added to the sample to facilitate homogenization.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt=B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.10.5 Sample Digestion

All digestion beakers were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid and then refluxed with hot concentrated nitric acid for 10 min. Sample digestion was conducted in the following manner.

1. A 4-gram sample was weighed (equivalent to approx. 1 gram of biological sample) and placed into a pre-cleaned 50 or 100 ml pyrex glass beaker.

2. Ten ml of concentrated nitric acid was added.

3. The sample was digested at 90 to 95°C until complete dissolution of solid material was achieved; this required 3 to 4 hours.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion. If, at this time, solid material remained, further drop wise additions of concentrated nitric acid were added.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

4.11 Composite Earthworm Samples

All earthworms collected from a site location were transferred into a glass sample jar and submitted to PSC as one composite sample.

4.11.1 Summary of Method

1. Composite earthworm samples were pulverized in the glass jar using a hand held domestic mixer.

2. A portion of each composite sample was removed for moisture determination.

3. Digestion was accomplished using nitric acid and hydrogen peroxide at 95°C.

4. The results were reported on a dry weight basis following correction for moisture content.

4.11.2 Sample Handling and Storage

Composite samples were submitted to the lab in labeled containers. Samples were frozen and thawed immediately prior to preparation.

4.11.3 Sample Preparation

1. Composite earthworm samples were submitted in labeled glass containers.

2. Upon receipt, the contents of the containers were weighed.

3. After placing the sample in a glass beaker used for digestion, it was homogenized using a hand held mixer until a visually homogeneous mixture was obtained.

4. The mixer was rinsed into the pyrex glass beaker with a small volume of dilute HNO_3 to minimize sample loss.

5. A sub-sample was weighed and set aside for moisture determination. In addition, as part of routine QA/QC at PSC, replicate analysis was preformed on 10 % of the samples submitted. Hence, a second sub-sample was weighed and set aside for replicate analysis for one in 10 samples prepared (10% replication).

6. The weight of the sample digested was equal to the sample weight minus the weight of the removed sub-samples.

4.11.4 Moisture Determination

Moisture content was determined on the aliquot set aside for moisture calculations.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt=B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.11.5 Sample Digestion

All digestion beakers were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid and then subjected to a hot concentrated nitric acid reflux for 10 min. Sample digestion was conducted in the following manner.

1. The sample (approximately 1 gram of biological material) was weighed and placed into a pre-cleaned 50 or 100 ml beaker.

2. Ten ml of concentrated nitric acid were added to the beaker.

3. The sample was digested at 90 to 95°C until complete dissolution of solid material was achieved; this required 3 to 4 hours.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

Note: If the total sample weight was less than 0.5 grams, the final digestate volume was made up to 25 ml rather than 50 ml to maintain more consistent reporting limits.

4.12 Composite Caterpillar Samples

4.12.1 Summary of Method

1. Composite caterpillar samples were pulverized using a hand held domestic mixer.

2. A portion of each composite sample was removed for moisture determination.

3. Digestion was accomplished using nitric acid and hydrogen peroxide at 95°C.

4. Results were reported on a dry weight basis following correction for moisture content.

4.12.2 Sample Handling and Storage

Composite samples were submitted to PSC in labeled containers. Samples were frozen and thawed immediately prior to preparation.

4.12.3 Sample Preparation

1. Composite caterpillar samples were submitted in pre-weighed numbered plastic snap cap vials.

2. Upon receipt the contents in the containers were weighed and the sample weight determined by the difference of pre-weighed vial weight.

3. After placing the sample in a glass beaker used for digestion, it was homogenized using a hand held mixer until a visually homogeneous mixture was obtained.

4. The mixer was thoroughly rinsed with a small volume of dilute HNO_3 to minimize sample loss.

5. A sub-sample was weighed and set aside for moisture determination. In addition, as part of routine QA/QC at PSC, replicate analysis was preformed on 10 % of the samples submitted. Hence, a second sub-sample was weighed and set aside for replicate analysis for one in 10 samples prepared (10% replication).

6. The weight of the sample digested was equal to the sample weight minus the weight of the removed sub-samples.

4.12.4 Moisture Determination

Moisture content was determined on the aliquot set aside for moisture calculations.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt=B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.12.5 Sample Digestion

All digestion beakers were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid then subjected to a hot concentrated nitric acid reflux for 10 min.

1. The sample (up to approximately 1 gram of biological material) was accurately weighed and placed into a pre-cleaned 50 or 100 ml beaker.

2. Ten ml of concentrated nitric acid were added.

3. The sample was digested at 90 – 95°C until complete dissolution of solid material was achieved, this required 3 to 4 hours.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

Note: If the total sample weight was less than 0.5 grams, the final digestate volume was made up to 25 ml rather than 50 ml to maintain more consistent reporting limits.

4.13 Maple Leaf Samples

4.13.1 Summary of Method

1. Composite maple leaf samples were pulverized using a hand held domestic mixer.

2. A portion of each composite sample was removed for moisture determination.

3. Digestion was accomplished using nitric acid and hydrogen peroxide at 95°C.

4. The results were reported on a dry weight basis following correction for moisture content.

4.13.2 Sample Handling and Storage

Composite samples were submitted to PSC in labeled plastic bags. Samples remained refrigerated in order to maintain freshness until preparation for analysis began at PSC.

4.13.3 Sample Preparation

1. Composite maple leaf samples were submitted in labeled plastic zip lock bags.

2. Upon receipt the contents of the bags were weighed and the sample weight determined.

3. After placing the entire sample in a large glass beaker, it was homogenized using a hand held mixer until a visually homogeneous mixture was obtained.

4. A sub-sample was weighed and set aside for moisture determination. In addition, as part of routine QA/QC at PSC, replicate analysis was preformed on 10 % of the samples submitted. Hence, a second sub-sample was weighed and set aside for replicate analysis for one in 10 samples prepared (10% replication).

5. The weight of the sample digested was equal to the sample weight minus the weight of the removed sub-samples.

4.13.4 Moisture Determination

Moisture content was determined on the aliquot set aside for moisture calculations.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt=B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.13.5 Sample Digestion

All digestion beakers were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid then subjected to a hot concentrated nitric acid reflux for 10 min. Sample digestion was conducted in the following manner:

1. A sample was weighed (up to approximately 1 gram of biological material) and placed into a pre-cleaned 50 or 100 ml pyrex glass beaker.

2. Ten ml of concentrated nitric acid were added to the beaker.

3. The sample was digested at 90 to 95°C until complete dissolution of solid material was achieved. This required 3 to 4 hours.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

Note: If the total sample weight was less than 0.5 grams, the final digestate volume was made up to 25 ml rather than 50 ml to maintain more consistent reporting limits.

4.14 Maple Cotyledons

4.14.1 Summary of Method

1. Composite maple cotyledon samples were crushed using a glass rod.

2. A portion of each composite sample was removed for moisture determination.

3. Digestion was accomplished using nitric acid and hydrogen peroxide at 95°C.

4. Results were reported on a dry weight basis following correction for moisture content.

4.14.2 Sample Handling and Storage

Composite samples were submitted to PSC in labeled plastic bags. Samples remained refrigerated in order to maintain freshness until preparation for analysis began at PSC.

4.14.3 Sample Preparation

1. Composite maple cotyledon samples were submitted in labeled plastic zip lock bags.

2. Upon receipt the contents of the bags were weighed and the sample weight determined.

3. The sample was placed in a glass beaker and homogenized using the blunt end of a glass rod until a visually homogeneous mixture was obtained.

4. A sub-sample was weighed and set aside for moisture determination. In addition, as part of routine QA/QC at PSC, replicate analysis was preformed on 10 % of the samples submitted. Hence, a second sub-sample was weighed and set aside for replicate analysis for one in 10 samples prepared (10% replication).

5. The weight of the sample digested was equal to the sample weight minus the weight of the removed sub-samples.

4.14.4 Moisture Determination

Moisture content was determined on the aliquot set aside for moisture calculations.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt=B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.14.5 Sample Digestion

All digestion beakers were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid then subjected to a hot concentrated nitric acid reflux for 10 min. Sample digestion was conducted in the following manner:

1. A sample was weighed (up to approximately 1 gram of biological material) and placed into a pre-cleaned 50 or 100 ml pyrex glass beaker.

2. Ten ml of concentrated nitric acid were added.

3. The sample was digested at 90 to 95°C until complete dissolution of solid material was achieved. This required 3 to 4 hours.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

Note: If the total sample weight was less than 0.5 grams, the final digestate volume was made up to 25 ml rather than 50 ml to maintain more consistent reporting limits.

4.15 Maple Tree Wood Cores

4.15.1 Summary of Method

1. Wood cores were crushed using a glass rod.

2. Digestion was accomplished using nitric acid and hydrogen peroxide at 95°C.

3. Results were reported on a dry weight basis.

4.15.2 Sample Handling and Storage

Samples were allowed to dry prior to submission to PSC. Samples were submitted to PSC in labeled plastic vials.

4.15.3 Sample Preparation

1. Wood core samples were submitted in labeled plastic tubes.

2. Upon receipt the contents of the tubes were weighed and the sample weight determined.

3. After placing the entire sample in a glass beaker, it was crushed using the blunt end of a glass rod.

4. A sub-sample was weighed and set aside for moisture determination. No sub-samples were removed for replicate analysis since sample weights were inadequate.

Note: Since wood cores were relatively dry, the glass rod used for homogenization was not a source of sample loss and therefore was not rinsed with HNO_3.

4.15.4 Moisture Determination

Moisture content was determined on the aliquot set aside for moisture calculations.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt=B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.15.5 Sample Digestion

All digestion beakers were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid then subjected to a hot concentrated nitric acid reflux for 10 min. Sample digestion was conducted in the following manner:

1. A sample was weighed (up to approximately 1 gram of biological material) and placed into a pre-cleaned 50 or 100 ml pyrex glass beaker.

2. Ten ml of concentrated nitric acid was added.

3. The sample was digested at 90 to 95°C until complete dissolution of solid material. This typically took 3 to 4 hours to complete.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

Note: If the total sample weight was less than 0.5 grams, the final digestate volume was made up to 25 ml rather than 50 ml to maintain more consistent reporting limits.

4.16 Corn Kernels

4.16.1 Summary of Method

1. Corn kernel samples were homogenized using a hand held domestic mixer.

2. A portion of each sample was removed for moisture determination.

3. Digestion was accomplished using nitric acid and hydrogen peroxide at 95°C.

4. Results were reported on a dry weight basis following correction for moisture content.

4.16.2 Sample Handling and Storage

Samples were submitted to PSC in labeled paper bags. Samples remained refrigerated in order to maintain freshness until preparation for analysis began at PSC.

4.16.3 Sample Preparation

1. Cobs of corn were submitted in labeled paper bags.

2. Kernels from individual cobs were removed and the cob discarded.

3. The sample was homogenized using a grinding apparatus to achieve a visually homogeneous mixture.

4. The weight of this homogenized sample was determined.

5. A sub-sample was weighed and set aside for moisture determination. In addition, as part of routine QA/QC at PSC, replicate analysis was preformed on 10 % of the samples submitted. Hence, a second sub-sample was weighed and set aside for replicate analysis for one in 10 samples prepared (10% replication).

6. The weight of the sample digested was equal to the sample weight minus the weight of the removed sub-samples.

4.16.4 Moisture Determination

Moisture content was determined on the aliquot set aside for moisture calculations.

1. The appropriate drying container (pyrex glass dish / tray) was weighed, (wt = A).

2. A sub-sample of homogenate was added to the dish and the combined weight determined (wt=B).

3. The weight of the homogenate was determined: wt C = B – A.

4. The "wet weight" (as received by PSC) of biomaterial (wt = D) in the homogenate was equivalent to 25% of wt C, D = 0.25 x C.

5. The specimen was dried at 105oC to a constant weight (wt = E).

6. The weight of the dry material (wt = F) is equal to E –A.

7. The percent moisture was determined using the following formula:

$$\text{Percent Moisture} = ((D - F) / D) \times 100$$

4.16.5 Sample Digestion

All digestion beakers were pre-cleaned with detergent, rinsed with RODI water and dilute nitric acid then subjected to a hot concentrated nitric acid reflux for 10 min. Sample digestion was conducted in the following manner:

1. A sample was weighed (up to approximately 1 gram of biological material) and placed into a pre-cleaned 50 or 100 ml pyrex glass beaker.

2. 10 ml of concentrated nitric acid was added.

3. The sample was digested at 90 to 95°C until complete dissolution of solid material was achieved. This required 3 to 4 hours.

4. 0.5 ml of 30% hydrogen peroxide was added dropwise after 1 hour and repeated again after two hours of digestion.

5. The final volume of the digestate was reduced to approximately 1 ml. The sample was not allowed to dry.

6. The digestate was transferred to a 50 ml polypropylene centrifuge tube with several small volume rinses of RODI water and made up to a final volume of 50 ml.

7. The polypropylene centrifuge tube was capped and shaken.

8. Sample was submitted for analysis (see Section 5.).

Note: If the total sample weight was less than 0.5 grams, the final digestate volume was made up to 25 ml rather than 50 ml to maintain more consistent reporting limits.

5. ANALYTICAL METHODS AND INSTRUMENTATION

Analytical methods and instrumentation are indicated in Table 1. A summary of the method was as follows. After sample digestion was complete, material (the digestate) was introduced into the argon based, high-temperature plasma. As the sample stream flowed into the plasma, high temperatures from the plasma caused desolvation, atomization, and ionization of target elements. Ions generated by these energy-transfer processes were extracted from the plasma, through a differential vacuum interface. Using a mass spectrometer, these ions were separated on the basis of their mass-to-charge ratio as they passed through the differential vacuum interface. The ions passing through the mass spectrometer were then counted, usually by an electron multiplier detector. The resulting information was processed by a computer-based data handling system in order to quantify concentrations of all parameters in the digestate (Standard Methods Committee 1997). Parameters quantified in biological tissues are summarized in Table 1, along with the instrumentation and analytical methodology used.

Table 1: Analytical Methods and Instrumentation

Parameter	Analytical Methodology	Instrumentation
Metals (Ag, Al, As, Ba, Be, Bi, Cd, Cu, Co, Cr, Fe, Mn, Mo, Ni, P, Pb, Sb, Se, Sr, Tl, Ti, U, V and Zn)	U.S. EPA Method No. 6020 (Modified)	PE Sciex ELAN 6000 Inductively Coupled Plasma- Mass Spectrometer

5.1 Estimated Quatitation Limits

Estimated quantitation limits (EQL's) are the minimum value of any given parameter that can be measured (quantified) with confidence in a biological tissue using the method described in Section 5. In situations where arsenic in a biological tissue, for example, was less than the EQL, it was not quantified. In this case, "nd" was stated in place of a value that would otherwise be reported if above the EQL. Table 2 summarizes the EQL's for all parameters quantified for the different biological tissues analyzed. Keep in mind that these values are a general guideline. High moisture contents or inadequate sample size of biological materials elevated EQL's in some situations. In these cases, the elevated EQL would appear in brackets next to the "nd" on the data sheets.

Table 2: Estimated Quantitation Limit (EQL) relative to biological samples.

Parameter	Vegetation (mg/Kg)	Frogs, GI tracts, Liver, and Vole Liver (mg/Kg)	Tadpoles and GI tracts (mg/Kg)	Vole excluding Liver, Deer/ Rabbit Meat (mg/Kg)	Caterpillars and Earthworms (mg/Kg)	Insects and Spiders (mg/Kg)	Fish Meat (mg/Kg)
Aluminum	0.5	1.8	2.4	1.5	2.4	1.2	1.5
Antimony	0.05	0.18	0.24	0.15	0.24	0.12	0.15
Arsenic	0.2	0.6	0.8	0.5	0.8	0.4	0.5
Barium	0.5	1.8	2.4	1.5	2.4	1.2	1.5
Beryllium	0.1	0.3	0.4	0.25	0.4	0.2	0.25
Bismuth	0.1	0.3	0.4	0.25	0.4	0.2	0.25
Cadmium	0.01	0.03	0.04	0.025	0.04	0.02	0.025
Chromium	0.5	1.8	2.4	1.5	2.4	1.2	1.5
Cobalt	0.01	0.03	0.04	0.025	0.04	0.02	0.025
Copper	0.05	0.18	0.24	0.15	0.24	0.12	0.15
Iron	5	18	24	15	24	12	15
Lead	0.05	0.18	0.24	0.15	0.24	0.12	0.15
Manganese	0.5	1.8	2.4	1.5	2.4	1.2	1.5
Molybdenum	0.1	0.3	0.4	0.25	0.4	0.2	0.25
Nickel	0.1	0.3	0.4	0.25	0.4	0.2	0.25
Phosphorous	5	18	24	15	24	12	15
Selenium	0.2	0.6	0.8	0.5	0.8	0.4	0.5
Silver	0.01	0.03	0.04	0.025	0.04	0.02	0.025
Strontium	0.1	0.3	0.4	0.25	0.4	0.2	0.25
Thallium	0.005	0.018	0.024	0.015	0.024	0.012	0.015
Titanium	1	3	4	2.5	4	2	2.5
Uranium	0.01	0.03	0.04	0.025	0.04	0.02	0.025
Vanadium	0.05	0.18	0.24	0.15	0.24	0.12	0.15
Zinc	0.5	1.8	2.4	1.5	2.4	1.2	1.5

6. DISCUSSION

6.1 Reporting Procedures for Replicate/Triplicate analysis

Where replicate or triplicate analyses were performed on a sample, the mean of those values was calculated and reported. Providing QA/QC was met (see Section 7), the mean of replicates was reported and used in statistical analysis and risk calculations.

7. QUALITY ASSURANCE/ QUALITY CONTROL

Samples were processed in batches not exceeding a total of 15 samples. With each batch the following Quality Control samples were processed.

Standard Reference Materials: Standard Reference Materials (SRM) were analyzed on a per batch basis to evaluate analytical performance by comparing actual results to SRM certified results. Bovine Liver and Oyster Tissue SRM were used for animal tissues, and Spinach SRM was used in conjunction with vegetation samples. Details of the SRM used are described below.

- SRM. 1577b Bovine Liver, National Bureau of Standards.
- SRM. 1566b Oyster Tissue, National Bureau of Standards.
- SRM. 1570a Trace Elements in Spinach Leaves, National Institute of Standards and Technology.

In order for QA/QC to be met, SRM were analyzed and those results were to be within 30% of the published or expected values presented by the National Bureau of Standards or National Institute of Standards and Technology (CCME 1993). The Certificate of Analysis, indicating the expected values for each of the Standard Reference Materials, is provided in Appendix A.

Matrix Spikes Prior to digestion, a sub-sample was fortified with a known level of target analytes. The sample was processed and the percent recovery of analytes determined. In some cases, a lack of samples precluded the use of complete matrix spike, in such cases, sample values were considered unsubstantiated. Results of Matrix Spikes are summarized in every Certificate of Analysis provided by PSC. To view these reports, refer to tabs 26 through 47 in Volume V.

Spikes An aliquot of the digestate was removed and fortified with a known level of target analytes. Differences from expected values provided a measure of matrix suppression or enhancement effects.

Spikes are part of the QA/QC procedure at PSC and were included as part of biomaterial analytical methodology.

Replicate Analysis involved analysis of a sample more than once, to provide a measure of sample and analytical variance. If sufficient sample was available, replicate analysis was routinely conducted on a minimum of 10 percent of the samples analyzed. Results of replicate analysis are provided in the Quality Assurance and Quality Control Report of Volume V tabs 1 through 25. An acceptable degree of variance between a sample and its replicate was less than 30% (CCME 1993). Some differences greater than 30% were acceptable provided that these departures were not unusual for the matrix being analyzed and that the general data set for that matrix were less than 30%.

8. REFERENCES

Canadian Council of Ministers of the Environment, December 1993, Guidance Manual on Sampling, Analysis, and Data Management for Contaminated Sites, Volumes I and II.

Standard Methods Committee, 1997. Metals by Inductively Coupled Plasma/Mass Spectrometry: ICP/MS Method. Section 3125 B.

U.S. EPA 6020 (Modification). Analysis of trace metals in biological material by Inductively Coupled Plasma Mass Spectrophotometry. U.S. EPA Method No. 6020 (Modification).

APPENDIX A

STANDARD REFERENCE MATERIAL

Certificate of Analysis

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1566b

Oyster Tissue

This Standard Reference Material (SRM) is intended primarily for use in evaluating analytical methods and instruments used for the determination of the concentrations of selected elements and proximates, selected fatty acids, total dietary fiber, as well as the caloric content in marine bivalve tissue, foods, or similar materials. A unit of SRM 1566b contains approximately 25 g of freeze-dried oyster tissue.

Certified Concentrations of Constituent Elements: Certified values for 22 elements and for methylmercury are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been fully investigated or accounted for by NIST. Certified values are based on results obtained by a single primary method with confirmation by other methods, or with two or more critically evaluated independent methods [1]. The certified value for sulfur is the result of a single NIST method. All other certified values are weighted means of results obtained using two or more independent methods.

Reference Concentration Values: Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification. Such values are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. Reference values for elemental concentrations are provided in Table 2. Reference values for proximates, nitrogen, total dietary fiber, selected fatty acids, and caloric content are provided in Table 3.

Information Concentration Values: Information concentration values for selected fatty acids are provided in Table 4. These are noncertified values with no reported uncertainties as there is insufficient information to assess uncertainties. The information values are given to provide additional characterization of the material, and are not recommended for use to monitor or assess analytical performance.

Expiration of Certification: The certification of this SRM lot is valid until **01 June 2010**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is nullified if the SRM is contaminated or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of certification. If substantive changes occur that affect the certification prior to the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

The coordination of the technical measurements leading to the certification of this SRM was performed by R.R. Greenberg of the NIST Analytical Chemistry Division. Coordination of technical measurements of organic compounds and methylmercury leading to certified, reference, and information values was performed by K.E. Sharpless and S.A. Wise of the NIST Analytical Chemistry Division.

The NIST analysts and cooperating laboratories that participated in the characterization of this SRM are listed in Appendix A.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert.

Willie E. May, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 17 January 2001

Nancy M. Trahey, Chief
Standard Reference Materials Program

Preparation of the oyster tissue material was performed by B.J. Porter of the NIST Analytical Chemistry Division and M.P. Cronise, C.N. Fales, and D.G. Friend of the NIST Standard Reference Materials Program.

The statistical analysis of the data was performed by J.H. Yen, L.M. Gill, and M.S. Levenson of the NIST Statistical Engineering Division.

NOTICE AND WARNING TO USERS

Storage: The material should be kept in its original bottle, tightly closed, and stored in a desiccator over magnesium perchlorate $Mg(ClO_4)_2$, at temperatures between 10 °C to 30 °C. It should **NOT** be exposed to intense sources of radiation, including ultraviolet light or sunlight.

Use: A minimum sample mass of 250 mg of material is necessary for any certified value in Table 1 to be valid within the stated uncertainty. This amount of material should be on a dry-mass basis (see Instructions for Drying). The contents of the bottle should be shaken well before each use, closed tightly **immediately** after use, and stored as described above.

Instructions for Drying: Prior to removal of subsamples for elemental analysis, the contents of the bottle should be thoroughly mixed. Before the mass determination, samples of SRM 1566b must be dried to constant mass by one of the following procedures:

Drying at room temperature for *at least* 5 d over $Mg(ClO_4)_2$ in a desiccator.
Vacuum drying at room temperature for *at least* 24 h at a pressure of approximately 30 Pa (0.2 mm Hg) using a cold trap.
3. Freeze drying for *at least* 5 d at a pressure of approximately 30 Pa (0.2 mm Hg).

The analyst should ascertain that the material has indeed reached constant mass. Although the above procedures have been generally sufficient, in a few instances the time needed to reach constant mass was longer than listed above. If the constituents of interest are volatile, a separate subsample of the oyster tissue should be removed from the bottle at the time of analysis and dried to determine the concentration on a dry-mass basis.

SOURCE, PREPARATION, AND ANALYSIS[1]

Source and Preparation of Material: The oysters (*Crassostrea virginica*, an American Eastern oyster) used for the preparation of SRM 1566b were purchased from Bon Secour Fisheries, Inc., Bon Secour, AL. The oysters were collected from the Gulf of Mexico, shucked, rinsed twice to remove sediment and shells, packed and sealed in polyethylene bags, and frozen. The frozen oysters and fluids were shipped in styrofoam coolers containing dry ice to NIST. At NIST, the oysters and fluids were ground in a Robot-Coupe Vertical Cutter Mixer that was equipped with a stainless steel bowl and titanium blades. The oyster tissue was blended for 100 s into a slurry; approximately 5 kg of slurry was poured into each of 40 specially cleaned aluminum trays outfitted with temperature probes, and frozen at -20 °C. The trays were taken to a large freeze-drying facility at the Frederick Cancer Research and Development Center, Natural Products Group in Frederick, MD. The freeze-dryer's initial temperature was -45 °C and gradually increased to a temperature of 10 °C over a period of five days. The freeze-dried material was stored at -20 °C, then broken into smaller pieces, blended in the Robot-Coupe Mixer, jet milled, and homogenized in a V-blender for 30 min to 40 min. The material was radiation sterilized (^{60}Co) at Neutron Products, Inc., Dickerson, MD, for approximately 5 h at 3 Mrad and then aliquoted into amber bottles.

Description of Calculations Used in Value Assignment

A. Certified Values and Their Uncertainties

Sulfur

The certified value of sulfur is the result of a single NIST method, thermal ionization mass spectrometry (TIMS), with confirmation by a second NIST method. Its uncertainty is expressed as an expanded uncertainty, U, and is calculated as $U = ku_c$. The quantity, u_c, is the combined standard uncertainty calculated according to the ISO Guide [2] that accounts for the combined components of uncertainty for the method at one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to five degrees of freedom and a 95 % prediction interval.

[1]Certain commercial equipment, instrumentation, or materials are identified in this certificate to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Methylmercury

The results for methylmercury are expressed as mg/kg mercury. The certified value is the mean of results from four different laboratory analyses of SRM 1566b using four different analytical methods. The expanded uncertainty in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the ISO Guide [2] and k is the coverage factor. The value, u_c, is intended to represent, at the level of one standard deviation, the combined effect of all the uncertainties in the certified value. Here, u_c is given by the standard error of the mean of the four analyses. The coverage factor, k, is determined from the Student's t-distribution corresponding to three degrees of freedom and 95 % confidence for each analyte.

All Other Elements

All other certified values are weighted means of results from two or more analytical methods. For these certified values, the uncertainty is calculated as $U = ku_c + B$. The quantity, u_c, is the combined standard uncertainty calculated according to the ISO Guide [2], which accounts for the combined effect of the within variance for all methods at one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each element. The term, B, is a bias adjustment for the difference between methods which is the maximum difference between the certified value and the method means [3]. Because of heterogeneity, the uncertainty associated with calcium and thorium takes the form of a prediction interval.

Table 1. Certified Concentration Values of SRM 1566b[1]

Element	Mass Fraction (%)			Element	Mass Fraction (%)		
Calcium[c,g]	0.0838	±	0.0020	Potassium[g,i,k]	0.652	±	0.009
Chlorine[g,i]	0.514	±	0.010	Sodium[b,g]	0.3297	±	0.0053
Magnesium[c,g]	0.1085	±	0.0023	Sulfur[d,i]	0.6887	±	0.0140

Element	Mass Fraction (mg/kg)			Element	Mass Fraction (mg/kg)		
Aluminum[g,l]	197.2	±	6.0	Mercury[a,n] (total)	0.0371	±	0.0013
Arsenic[g,j]	7.65	±	0.65	Methylmercury[o,p,q,r] (as mercury)	0.0132	±	0.0007
Cadmium[e,h]	2.48	±	0.08	Nickel[e,j,k]	1.04	±	0.09
Cobalt[e,g]	0.371	±	0.009	Rubidium[e,g]	3.262	±	0.145
Copper[e,g,h]	71.6	±	1.6	Selenium[g,j]	2.06	±	0.15
Iron[g,k,m]	205.8	±	6.8	Silver[e,g]	0.666	±	0.009
Lead[e,e]	0.308	±	0.009	Thorium[e,g]	0.0367	±	0.0043
Manganese[e,g]	18.5	±	0.2	Vanadium[e,g]	0.577	±	0.023
				Zinc[g,k]	1 424	±	46

Dry-mass basis

Analytical Methods:

[a] Cold vapor atomic absorption spectrometry at NIST
[b] Flame atomic emission spectrometry at NIST
[c] Isotope dilution inductively coupled plasma mass spectrometry at NIST
[d] Isotope dilution thermal ionization mass spectrometry at NIST
[e] Inductively coupled plasma mass spectrometry at NIST
[f] Inductively coupled plasma optical emission spectrometry at NIST
[g] Neutron activation analysis (instrumental) at NIST
[h] Neutron activation analysis (radiochemical) at NIST
[i] Prompt gamma activation analysis at NIST
[j] Electrothermal atomic absorption spectrometry at National Research Council of Canada (NRCC)
[k] Isotope dilution inductively coupled plasma mass spectrometry at NRCC
[l] Inductively coupled plasma mass spectrometry at NRCC
[m] Inductively coupled plasma optical emission spectrometry at NRCC
[n] Neutron activation analysis (radiochemical) at Jožef Stefan Institute, Ljubljana, Slovenia
[o] Gas chromatography with atomic emission detection at NIST
[p] Gas chromatography with atomic fluorescence detection at Jožef Stefan Institute, Ljubljana, Slovenia
[q] Cold vapor atomic absorption spectrometry at Jožef Stefan Institute, Ljubljana, Slovenia
[r] Cold vapor atomic absorption spectrometry at Research Centre Jülich, Jülich, Germany

Table 2. Reference Concentration Values of SRM 1566b[1]

These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification; therefore unrecognized bias may exist for some measurands in this matrix.

Element	Mass Fraction (%)
Nitrogen[a]	7.6 ± 0.4

Element	Mass Fraction (mg/kg)	Element	Mass Fraction (mg/kg)
Antimony[b]	0.011 ± 0.002	Strontium[c]	6.8 ± 0.2
Barium[d]	8.6 ± 0.3	Tin[d]	0.031 ± 0.008
Boron[a]	4.5 ± 1.9	Uranium[d]	0.2550 ± 0.0014
Hydrogen[a]	7.2 ± 0.4		

[1] Dry-mass basis

[2] Analytical Methods:

[a] Prompt gamma activation analysis at NIST
[b] Neutron activation analysis (instrumental) at NIST
[c] Inductively coupled plasma mass spectrometry at NIST
[d] Isotope dilution inductively coupled plasma mass spectrometry at NRCC, Ottawa, Canada

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 1577b

Bovine Liver

This Standard Reference Material (SRM) is intended primarily for use as a control material and in evaluating analytical methods for the determination of major, minor, and trace elements in animal tissue and other biological matrices.

Certified Values of Constituent Elements: The certified values for the constituent elements are given in Table 1. Certified values are based on results obtained by definitive methods of known accuracy; or alternatively, from results obtained by two or more independent analytical methods. Noncertified values are provided for information only in Table 2. Analytical methods used for the analysis of SRM 1577b, including certified and information values, are given in Table 3.

Notice and Warnings to Users:

Expiration of Certification: This certification is invalid after 5 years from the date of purchase and shipment from NIST. Should any of the certified constituents change within a 5-year period purchasers will be notified by NIST.

Stability: The SRM should be kept in its original bottle and stored between 10-30 °C. It should not be exposed to intense sources of radiation. The bottle should be kept tightly closed and stored in a desiccator away from direct sunlight.

Use: A minimum sample of 250 mg of the dried material (see Instructions for Drying) should be used for any analytical determination to be related to the certified values of this Certificate.

Dissolution procedures should be designed to effect complete solution, but without losses of volatile elements, such as mercury. Dissolution for these determinations should be carried out in a closed system.

Statistical consultation was provided by S.B. Schiller of the Statistical Engineering Division.

The overall direction and coordination of the analyses leading to this certification were under the chairmanship of J.R. DeVoe, Chief of the Inorganic Analytical Research Division.

The technical and support aspects involved in the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Standard Reference Materials Program by T.E. Gills.

Gaithersburg, MD 20899
August 27, 1991

William P. Reed, Chief
Standard Reference Materials Program

(over)

Table 1. Certified Values of Constituent Elements

Element	Content (wt. percent)
Chlorine	0.278 ± 0.006
Phosphorus	1.10 ± 0.03
Potassium	0.994 ± 0.002
Sodium	0.242 ± 0.006
Sulfur	0.785 ± 0.006

Element	Content (μg/g)	Element	Content (μg/g)
Cadmium	0.50 ± 0.03	Molybdenum	3.5 ± 0.3
Calcium	116 ± 4	Rubidium	13.7 ± 1.1
Copper	160 ± 8	Selenium	0.73 ± 0.06
Iron	184 ± 15	Silver	0.039 ± 0.007
Lead	0.129 ± 0.004	Strontium	0.136 ± 0.001
Magnesium	601 ± 28	Zinc	127 ± 16
Manganese	10.5 ± 1.7		

The estimated uncertainty of a certified value is the sum of the half-width of a 95% confidence interval for the mean of results and an allowance for systematic error, except for the uncertainty associated with sulfur which is based on scientific judgment and is roughly equivalent to one percent of the certified value. The systematic error is estimated by the maximum absolute deviation between a single method mean and the grand mean. The grand mean was computed using the weighting scheme of Paule and Mandel (NBS Journal of Research 87, pp 377-385).

Table 2. Noncertified Values of Constituent Elements

Element	Content (wt. percent)
Nitrogen	(10.6)

Element	Content (μg/g)	Element	Content (μg/g)
Arsenic	(0.05)	Cobalt	(0.25)
Aluminum	(3)	Vanadium	(0.123)
Antimony	(0.003)		
Bromine	(9.7)		
Mercury	(0.003)		

Instructions for Drying: Samples of this SRM must be dried before weighing according to the following procedure: Dry for 24 hours at 20 to 25 °C in a vacuum oven at a pressure not greater than 30 Pa (0.2 mm Hg).

Source and Preparation of Material:

The bovine liver for this standard was obtained in the Portland, Oregon area. The gross fat, major blood vessels, and "skin" were removed and the liver was ground. The ground liver was then mixed, transferred to polyethylene-lined trays, and lyophilized by Oregon Freeze Dry Foods, Inc., Albany, Oregon. After lyophilization, the liver was powdered in a Tornado mill, packaged in moisture-proof bags, and then transported to the National Institute of Standards and Technology.

Quality Assurance and Quality Control Protocol For Data of the Ecological Risk Assessment Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

Quality assurance and quality control (QA/QC) is essential in order to assess variability between samples collected in the field (in this report referred to as duplicate samples), aliquots (in this report referred to as replicate samples) analysed in the laboratory, and to compare analytical results of Standard Reference Material (SRM's) with certified expected values. This protocol outlines the methods used to calculate percentage difference between analytical results of duplicate samples from the field, replicate analysis of samples in the laboratory, and obtained and expected results of SRM's. In addition, a description of how replicate and triplicate values on a particular sample were averaged to yield a mean for use in the Ecological Risk Assessment (ERA) is also provided. The procedures mentioned here are those that were followed by Jacques Whitford to calculate percent differences between replicate samples, duplicate samples and Standard Reference Materials (SRM). QA/QC procedures conducted by Philips Analytical Services Inc. (PSC) in the laboratory, including process blanks, process % recovery and matrix spikes are listed on every certificate of analysis in volume V.

2. OBJECTIVES

1. To outline the accepted method for calculating percent differences between replicate samples, duplicate samples and obtained and expected Standard Reference Material data.

2. To indicate when replicates and or triplicates were averaged to arrive at a mean for use in ERA calculations.

3. PERCENTAGE DIFFERENCE CALCULATION FORMULA

As a means of determining the reproducibility or variability related to analytical procedures, Jacques Whitford's calculated the percentage differences between analysed values for the original and duplicate, replicate and SRM samples. In situations where more than one replicate was analysed, results with the largest variance were used to calculate percentage difference in order to acquire a conservative measure of variance.

For sample reproducibility calculations, percentage differences were calculated for those chemical parameters with analytical values greater than 3 X EQL (EQL is the estimated quantitation limit, i.e., the lowest level of a parameter that can be identified with confidence by an analytical laboratory).

Percentage differences will be determined using the following formula:

$$Percentage\ difference\ of\ \ Analyte\ A = \frac{(Analyte\ A\ in\ test\ 1 - Analyte\ A\ in\ test\ 2)\ x\ 100}{(Analyte\ A\ in\ test\ 1 + Analyte\ A\ in\ test\ 2)\ /\ 2}$$

In some instances, percent differences were not calculated due to data being less than three times the EQL or results being reported as "nd" (non-detect) by PSC. For these situations, no percent difference was calculated and "NC" (not calculated) was presented in the QA/QC report in Volume V. For the purposes of reporting in Volume V, results of Test 1 of a sample are considered "Original" and results of Test 2 are considered "Duplicate" or "Replicate" results. Calculating the percent difference between expected and observed values for SRM's used the same formula as shown above. Expected values were considered test 1, and observed values were considered test 2.

4. QUALITY ASSURANCE/QUALITY CONTROL LABORATORY DUPLICATE, REPLICATE AND TRIPLICATE ANALYSIS

For soils, percent differences between two duplicate samples or two replicate samples less than 70 were considered acceptable. For all other matrixes, a percent difference of less than 30 was considered acceptable (CCME 1993; *pers. comm.* Bishop 2002). The acceptable range for soils is greater due to the inherent variability with this heterogeneous matrix. Where duplicate analyses were performed on two samples or where replicate or triplicate analysis was done on the same sample, the mean of the respective analyses contributed to the ERA report.

5. CONCLUSION

This protocol has outlined the methods that were followed to assess data as to its variability before it was used in various components of the ERA. Results of Jacques Whitford's QA/QC procedure are presented in the Ecological Risk Assessment-Natural Environment, Volume 5.

6. REFERENCES

Bishop, J. Senior Consultant, Stantec. Personal communication, telephone. 2002.

Canadian Council of Ministers of the Environment, December 1993, Guidance Manual on Sampling, Analysis, and Data Management for Contaminated Sites, Volumes I and II.

Soil/Sediment Sampling and Analysis Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

Site specific chemical analysis of soils, including the four CoCs, were obtained in the general location where biological specimens were collected as part of the Ecological Risk Assessment (ERA) in Port Colborne. Sediment samples were collected from ponds where tadpoles and frogs were sampled. Quantifying CoCs in soils and sediments in the general location where biological specimens resided was a necessary and critical step in the ERA process to assess the relationships between soil/sediment CoCs concentrations and biological samples.

Methods for the collection of receptors for this ERA dictated the pattern in which soils and sediments were collected. For example, insects were collected along 100 meter transects whereas earthworms were collected from 1x1 meter quadrates. Therefore, depending on the receptor being sampled, different methods were used to obtain a representative soil or sediment sample for the site. These methods are outlined in the respective data collection protocols.

The depth at which soils were sampled for the ERA remained consistent, 0 to 5 centimeter depth, throughout the study since all receptors collected in the study had exposure to this profile of soil. Additionally, this profile of soil was found to have the highest level of CoCs compared with the profile ranging from the 5 to 15 centimeter depth (as indicated by maple sap soil data, Volume V) and therefore would generally provide a conservative estimate of CoC exposure from soils.

2. OBJECTIVES

This soil/sediment sampling and analysis protocol has two objectives:

1. To outline the equipment and sampling procedures that were used to collect soils at the 0-5 centimeter depth.

2. To illustrate the analytical methods that were used by Philip Analytical Services Inc. (PSC) to quantify CoCs in the soils and sediments collected.

3. SOIL/SEDIMENT SAMPLING METHODS

Samples of soil/sediment were collected from the 0-5 cm depth at the general location where biological specimens were collected. For the location of biological sample collection and corresponding soil/sediment sample areas, please refer to the sample location maps (Figure 1-1 and Figure 1-2) at the back of this volume. Each sample consisted of approximately 10 soil cores distributed uniformly across the sample area. Please refer to the respective sampling protocols for a description on how core sampling was distributed across the sample area for each biological sample collected. Cores were taken by a stainless steel Oakfield® soil corer at a depth of 0-5 cm (except those collected at the location of maple sap collection, 0-5 and 5-15cm depth) and were transferred to a labeled container that was submitted for laboratory analysis at PSC. All samples remained on ice in a cooler until being received at PSC, at which time they were refrigerated until sample preparation.

4. LABORATORY SAMPLING METHODS

Once collected, samples were submitted to PSC for analysis. PSC is accredited by the Standards Council of Canada/Canadian Association of Environmental Analytical Laboratories (CAEAL) program. Therefore all samples were handled, processed, and analysed in accordance with CAEAL procedures. All soil and sediment samples were analysed for 19 inorganic parameters including the CoCs. Parameters included all those identified in the Ministry of Environment "Guideline for Use at Contaminated Sites in Ontario" (Guideline) and other parameters included in the Inductively Coupled Plasma Emission (ICP–ES) and hydride generation analytical (AAS) process:

1. by ICP-ES - Ni, Co, Cu, Fe, Pb, Zn, Cr, Al, Ag, Ba, Be, Cd, Mo, P, Ti, V, Mn.

2. by AAS - As, Se.

5. QUALITY ASSURANCE/ QUALITY CONTROL

For quality assurance, a representative of Stantec was allowed to monitor all soil and sediment sampling. Jacques Whitford took an additional 15 percent of samples in order to have duplicate samples that could be used to assess the variance in data from 15 percent of the sites sampled. In addition, the PLC's consultant took an additional 15 percent of soil/sediment samples for QA/QC purposes.

Surface Water Sampling Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

As part of the Ecological Risk Assessment (ERA) for the Community Based Risk Assessment (CBRA), to address the potential for ecological risk for chemicals of concern (CoCs) in the aquatic environment, a surface water sampling program was undertaken in the spring and summer of 2001. The results of this program will be used to assess potential risks to specific aquatic receptors in the environment by providing concentration values of CoCs that occur in surface water in the study area. The following details a protocol for the collection and analysis of surface water in and around the Port Colborne Inco Refinery site.

2. GENERAL STUDY APPROACH

For the purpose of this program, surface water is defined as standing water in permanent and temporary ponds, municipal/agricultural drains and ditches which are found in the Study Area (as defined by soil nickel concentrations, MOE, 2000a; MOE, 2000b). The aquatic environment in and along the shore of Lake Erie were not included in this program. In addition, collection of surface water from stormwater and/or treatment ponds were not included in the program. The location of sample sites was determined during an amphibian breeding survey which was conducted by Jacques Whitford Environment Limited (Jacques Whitford) in April, May and June of 2001. For the assessment of CoCs in the aquatic environment, surface water samples were collected from the following areas:

1. The Primary Study Area - area with reported high soil nickel between 500-3000 ppm (MOE, 2000a; MOE, 2000b).

2. The Secondary Study Area - area with reported moderate soil nickel between 200-500 ppm (MOE, 2000a; MOE, 2000b).

3. Reference Areas located west of the City of Port Colborne.

Sample collection was undertaken from April through to August of 2001.

For the field collection program, general sampling methodology followed the methods and protocols developed by the Ontario Ministry of the Environment Guidance on Sampling and Analytical Methods for Use at Contaminated Sites in Ontario, Section 5.3 Surface Water Sampling (MOE 1996), and MOE Guide to the Collection and Submission of Samples for Laboratory Analysis (MOE 1993).

Samples were collected using the manual grab sampling technique. A single grab sample is used to represent surface water at a given point of time as opposed to a composite sample that represents conditions over a longer time period (i.e., 24 hours). Given the shallow nature of surface water bodies in the local area, grab samples were collected by hand by Jacques Whitford field staff. A grab method was used (MOE 1996), where the water sample was collected directly into the laboratory container provided by PSC, with no transfer of the sample from a collection container to a laboratory container.

3. SAMPLING METHODOLOGY

3.1 Collection Method

Following the identification of a surface water body from which samples were to be taken, the specific location for the collection of a sample was documented with the station located near the central area of the water body. For specific sample site locations, please see the sample location maps (Figures 1-1 and 1-2) at the back of this Volume.

Grab samples were collected in 500-ml plastic sample containers that were provided by PSC. Sample containers were labeled with the location and date and time prior to sample collection. Prior to taking the sample, the container was rinsed at source three times with the water to be collected. Samples were collected by submerging the container, with cap on, to a central position in the water column. The container's cap was then removed and water allowed to flow into the container. Once full, the container was removed in an upright position and capped. Sample preservation requirements (analytical grade Nitric Acid of pH <2.0) was added to the container in the field immediately after collection of the sample. As part of the analytical program, travel and field blanks were provided by PSC prior to sampling in the field. The cap of the field blank was removed during the water sampling procedure at a sample location. The samples blanks were stored in a cooler on ice packs and maintained at temperature between 4 and 9°C. Following collection, samples were delivered to the laboratory within 24hr for analysis.

During sample collection, care was taken to prevent disturbing sediments in order to reduce particulate suspension into the water column from which the sample was collected. For watercourses where a current was present, sampling within the stream proceeded from a downstream location to an upstream location to prevent disturbed sediments from being collected in the sample. Throughout all sample collection procedures, latex or PVC unpowdered gloves were worn by the collector(s).

In addition to the collection of water samples, at each sample station the following was recorded:

- Data of Sampling
- Time of Sampling
- Water temperature
- pH
- Water depth (cm)
- UTM co-ordinates
- Description of Substrate (i.e., organic, sand, gravel, clay, detritus etc)

The pH was determined with a Fisher Scientific portable Accumet pH meter (model AP61). Temperature was recorded to the nearest degree Celsius. pH and the other physical parameters were determined at each station following the collection of the water sample to reduce sediment contamination in the sample. For each sample station, data was recorded on a standardized field data sheet (attached).

4. ANALYTICAL PROGRAM

Once collected, surface water samples were submitted to PSC, accredited by the Standards Council of Canada/Canadian Association of Environmental Analytical Laboratories (CAEAL) program. Samples were analyzed for 30 parameters, including ICP-MS metals (17), and Arsenic and Selenium at detection levels below Provincial Water Quality Objectives (PWQO) for CoCs. Details regarding the laboratory testing guidelines and procedures are provided in a separate protocol developed by Jacques Whitford for the CBRA (Jacques Whitford 2001).

5. QUALITY ASSURANCE/QUALITY CONTROL

As part of an overall quality assurance/quality control (QA/QC) program to monitor and evaluate the accuracy of the Jacques Whitford data, a representative of the Public Liaison Committee (PLC) provided third party verification in the field program during the collection of water samples. A sub set (20%) of additional duplicate samples were collected by the PLC representative for their submission for chemical analyses. For Jacques Whitford's QA/QC during the field program, a duplicate sample was taken for every 5 samples collected and submitted to the laboratory as a blind duplicate. Trip/field and spiked blanks were included in the sampling program. Field and laboratory analysis quality control procedures

(including the use of certified reference materials and standards with analytical runs) are detailed in specific protocol developed for the CBRA (Jacques Whitford 2001).

6. INTERPRETATION OF DATA AND REPORTING

Surface water analytical data was compared to the Water Management – Policies Guidelines Provincial Water Quality Objectives (PWQO) (MOE 1994). In addition, in the absence of a PWQO, data comparisons were made to relevant criteria found in the literature, where available. Data collected from surface water sampling program was also integrated with the frog and tadpole data collected as part of the ERA program.

7. REFERENCES

Jacques Whitford, 2001. Sampling and Analysis: Quality Assurance and Quality Control. Port Colborne Community Based Risk Assessment. Jacques Whitford's July 9, 2001 Protocol, Appendix C5 in Report Entitled "Potential CoC Identification Using Soil Chemical Concentration Data In Exceedance of MOE Generic Guidelines, Port Colborne Community Based Risk Assessment, Port Colborne, Ontario. Draft document prepared for Inco Ltd., November 23, 2001.

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MOE, 2000b. Phytotoxicity Soil Investigations: Inco – Port Colborne (1999). Ontario Ministry of the Environment. July 2000.

PORT COLBORNE CBRA
SURFACE WATER COLLECTION
FIELD DATA SHEET

Date (dd-mm-yr) _____

Time _____

Station Indentification Number _____

UTM co-ordinants _____

Water Temperature ^{o}C _____

Water Depth (at sample site) _____

Water pH (at sample site) _____

Substrate _____

_____

_____

_____

Additional Comments _____

_____

Amphibian Survey Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

The Ecological Risk Assessment (ERA) component of the Community Based Risk Assessment (CBRA) for Port Colborne involves identifying important ecosystem components and determining whether they are at risk as a result of Chemicals of Concern (CoCs). Amphibians are important components of aquatic and terrestrial ecosystems because considerable evidence suggests that they are sensitive to environmental stressors. There are potentially nine species of frogs and toads (anurans) and six species of salamanders and newts in the Port Colborne area. Jacques Whitford Environment Limited (JWEL) chose to investigate the distribution and abundance of frogs and toads as potential Valued Ecosystem Components (VECs) in the Study Area (as defined by soil nickel concentrations, MOE, 2000a, MOE, 2000b). Though considered, conducting structured surveys for salamanders and newts in the study area was not undertaken as these species are not easily detected in a landscape when compared to frog and toads. Frogs and toads can be surveyed by listening to, and counting, the calls of males during the breeding season. Each species has a distinctive call and tape recordings are available of these calls. In addition, methods for surveying frogs and toads is well established (Heyer *et al*. 1994, MMP 2001, NAAMP 2001).

2. OBJECTIVES

The primary objectives of the 2001 frog survey were:

1. To undertake a frog and toad survey using anuran calls to determine their distribution and relative abundance in the Port Colborne Study Area.

2. To establish the breeding status of nationally rare, provincially rare and locally rare species [including Vulnerable, Threatened and Endangered (VTE) species] that could potentially occur in the Study Area.

3. To select individual anuran species as VECs for the ERA.

The survey methods detailed below allowed for the assessment of anuran breeding occurrence and abundance for one breeding season. No comparative year-to-year data were collected due to the time limitation of the ERA study.

3. GENERAL STUDY APPROACH

JWEL's approach in conducting the amphibian survey is to determine what species occur in the study area and to undertake an assessment of their abundance. The survey was undertaken following accepted methods as described by protocols developed by the Marsh Monitoring Program (MMP 2001) and the North American Amphibian Monitoring Program (NAAMP 2001). The protocols developed by these programs have been modified for the Port Colborne area.

The survey uses stations at approximately 0.8 km intervals along the road network. This survey method does not specifically target survey stations that are located in or near potential frog breeding areas. In this way, the general distribution and abundance of breeding frogs can be assessed at a landscape level. The primary focus of the road survey was to undertake night surveys in the spring and early summer to document the breeding calls of male frogs and toads in the Study Area. Existing atlas survey data for Ontario (Weller and Oldham 1988, Plourde *et al*. 1989; Oldham and Weller 2000) provide a list of the species that could occur and their peak times for calling during the breeding season in the Niagara area (Table 1).

Table 1: Species of Frogs and Toads in the Port Colborne Area

Species	Peak Calling Period
American Toad	Second week of April through first week in May
Fowler's Toad	Last two weeks of May
Grey Treefrog	Last two weeks of May through first week in June
Chorus Frog	Second week of April through first week in May
Spring Peeper	Third and fourth week of April
Wood Frog	Second week of April through first week in May
Leopard Frog	Second week of April to second week in May
Green Frog	Third and fourth week of May
Bullfrog	Last week of May and first week of June

4. SURVEY METHODOLOGY

4.1 Calling Survey Method

Calling surveys require listening for the distinctive calls of male frogs and toads during the breeding season. At each survey station, a listening period of four minutes was used. A standardized index of abundance was recorded for each species according to the following code system:

Code	Criteria
0	No calls can be heard
1	Individual calls heard and not overlapping
2	Calls are overlapping; but individuals are still distinguishable
3	The number of individuals calling is so large that the chorus is constant and individual calls are overlapping

Calling surveys were conducted over one night for four survey periods as follows:

Survey 1 – April 12 (night air temp 5-7°C)

Survey 2 – April 23 (night air temp variable but above 10°C and as high as 18°C)

Survey 3 – May 7 (night air temp 14-17°C)

Survey 4 – May 17 (night air temp above 14-17°C)

4.2 Road Survey

The surveys began one hour after sunset, and station stops were made every 0.8 km. At each station, the car was stopped on the side of the road and the surveyor exited the vehicle. After one minute, a four-minute listening period was started. During this period, a call code was assigned for each species heard. A tape recorder and parabolic reflector with high quality microphone was used to record the frog calls and chorus at selected sites.

For the route, each station was given a specific identification number and the following was recorded on a field data collection sheet:

- Station Number.
- Start time of survey period.
- Air temperature, wind and cloud cover.
- Species calling and calling code for each species.

(See attached field sheet)

The route for the road survey was as follows:

- Starting at Chippawa and Second Concession, eastward on the Second Concession to Miller Road. Miller Road south to Killaly East.
- Killaly East to Pinecrest.
- South on Pinecrest to Firelane #2, westward on Fireline#2 to Lakeshore E. to Reuter Road.
- North on Reuter Road.
- All other north-south roads between the Second Concession and Lakeshore/Firelane #2 were surveyed also, including Elizabeth St/Highway 140, Lorraine/Babian Road, and Weaver Road. For each of these roads, the starting point was at the Killaly East intersection. From the Killaly East intersection, surveys were conducted both northward and southward from the intersection.

Finally, two survey stations were undertaken west of the Inco Refinery site in the City of Port Colborne, at the intersection of Rodney Street and Welland, and at the intersection of Rodney Street and Davis.

Based on this route, a total of 29 stations was surveyed for the program. For the specific location of each survey station, please see the sample location map (Figure 1-1) at the back of this Volume.

To provide an element of standardization, surveys were undertaken during optimal conditions:

- Air temperature above minimum listed for each survey period.
- High humidity, light rain.
- Wind scale 0-2 (Beaufort Wind Scale).
- Cloudy 1-2 (Sky code).

Surveys were not performed during nights with cold temperatures, high winds and rain/shower conditions. During the survey periods, weather conditions were recorded.

4.3 Rare Species Survey

The nationally and provincially "Threatened" Fowler's Toad has been recorded from many locations along the Lake Erie shoreline and in the Nickel Beach area (Plourde et al. 1989, Oldham and Weller 2000). Field surveys undertaken by JWEL in 2000, as part of the ERA site characterization, identified

potential habitat for this species along the lakeshore in the Nickel Beach area (JWEL, 2001). Therefore, due to the significance of this species, a specific foot survey for Fowler's Toad was performed by JWEL during 2001.

The survey focussed on backshore areas of Nickel Beach (on the Inco Refinery Site) and along Lakeshore East and Firelane #2 eastward to Lorraine Road. A daytime survey was undertaken to locate potential breeding ponds and pools, followed up by nighttime surveys.

Breeding sites for Fowler's Toad identified during the survey were visited during the day to document the existing breeding habitat. Breeding sites were visited from May to July 2001 to document progress of the tadpole development, final metamorphosis to the young toad stage and emigration from the breeding pool. Surface water and sediment samples were collected from Fowler's Toad breeding ponds. Information regarding breeding numbers and locations will be forwarded to the Natural Heritage Information Centre so that current breeding sites for this rare species are recorded for Ontario.

5. TREATMENT OF DATA

Following the completion of the survey, a general assessment of the study area's amphibian populations was undertaken. Data collected during the survey was compared to existing information on file with the Ontario Herpetofaunal Summary at the Natural Heritage Information Centre and the Ontario Marsh Monitoring Program. For assessment of breeding occurrence against soil CoCs, analysis was based on the highest calling code for each species during the survey period. The incidence of species at a survey station was related to an assessment of the survey area's anuran breeding habitat for an area up to 300 m from the station, based on aerial photograph interpretation. In addition, important breeding ponds for the species present were identified and mapped for the Study Area. The selection of VEC's was made after the amphibian survey was completed and potential areas for the collection of frogs, toads and tadpoles for tissue analysis were identified as a result of the survey.

5.1 Quality Assurance/Quality Control

For quality assurance, a representative from the Public Liaison Committee's (PLC's) consultant was present during the surveys. For the Fowler's Toad the PLC's consultant undertook a sound recording of the call and photographs of a breeding pond was taken for documentation of the occurrence for the Ontario Natural Heritage Information Centre data base.

6. REFERENCES

JWEL 2001. Ecological Risk Assessment, Natural Environment, Part of the Port Colborne CBRA. Draft Report. Prepared by Jacques Whitford Environment Limited, Markham, Ontario.

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PORT COLBORNE CBRA
Amphibian Survey Field Data Collection Sheet

Visit #1 **Date (dd-mm-yr):** _____ **Start time** _____

Wind Scale No: _____ **Cloud Cover (10^{ths})** _____ **Air Temp. (°C or °F)** _____

		Station _____		
Species	Code	Chorus Code	Number of Individuals Counted	In*
Chorus Frog	CHFR			
Spring Peeper	SPPE			
Wood Frog	WOFR			
American Toad	AMTO			
Fowler's Toad	FOTO			
Northern Leopard	FLFR			
Green Frog	GRFR			
Bullfrog	BULL			
Gray Treefrog	GRTR			

**Individuals within 120 meters of recorder.*

Additional Comments: _____

JWEL Recorder: _____ **PLC Recorder:** _____

Bird Survey Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

Initial site characterisation for the Ecological Risk Assessment (ERA) for the Port Colborne Community Based Risk Assessment (CBRA) was undertaken in July 2000 (JWEL, 2001). These investigations identified and characterised the avifauna for a section of the total study area (primarily the lands in and around the Inco Port Colborne Refinery, to just easterly of Snider Road). The report identified that because the bird survey was conducted during the mid-summer period, the breeding status of a number of rare species (Hooded Warbler, Prothonotary Warbler, Red-headed Woodpecker, Yellow-breasted Chat) could not be determined.

The identification of the avifauna in the Study Area (as defined by soil nickel concentrations, MOE 2000a,b) and presence of rare breeding bird species, is important for the selection of receptors and/or Valued Ecological Components (VECs). Therefore, additional bird surveys were undertaken during the primary breeding season in 2001 for the Study Area.

2. OBJECTIVES

The primary objectives of the 2001 bird survey were:

1. To compile a species list of the study area's avifauna which can be compared to other lists for the area.

2. To find out whether any Vulnerable, Threatened or Endangered (VTE) species are breeding in the study area.

3. To select the most appropriate bird species to be used as receptors/VECs in the ERA to track potential risks of Chemicals of Concern (CoCs).

Several VTE species are recorded from the Port Colborne area and they could occur and breed in wetlands and woodlands in the area. The field studies were more intensive than those carried out in 2000 and were undertaken when the majority of woodland and wetland species were singing and establishing territories. Information on the status of VTE species can be found in Austen *et al*. (1994) or on the NHIC website at http://www.mnr.gov.on.ca/MNR/nhic/nhic.html.

The species list developed in 2000 for the Study Area was augmented by including other species encountered during transect walks through the major habitat types in 2001. In 2001 the general distribution and abundance of each species was recorded during the transect walks to ensure that the receptors/VECs selected to represent the avifauna were indeed present during the breeding season and representative of the study areas bird community.

3. SURVEY APPROACH

Three survey methods are typically used to census birds: point counts, line transects and territory mapping (Bibby *et al*. 1992). A modified open-ended transect method was used for this study. This method is the most efficient of all general survey methods in terms of data gathered per unit effort. The survey was conducted to detect species occurrence and frequency. Bird song play back was used to facilitate the detection of rare birds. In habitat suitable for target species (Red-headed Woodpecker, Yellow-breasted Chat, Hooded Warbler and Prothonotary Warbler), calls were broadcast using a portable tape recorder four to five times with a period of approximately one minute of silence for detection. Squeaking and "pishing" were used also to attract responses from otherwise elusive birds.

Transects were walked through woodlots and fields and six transects traversed several habitat types. Transect length varied based on the area of the woodlots and fields in the given areas. Transects were not walked at a fixed rate or for a specific time period. Effort along transects varied, with more effort (play back, prolonged stopping for visual and audio detection) in suitable habitat for rare species. Evidence of the breeding status of a species followed Cadman *et al*. (1987) and was divided into (i) possible breeding, (ii) probable breeding and (iii) confirmed breeding. For each transect, bird species observed were recorded as they were encountered. The breeding status of a recorded species was revised during the survey as additional information on breeding status was accumulated during observations. In addition, for each species noted, the numbers of individuals encountered along a transect was recorded.

For 2001, effort was concentrated on surveying diurnal land birds. No specific night surveys for nocturnal birds, such as owls, were undertaken.

The surveys conducted in 2000 and 2001 were performed along the following transects:

- Transect 1 - Mature upland dune forest along Nickel Beach;
- Transect 2 - Swamp forest on the Inco Refinery Site west of Reuter Road;
- Transect 3 - Wetland and field habitat on the Inco Refinery Site;
- Transect 4 - Swamp forest east of Reuter Road;
- Transect 5 - Woodlot west of Elizabeth Street;

- Transect 6 - Miller Road, south of Killaly East;
- Transect 7 - Snider Road, south of Killaly East;
- Transect 8 - Woodlot east of Snider Road;
- Transect 9 - CN railway bed, from Pinecrest Road west to Snider Road;
- Transect 10 - Woodlot east of Weaver Road, just south of Second Concession;
- Transect 11 - Woodlot and field west of Weaver Road, just south of Second Concession; and,
- Transect 12 - Woodlot and field east of Miller Road, between Second Concession and Highway 3.

For more specific details on the extent of the transect routes, please see the sample location map (Figure 1-1) at the back of this Volume.

To ensure consistency of data collection conditions, surveys were only undertaken within the following parameters:

- No precipitation (except lightest drizzle);
- Wind force of 3 or less on the Beaufort Scale;
- Air temperature not deviating more than 5^{o}C from the average for the time of day and date;
- Morning start time of 30 minutes post-dawn and finished by 11:00 a.m.; and,
- Surveyor must be able to visually identify all bird species that may occur in the area, and be able to identify by song at least 90% of all bird species that may occur in the area.

4. SCHEDULE

In 2000, surveys were conducted from July 10 through to July 13. Survey dates for each transect, were as follows:

- July 2000 Transect 1, Transect 2, Transect 3, Transect 4, Transect 8 and Transect 12
- May 24, 2001 Transect 5, Transect 6, and Transect 7

- May 31, 2001 Transect 1, and Transect 2
- June 1, 2001 Transect 9, Transect 10 and Transect 11

In 2001 transect surveys were conducted during the last two weeks of May and first week of June 2001.

5. TREATMENT OF DATA

The occurrence and breeding status of each species were documented for each transect as per Cadman et al. (1997). A summary list of species occurrence and their breeding status for the years 2000 and 2001 was compiled for the study area. Species rarity at the provincial level is based on the NHIC database (NHIC, 2001). The bird species list for the study area was compared to species documented for the Niagara Region and surrounding area (Cadman et al. 1987). In addition, the provincial coordinator for the Ontario Breeding Birds Atlas (Michael Cadman) was contacted to acquire data collected for the atlas in 2001 for the Port Colborne area.

5.1 Quality Control/ Quality Assurance

For quality assurance, a representative of the Public Liaison Committee's (PLC's) consultant was allowed to monitor all transect surveys undertaken in 2001.

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Frog and Tadpole Collection Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

Initial site characterisation for the Ecological Risk Assessment (ERA) for the Port Colborne Community Based Risk Assessment (CBRA) was undertaken in 2000 (JWEL 2001). These investigations identified swamps and ephemeral ponds in woodlots represent the primary aquatic environments for lands in the Study Area (as defined by soil nickel concentrations, MOE, 2000a; MOE, 2000b). For the ERA, the need to address the potential risk of Chemicals of Concern (CoCs) to the aquatic environment has been identified. To assess this potential risk, frogs have been identified as a suitable receptor for the assessment of risk as they meet a number of important criteria, including:

- they have an aquatic phase (egg and larval stage) in their life cycle;
- adults frequent and inhabit ponds and watercourses throughout their adult life;
- adults also inhabit terrestrial environments;
- they represent an important prey for many other species in the Study Area;
- frogs have been identified as good indicators of the environmental health of aquatic ecosystems;
- there is a body of literature for the effects of metals on frogs; and,
- frogs are distributed throughout the Study Area.

Both adult and tadpole amphibians have been documented to accumulate metals (Beyer *et al,* 1985, Freda 1991, Lacki *et al*. 1992, Sparling and Lowe 1996). For metal CoCs, copper is the most widely studied metal for effects on amphibians (Herkovits and Helguero 1998). The assessment of toxic effects of metals on amphibians has been studied on eggs, tadpoles and adults. For eggs and tadpoles, the effects of water concentrations of chemicals has been, for the most part, conducted under controlled laboratory conditions (Jayaprakash and Madhysastha 1987, Khangarot and Ray 1987, Horne and Dunson 1995a, b, Herkovits *et al*. 1996). However, some studies have undertaken field assessments to document amphibian distribution with respect to pond water chemistry for a local area (Glooschenko *et al*. 1992).

For the assessment of risk to the amphibian populations in the Study Area, two other surveys have been conducted: an amphibian survey (JWEL, 2002a) and surface water sampling (JWEL, 2002b). These surveys (i) provide data on the distribution and relative abundance of frogs in the landscape and (ii) provide levels of CoCs in the ponds and watercourses. This protocol details the methods for the collection and analysis of amphibian specimens, adults and tadpole, for the purpose of assessing tissue CoCs levels.

2. OBJECTIVES

The primary objectives of the frog and tadpole field collection program were:

1. To collect both adults and tadpoles.

2. To determine levels of CoCs in tissues of adults and tadpoles.

3. To collect sediment and water samples from ponds where frogs and tadpoles are collected and determine CoCs.

4. To determine if CoCs levels in tissues are related to CoCs in sediments and surface water concentrations in the Study Area.

5. To provide values for CoCs concentrations in amphibians for the Quantitative ERA.

3. GENERAL APPROACH

Field investigations identified eight species of anurans (frogs & toads) that occur in the Study Area (Spring Peeper, Western Chorus Frog, Wood Frog, Northern Leopard Frog, Green Frog, Bullfrog, Fowler's Toad and American Toad). Of these eight species, the Western Chorus Frog and Spring Peeper are the most abundant and widely distributed but they are difficult to collect, as they are small and arboreal. Consequently, adult Northern Leopard Frog and Green Frog were chosen for analysis of CoCs in tissues. These species were chosen as they are associated with ponds and watercourse as adults, have sufficient body mass for analysis, and are easier to collect.

For the collection of tadpoles, Northern Leopard Frog and Green Frog were the target species because they have a large body mass and have an extended period in the aquatic tadpole stage (months to over a year). Tadpoles of other species are either very small or have a short aquatic stage (weeks to one month).

4. SAMPLE COLLECTION LOCATIONS

Frogs and tadpoles were collected at various locations. Within the Study Area 10 sample areas were identified and five sample areas where identified as reference sites located west of the City of Port Colborne. In the Study Area, frog and tadpole samples were collected from two general areas, (i) Primary study area with reported high soil nickel between 500-3000 ppm (MOE, 2000a; MOE, 2000b) and (ii) Secondary study area with reported moderate soil nickel between 200-500 ppm (MOE, 2000a; MOE, 2000b). For specific sample site locations, please see the sample location maps (Figures 1-1 and 1-2) at the back of this Volume.

5. COLLECTION METHODS

Adult frogs were collected by hand, and/or with the aid of a dip net, while walking the edge of ponds and watercourses. Tadpoles were collected from ponds and watercourses with a dip net. A minimum of five adults and five tadpoles of Northern Leopard Frog or Green Frog were collected from each site. At each collection site, surface water was sampled as detailed in the protocol (JWEL 2001b) Pond sediment (surface 0-5 cm) was collected from eight locations within each site, mixed and a sub-sample used for chemical analysis. Sediment sampling followed the soil sampling protocols developed by JWEL for the CBRA (JWEL 2001b; JWEL 2001c).

6. FIELD COLLECTION SCHEDULE

The collection of frogs and tadpoles was conducted over a period from the second week of June to the last week of August 2001.

7. PREPARATION OF SPECIMENS

Captured frogs were kept in separate canvas collection bags for each collection site. Tadpoles were kept in 1-l plastic bottles to which water from the sample pond/watercourse was added. Adult frogs were euthanized by double (brain and spinal cord) pithing. The gastrointestinal tract and liver were removed from each frog, weighed and placed in a separate plastic bag or vial. The eviscerated bodies and tissue samples of each individual were then placed in a plastic bag, label added and frozen to approximately -5^{o}C until analysis.

Following field collection, tadpoles were frozen at approximately -5^{o}C until analysis. Prior to analysis, the tadpoles where thawed and the GI tract was removed with the aid of a dissection microscope. The total wet weight for each tadpole was recorded (to 0.1 grams) prior to the removal of the GI tract, and following GI removal. This would allow for the determination of the wet weight fractions of the GI tract and remain body tissue for each tadpole. Following removal of the GI tract, the eviscerated bodies were

combined to form one composite sample for CoCs analysis. Similarly, the GI tract from each tadpole was combined to from one composite sample for CoCs analysis.

8. CHEMICAL ANALYSIS

Following the collection and preparation of specimens and tissue, frozen samples were delivered to the laboratory for analysis. For each specimen, tissue analysis was undertaken separately for the liver (adult frogs only), GI tract, and remaining body. Tissue and soil samples were analyzed for ICP metals (17), arsenic and selenium by an Canadian Association for Environmental Analytical Laboratories (CAEAL)-accredited laboratory Philips Analytical Services Inc. (PSC) and appropriate controls and blanks were used. JWEL, 2001c provides details for soil analysis. Detailed analysis procedures for tissues are presented in separate analysis protocols (JWEL, 2002c).

9. TREATMENT OF DATA

The procedures used for the statistical analysis of the data is detailed in a separate report prepared for the ERA (JWEL 2002d).

9.1 Quality Assurance/Quality Control

All testing was carried out in conformance to strict quality control/quality assurance methods, conforming to Jacques Whitford's QA/QC protocols and under the company's ISO 9001 registration. Representatives of the Public Liaison Committee's (PLC's) consultant were allowed to monitor field collection of specimens and could take a fraction of duplicate samples (20%) for analyses. Laboratory QA/QC is presented in a separate chemical analysis protocol (JWEL, 2002c).

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Maple Seed Greenhouse Trials Protocol
Greenhouse Dose-Response for Maple Seed Germination and Growth Response on CoCs-Impacted Soils
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

Initial site characterisation for the Ecological Risk Assessment (ERA) for the Port Colborne Community Based Risk Assessment (CBRA) was undertaken in 2000 (JWEL, 2001a). These investigations identified that many of the woodlots in and around the Inco Port Colborne Refinery have Red Maple (*Acer rubrum* L.), Silver Maple (*Acer saccharinum* L.) and their hybrid Freeman's Maple (*Acer rubrum X Acer saccharinum*) as a major components of the tree communities. In addition, in the local area, these species are found in woodlots occurring on both organic and clay soils. The majority of the maple trees in these woodlots are between 50 to 70 years of age and have therefore been exposed to Chemicals of Concern (CoCs) through airborne deposition and their accumulation in surface soils.

The existing mature trees in the local area's woodlots do not appear to demonstrate significant chronic injury as a result of an extended period of exposure to CoCs in the soil and air. However, the long-term health of these woodlots requires the regeneration of new trees to succeed old mature trees as they die off. Generally plants, including trees, in advanced stages of maturity are likely to be less responsive to environmental contamination than actively young growing plants (MOE, 1993). Therefore, the ability of woodlots in the areas east of the Inco Port Colborne Refinery to remain sustainable over time through the regeneration of new growth has been identified as requiring assessment for the ERA.

To assess soil CoCs Dose-Response effects on maple seed germination and seedling growth, greenhouse trials (designated greenhouse Dose-Response trials) were conducted in the summer of 2001. These trials were conducted using maple keys from the Silver Maple. This species has been selected as it represents a common forest tree in the area and has been identified as being sensitive to soil CoCs (Temple and Bisesar, 1981; MOE, 1994). In addition, Silver Maple is a species that produces seeds in May-June that naturally germinate shortly after falling (Hosie,1979). Therefore, using this species allows for greenhouse trials to be initiated in the summer of 2001.

2. OBJECTIVES

The primary objectives of the greenhouse Dose-Response trials was to:

1. Identify the critical levels of CoCs in organic and clay soils at which phytoxicity inhibits maple seed germination.

2. Identify the critical levels of CoCs in organic and clay soils at which phytoxicity effects seedling growth.

3. Identify CoCs levels in organic and clay soils at which visual injury to seedling plants occurs.

3. APPROACH

The approach to study design is as follows:

1. Collect maple keys from woodlots east of the Inco Port Colborne Refinery in areas with known high soil nickel concentrations (MOE, 2000a; MOE, 2000b) and from a reference area located west of the City of Port Colborne.

2. At maple key collection sites, sample soils to determine soil CoCs Concentrations.

3. Conduct analysis of maple key codoledons (both control and Inco site) to determine CoCs levels in mature keys.

4. Grow maple keys in clay and organic soils from the Port Colborne area which have been blended containing (using nickel as the indicator metal) approximately 500, 1,500 and 3,000 ppm, as well as control soils.

5. Conduct sequential, Dose-Response trials to monitor and assess maple key germination and seedling growth for a period of 56 days.

4. LOCATION

The greenhouse trials were carried out at a greenhouse located at the University of Guelph.

5. SOILS

The collection and treatment of soils for the testing are detailed in Soil Sampling Protocol: Year 2001 Green House & Field Trails (JWEL, 2001b) and Greenhouse Trials Protocol #1 (JWEL 2001c) developed for the CBRA. For this test, surface profile Clay 1 (Welland Clay) and organic soils were used.

6. SOIL METAL CONCENTRATIONS

Preparation of the surface profile soils, both treatment and control, are detailed in the Greenhouse Trials Protocol #1 developed for the CBRA (JWEL, 2001c). For the test, soil treatments that were used include:

1. Control Clay 1 and Control Organic soils.

2. Treatment Clay at 500, 1500 and 3000 ppm Ni.

3. Treatment Organic at 500, 1500 and 3000 ppm Ni.

7. GROWTH CONTAINERS

Testing was carried out in six-inch plastic pots in which approximately fifteen (15) centimeter thick treatment and control soils were added.

8. AMENDMENTS

For this test, during the blending and amendment process the soils were pH adjusted as detailed in the JWE greenhouse Dose-Response trials protocol (JWEL, 2001c). However, for this experiment no fertilizer or amendments (limestone) were added to the soils.

9. KEY COLLECTION AND PLANTING

One seed type was used, Silver Maple keys. Maple keys were collected from a tree at a control site (a yard tree located in the City of Welland) and from a tree east of the Inco Refinery along Snider Road (See Figures 1-1 and 1-2 at the back of this Volume for the sample site locations) where soil CoCs levels are reported to be high (500-3000 ppm, MOE, 2000a; MOE, 2000b). During the collection of keys, only keys that had fallen to the ground were collected for the experiment. This ensured that the keys were naturally "ripe" and ready for germination. Following collection in last week of May 2001, the keys were placed in labeled paper bags and stored in a refrigerator at approximately 5°C at the University of Guelph greenhouse facility until pots and soils had been prepared.

At each collection site, surface soil (0-5 cm) was collected from eight locations within each site, mixed and a sub-sample used for chemical analysis. Soil sampling followed the soil sampling protocols developed by JWEL for the CBRA (JWEL, 2001c; JWEL, 2001d).

For planting, for each soil type, in each pot, five (5) maple keys were planted. For each pot the soil was pressed firmly into the pots, over which the keys were laid. A cover layer of 1 cm of loose soil was then placed over the keys. Seeds and seedlings were then watered as required by greenhouse staff.

10. SEED VIABILITY TEST

To determine the natural viability (germination success) of Maple keys from the local area a test sample of one hundred maple keys collected from the control site were planted in a flat with control organic soil. The success rate, as a percentage, of maple key germination for the control keys in the control soil could then be used to standardize the germination success of Maple keys in the treatment soils.

11. SCHEDULE

The greenhouse testing was conducted over a three-month period, July through September 2001. Actual plant growth duration was 56 days (8 weeks) from time of plant emergence.

12. TREATMENTS

The Maple key viability test would consist of up to 3 three trials, involving:

1. Control Organic Soil.

2. One seed type: One Hundred Control Maple keys.

3. Up to three replicates.

Should the first trail result in a germination success of 80% or greater, then additional trials would not be undertaken.

The greenhouse Dose-Response trials consisted of 48 tests, involving:

1. Two Soils: 1] Organic soil, 2] Clay 1 soils.

2. Four Blended Levels of Soil CoCs: Control, 500, 1,500, and 3,000 ppm Ni.

3. Two Seed Types: Silver Maple keys 1] Control site, 2] Inco Refinery Site.

4. Three replicates per soil treatment (i.e., three pots with organic soil with 500 ppm Ni).

5. Five keys per pot.

13. PHYSICAL AND CHEMICAL ANALYSES

Maple Key CoCs

Samples of collected Maple keys (3 composite samples of 5 keys) from the control site and area east of the Inco Refinery were analyzed for CoCs as follows:

1. Only the bare codolendons of the maple key was analyzed for CoCs. Prior to submission to the laboratory, each individual maple key had the "wing" removed and the paper tissue surrounding the codoledons was removed.

2. Codoledons were washed and then oven-dried at (80^{B} C +/-5^{B} C) to a constant weight, ground, weighed and ashed in a muffle furnace at 450^{B} C for 16 hours. The ash was digested with nitric acid using a modification of AOAC method No. 985 (Isaac, 1990).

3. Samples were analysed for 17 metals, arsenic and selenium by ICP-MS (see JWEL, 2001d).

4. Quality control included analysis of laboratory plant standards, sample duplicates, and reagent and preparation blanks with each set of samples.

Maple Key Viability

Maple key germination was scored when the length of the emerging radicle was equal to the diameter of the seed (MOE, 1993). Pots were monitored for 3 weeks following the emergence of the first seedlings.

Greenhouse Dose Response Trials

For the greenhouse Dose-Response trails the assessment of seeds and seedlings was undertaken as follows:

Germination	- Scored when length of the emerging radicle (i.e. root) is equal to the diameter of the seed (MOE, 1993)
Evaluation of Injury	- Leaf drop - Visual folliar injury rating (MOE, 1993)
Growth Parameters	- Plant height (MOE, 1993) - Number of leaves
Leaf Tissue CoCs	- collect first two basal leaves from seedling at the end of the trails - conduct CoCs analysis of basal leaves

Following the germination assessment, an evaluation of injury and growth was conducted on one seedling per pot. For each test pot, following germination, the most viable of the seedlings, as measured by total height, was selected to be carried forward for the Dose-Response trial. At the end of 56 growing days, the total height of the seedling was measured following MOE methods (MOE1993). In addition, assessment of folliar injury following MOE 1993 was also undertaken at the end of the trial. Finally leaf drop was recorded throughout the trial period.

Collection of basal leaves was conducted at the end of the trial period. Following collection, treatment and analysis of basal leaves was undertaken following JWEL protocol developed for vegetation tissue analysis for the CBRA (JWEL, 2001d).

14. TREATMENT OF DATA

The procedures used for the statistical analysis of the data is detailed in a separate report prepared for the ERA (JWEL 2002).

15. QUALITY ASSURANCE/QUALITY CONTROL

All testing and collection of samples was carried out in conformance to strict quality control/quality assurance methods, conforming to Jacques Whitford's QA/QC protocols under the company's ISO 9001 registration. Representatives of the Public Liaison Committee's (PLC's) consultant were allowed to monitor all soil and pot preparations and collection of maple keys. Laboratory QA/QC is presented in a separate chemical analysis protocol (JWEL 2001d).

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Earthworm Toxicity Tests and Field Sampling Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

Invertebrate soil faunas are important to the flow of energy and nutrients through the food web (Griffiths and Bardgett 1999). Earthworms may comprise up to 90% of the biomass of soil invertebrates and are therefore very important in the total ecosystem (ASTM 1998). Earthworms improve soil structure by increasing soil aeration through burrowing and also increase soil fertility by breaking down organic matter (i.e., leaf litter) and releasing nutrients. Chemicals of Concern (CoCs) in soils may have adverse effects on earthworms in high concentrations and may be absorbed into their bodies through their skin or intestinal wall. In addition, worms are a common food for many birds and therefore the amount of CoCs in worms may pose a risk to birds, mammals and their predators.

Two methods have been chosen which can provide an indication of the potential risk of exposure of earthworms to chemicals in soils: (i) Toxicity testing to identify survival and reproduction when exposed to soils with varying concentrations of CoCs; and (ii) Field assessment to determine the distribution and abundance of earthworms in soils with different concentrations of CoCs in the Port Colborne area. As part of the field assessment, the analysis of whole worms would provide CoCs concentrations for the Ecological Risk Assessment (ERA).

In Ontario, 19 species of worms have been recorded (Reynolds 1977). The distribution and abundance of worms is related to historical, agricultural and ecological factors including; soil texture, pH, moisture, depth, and food supply. Due to these factors, the occurrence of only one to four species of worms is typical in any local area, with one or two species often representing the majority of worms present (Fox pers. comm., Reynolds pers. comm.). Literature values for earthworm population density are reported to range from 831 worms/m^2 in pastureland to 32 worms/m^2 in forest soils. Tree species and their associated leaf litter play a role in the abundance and distribution of worms (Reynolds 1972). In Ontario, worms prefer maple and elm leaves, followed by ash, with the least preferred species being oak and beech. For the ERA, a leaf litter accumulation study and worm density study was undertaken.

2. OBJECTIVES

The objectives of these studies were:

1. To determine the distribution and abundance of earthworms in the Port Colborne Study Area.

2. To relate earthworm species richness, breeding success (based on the ratio of adults to juveniles), density and biomass to soil CoCs.

3. To determine the effects of soil CoCs on survivorship and reproduction of earthworms in a laboratory setting.

4. To determine the concentrations of CoCs in earthworms caught at stations throughout the Port Colborne Study Area.

3. EARTHWORM TOXICITY TESTS

Laboratory toxicity studies were conducted by ESG International Inc., in Guelph Ontario. ESG is a Canadian Association for Environmental Analytical Laboratories (CAEAL)-certified laboratory specializing in toxicology and soil science, which performs toxicity testing using standard biological test methods (Environment Canada, 2001).

The toxicity experiments performed for the ERA tested the response of earthworms to soil nickel concentrations in the range of 50 to 10,000 ppm. The bioavailability of metals in soils varies depending on soil parameters including: cation exchange capacity, pH, organic matter content, porosity and other soil factors. Therefore, the toxicity studies, local Port Colborne clay and organic soils were collected and processed for the tests.

For both acute and chronic testing, the test worm species was *Eisenia andrei* (Compost Worm or Red Wiggler). *Eisenia andrei* is one of three species of earthworms recommended for toxicity testing in the Environment Canada (2001) test protocol. The other species are *Lumbricus terrestris* (Night Crawler or Dew Worm) and *Eisenia fetida* that is very similar to *E. andrei* and can only be distinguished using electrophoretic patterns or other molecular tests. ESG International Inc. has all three earthworm species in culture at their laboratory. *Lumbricus terrestris* cannot be used in reproduction tests because it does not reproduce under laboratory conditions. In contrast both *E. fetida* and *E. andrei* reproduce in the laboratory and are amenable to both acute and chronic testing; and reach reproductive maturity within 38 days, produce cocoons every 48-92 hours with up to 13 neonates per cocoon. For these reasons, earthworm reproduction testing used *Eisenia andrei* in this study.

Though *Eisenia andrei* does not occur naturally in the fields and woodlots in and around the Inco Port Colborne Refinery, surrogate test species are commonly used in both aquatic and terrestrial toxicity testing. There are advantages to using surrogate species with standard test methods and established performance criteria that are available in the literature. *Eisenia andrei* is considered as a suitable test species because reviews have shown that for the most part, the sensitivity of different species of earthworms is comparable (Heimbach 1985).

3.1 Experimental Concept and Design

The following experimental design was used for the earthworm toxicity testing. The methods described below are based on ASTM (1998) protocol and were used to assess the effects of earthworms exposed to various soils from the Port Colborne area with different levels of CoCs. Soils from four areas were collected for the study: clay and organic soils from area with known high nickel (just east of the Inco refinery) and clay and organic soils from a reference area west of Port Colborne where nickel concentrations are less than 50 ppm (See sampling locations in sample location map in main report).

Soils collected and processed for the Community Based Risk Assessment's (CBRA's) crop greenhouse experiments were also used for earthworm toxicity studies. Details for the greenhouse soil collection and sampling procedures are provided in a separate protocol (JWEL, 2001a). In summary, following the collection of the four test soils, their pH was adjusted with a mixture of reagent grade calcium carbonate and magnesium carbonate to raise the clay soils close to a pH of 7.0 and the organic soils close to a pH of 6.5 (JWEL 2001b). Additionally, a laboratory standard control soil (hereafter, "control soil") collected by ESG from Alberta (black chernozem, free of contaminants) provided QA/QC data on test organism health and experimental conditions.

A phased approach was chosen for the laboratory toxicity tests. Three Phases were identified: i) Phase I – Acute Testing, ii) Phase I – Chronic Testing and iii) Phase II – Chronic Testing. Each of these phases is detailed below. Initial testing was conducted on clay soils with the highest CoCs concentrations plus clay soils from the reference area. For each Phase, toxicity testing followed the ASTM standard methods (ASTM, 1998) and/or Environment Canada's protocols (Environment Canada, 2001).

3.2 Phase I – Acute Testing

The acute testing was performed on clay and organic soils collected from the Port Colborne area with varying concentrations of Ni (see table 1). Chemical and physical analyses were conducted on sub-samples of the soils prior to commencement of the toxicity testing to determine the levels of CoCs and characterize the soil types. The soil was air-dried and sieved using a mesh size of 4 and 9 mm, to remove animals, stones, and other debris. Each batch of soil was hydrated to 70% of its water holding capacity and partitioned among the test units.

Table 1: Nickel Concentration (ppm) of Soils Collected from the Port Colborne area used in Earthworm Toxicity Tests

Clay Reference Soil	Clay High Nickel Soil	Organic Reference Soil	Organic High Nickel Soil
40	8660	50	1490

For the test, approximately 350 g of soil was placed in a 500-ml wide-mouthed glass jar. Jars were covered with perforated tin foil held by a metal screw ring to facilitate gas exchange for the earthworms. Soils were assessed for physico-chemical parameters including, % moisture, pH and conductivity. Five healthy worms were placed on the surface of the soil in each jar for each of the four test soils (High Ni Clay, Control Clay, Ni Organic, Control Organic) and ESG control soil. Six replicate test units were prepared for each test soil and the control, for a total of 30 test units across the experiment. Jars/soil were maintained throughout at 20 $^{\circ}$C and continuous fluorescent illumination (24-h). The experimental endpoint for the test was the number of live worms in each test chamber on Days 0, 7 and 14. All procedures were carried out as outlined by Environment Canada (2001).

3.3 Phase I – Chronic Testing

Following the Acute Testing, Chronic (63-day) toxicity testing was performed to examine the effects of the site soils on the survival and reproduction of earthworms to prolonged exposure. For this Phase, the same soil treatments (Table 1) and general experimental design used in the Acute Testing were used. Ten replicate test units were prepared for each test soil and the control, for a total of 50 test units across the experiment. Two reproductively mature (clitellated) earthworms were added to each test unit. Jars/soil were maintained throughout at 20 oC and continuous fluorescent illumination (24-h). At day 35 each test unit was inspected to determine the number and condition of adult worms. At the end of 63 days, each unit was assessed for juvenile production, juvenile wet mass, juvenile dry mass, number of hatched and number of unhatched cocoons. Following Phase I of the chronic toxicity test, Phase II was initiated.

3.4 Phase II – Chronic Testing

The Phase II Chronic Testing was undertaken to identify the range of exposure Ni concentrations that could provide a "safe" soil Ni value for earthworms in the Port Colborne area. For this test, earthworm survivorship was monitored in a series of clay and organic soils with decreasing concentrations of CoCs. Soil blends were prepared by mixing control soils with soils with high Ni concentrations. The resulting soil mixtures used for the test are provided in tables 2 and 3.

Table 2: Nickel Concentration (ppm) of Clay Soil Blends Use in Phase II – Chronic Testing

Blend Number	Blend Ratio (High : Reference)	Expected Soil Bend Concentration (ppm, nearest tenth)
6 (High Clay)	100 : 0	8660
5	80 : 20	6940
4	50 : 50	4350
3	25 : 75	2200
2	12 : 88	1070
1	5 : 95	470
7 (Reference Clay)	0 : 100	40

Table 3: Nickel Concentration (ppm) of Organic Soil Blends Use in Phase II – Chronic Testing

Blend Number	Blend Ratio (High : Reference)	Expected Soil Bend Concentration (ppm, nearest tenth)
14 (High Organic)	100 : 0	10400
12	80 : 20	8330
11	50 : 50	5230
10	25 : 75	2640
9	12 : 88	1290
8	5 : 95	570
13 (Reference Organic)	0 : 100	50

The experiment design was same as that conducted for Phase I. Two adult (fully clittelated) worms were placed in each test unit. Ten test units were prepared for each soil treatment. Jars/soils were illuminated with a fixed daily photoperiod, 16-h light and 8-h dark. Worms in each test unit were fed an identical quantity of food on days 0, 14, 28 and 42 (Environment Canada 2001). The measurement endpoints are the number of live and dead worms during each feeding day and the number and weight of live and dead juvenile and adult worms at the end of the test.

The statistical endpoints include the level of contamination that results in no adverse effects (NOAEC), the lowest level of contamination that results in an adverse effect (LOAEC), and the levels of contamination that result in a 20 or 50% reduction in survival and reproduction of earthworms.

4. EARTHWORM FIELD COLLECTION PROGRAM

4.1 Study Area and Control Site Assessment

A survey to determine the distribution and abundance of earthworms in the Port Colborne area was undertaken in the last week of September 2001 and in June 2002. The 2001 field program sampled earthworms using the following nested design:

1. Three sample areas based on MOE soil CoCs measurements (MOE, 2000a; MOE, 2000b)

    - Primary study area with reported high soil nickel between 500-3000 ppm.
    - Secondary study area with reported moderate soil nickel between 200-500 ppm.
    - Reference area located west of the City of Port Colborne.

2. Two habitat types (woodlot and grass field) within each sample area

3. Two woodlots and two fields per area.

4. Three sample plots per field/woodlot.

For the woodlots, earthworm collection locations were selected in the same general location as those for the leaf litter assessment program. In this way, data from worm sampling in the woodlots could be correlated to findings of the leaf litter study. For the fields, earthworm collection locations were selected in the same general location as those for vole and insect collection.

In 2002, a sampling program was undertaken to determine worm distribution (west to east) in two woodlots located adjacent to the Inco Port Colborne Refinery. For one woodlot located along Reuter road, worm samples were collected from five stations, moving west to east through the woodlot. In the second woodlot, located east of Snider Road, worm samples were collected from three stations, moving west to east through the woodlot.

In addition to the woodlot surveys, 15 worm samples on both clay and organic soils (a total of 30 sample stations) were collected to determine if there was a correlation between worm abundance and worm tissue concentration.

For sample site locations for the 2001 and 2002 field programs please see the sample location maps (Figures 1-1 and 1-2) at the back of this Volume.

4.2 Earthworm Sampling Method

Several methods are used for sampling earthworms. These include digging and sorting, chemical and electrical methods as well as collection at night under suitable moist conditions. A chemical method is recommended by Agriculture Canada (Fox pers. comm.) and details are available on a web site: http://res2.agr.ca/london/pmrc/english/faq/wormsoil.html. For the field program, for each field and woodlot sampled, worm samples were collected from three randomly selected plots 0.5 X 0.5 m in size. For this study, a chemical method using a hot mustard solution was used:

1. Twelve hours prior to sampling in the field, approximately 55 grams of Keen's dry mustard powder was added to a glass jar, to which 125 ml of water was added to make a thick paste.

2. At the sample site, the paste was mixed with 7 litters of water in a plastic pail.

3. The mustard solution was then transferred to a garden watering can with a fine spray for application.

4. A random sampling site was selected and an area of approximately 0.5 X 0.5 m was cleared of vegetation and leaf litter exposing bare soil. For fields, grass and vegetation was cut with grass clippers and for woodlots, leaf litter was removed, and any worms in the litter were included in the sample.

5. Following the clearing of the quadrat area, the quadrat frame was placed on the ground and approximately half of the mustard solution was sprayed evenly onto the soil within the quadrat. Emerging worms were picked up with forceps and placed in a labeled jar for a five-minute period.

6. After five minutes, the remaining mustard solution was then sprayed over and soil and emerging worms collected for another five minutes. Following the end of an additional 5-minute period, the collection of worms stopped. This sampling method resulted in a total 10 minute sampling period during which emerging worms were collected.

Each sample of earthworms was be placed in a labeled glass jar and stored on ice in a cooler. At the end of each day, worms were sorted to species and age class and the data recorded. Worms were aged following an age classification formula as either (i) juveniles, (ii) aclitellate adults, (iii) clitellate adults, and (iv) post-clitellate adults (e.g., a sample of 10 worms for a species could have a formula as 2-2-3-3) (Reynolds 1997). The identification and classification of earthworms into age classes was undertaken by Dr. John W. Reynolds.

For the 2001 field program and the 2002 woodlot surveys, the following information was recorded for each quadrat after samples were sorted by species and age class:

- Species;
- Number of individuals in each age class for each species;
- Total number of worms for each species;
- Total weight (wet-biomass) for each species; and,
- Total weight (wet-biomass), all species combined.

Weights were measured with an electronic balance to the nearest 0.1 gram. Following sorting, identification, aging and weighing, the worms of each sample were rinsed in distilled water to remove soil particles. The washed worms were then placed into a clean labeled sample jar to represent a composite species/age class sample. The combined sample was then frozen at approximately –5°C until submitted to the laboratory for analysis.

For the year 2002 program, for the 30 sample stations (15 on clay soil and 15 on organic soil) only the number of worms per sample quadrate was recorded prior to collection. In addition, at 12 sample stations two quadrate samples were collected. For the second duplicate samples, following counting and collection, the worms were taken from the field to be purged.

4.3 Purging of Earthworm

To determine the concentration of CoCs in worm tissue, twelve duplicate worm samples collected from the field were purged of soils prior to analysis in the laboratory. Following collection in the field, the worms were rinsed with distilled water and placed in a a 1 liter plastic Nalgene bottle which was filled two thirds with moist bran (Branflakes). Worms were left in the bran to purge existing soils in the gut for a period of 72 hr at room temperature. During the 72 hr period, the bran was replaced to prevent worms from re-ingesting soils. Following 72 hr, the worms were removed from the container, rinsed thoroughly, placed in a glass jar and frozen prior to be sent to the laboratory for chemical analysis.

4.4 Earthworm Tissue Analysis

Frozen composite earthworm samples were submitted to the laboratory for assessment of whole body CoCs concentrations. Tissue analysis was undertaken on whole earthworms including soil that may be in the gut. Homogenized samples were analyzed for ICP metals (17), arsenic and selenium by an CAEAL-accredited laboratory (PSC); appropriate controls and blanks were used. Detailed analysis procedures are presented in a separate chemical analysis protocol developed by JWEL for the CBRA (JWEL 2002a).

4.5 Soil Sampling and Analysis

For the field collection program, woodlot and field soils were sampled to determine the sample area's soils CoCs concentrations. For both fields and woodlots, surface (0-5 cm) soil cores were collected. Cores were combined and mixed and transferred to a labeled jar. Soil samples were analyzed for ICP metals (17), arsenic and selenium by a CAEAL-accredited laboratory (PSC) and appropriate controls and blanks were used. Detailed collection and analysis procedures for soils are presented in separate analysis protocols (JWEL, 2001b; JWEL, 2001c).

5. TREATMENT OF DATA

The procedures used for the statistical analysis of the data is detailed in a separate report prepared for the ERA (JWEL 2002b).

6. QUALITY ASSURANCE/ QUALITY CONTROL

All testing was carried out in conformance to strict quality control/quality assurance methods, conforming to Jacques Whitford's QA/QC protocols and under the company's ISO 9001 registration. For the field program, representatives of the Public Liaison Committee's (PLC's) consultant were allowed to monitor field collection of specimens and to take a fraction of duplicate samples (20%) for analyses. Laboratory QA/QC methods are presented in a separate chemical analysis protocols (JWEL, 2001a; JWEL, 2001b; JWEL 2002a).

For the laboratory toxicity studies, representatives of the PLC's consultant were allowed to monitor the field collection of soils and blending of soils used for the study. In addition, the PLC's consultant was provided with duplicate soil samples (20%) for independent analyses.

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Leaf Litter Decomposition Study Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

The general concept of element cycling in the natural environment is well established and is outlined in numerous texts (Bormann and Likens 1979, Clarke 1954, Colinvaux 1973). Mineral elements are accumulated from soil, water, and air, transferred through food chains, and may be returned to the soil or water via the process referred to as decomposition.

The decomposition process returns the elements that were incorporated in the tissues of plants and animals back to a form that can be used again by primary producers i.e. plants. In a more or less steady state, the amount of elements accrued by the organisms in the system is the same amount that is released through the decomposition process. The time required for nutrients to complete their cycling through the system is not known and varies within and between ecosystems.

Decomposition processes are controlled by physical factors such as temperature and moisture and biological factors such as ingestion by earthworms and breakdown by fungi and bacteria. The cycles are affected by daily and seasonal weather conditions and other factors such as fire, logging and grazing. Also certain shifts in the cycling occur due to changes such as succession.

Factors that disrupt decomposition ultimately influence the rate of natural processes. Without decomposition, the essential nutrients required by the plants and all other organisms that eventually depend on them may be bound up and rendered unavailable. In cases where the decomposition process is decreased, the amount of materials being formed and returned to the system (i.e., leaf litter fall) is greater than the amount being broken down or decomposing. Under conditions of decreased decomposition, the amount of litter on the surface of the ground may start to accumulate and nutrients would not be available to the vegetation (i.e., trees) in that area. If the disruption continued over a long period, the tree's growth might decrease accordingly.

The woodlots in the vicinity of Port Colborne have been subjected to historic aerial deposition of Chemicals of Concern (CoCs), including nickel, copper, and cobalt (Kuja et al. 2000a, Kuja et al. 2000b, Kuja et al. 2001). It has been suggested that the levels of CoCs derived from aerial fallout may have reached levels that are harmful to the vegetation growing in those woodlots (McIlveen and Negusanti 1994). Leaf litter decomposition may also have been negatively impacted by the accumulated CoCs. Metal contamination has been shown to influence the rate at which litter decomposes as well as impacting soil-dwelling organisms (Babich et al. 1982, Babich et al. 1983, Babich and Stotzky 1982, de Catanzaro 1983, Dumontet et al. 1992, Freedman and Hutchinson 1980, Gardenfors 1986, Giashuddin

and Cornfield 1978, McIlveen 1980, McIlveen 1999, Strojan 1978, Tyler 1975). For the present investigation, it can be assumed that nutrient and metal cycling has reached an equilibrium or steady state. It can also be assumed that any current inputs of CoCs would be minimal.

Measurement of leaf litter decomposition rates is traditionally measured using known amounts of litter confined in mesh bags placed *in situ* under field conditions (Freedman and Hutchinson 1980, McIlveen 1980, McIlveen 1999, McIlveen and McLaughlin 1993). Loss in weight of the litter over time is used as the indicator of decomposition rate. Because the decomposition rate is not rapid and involves sequential steps in the breakdown of the different types of material, as well as a variety of different types of microbes, small arthropods and other small animals, such studies often require two years or more to complete (McIlveen 1980, McIlveen 1999). Woody litter will take longer periods. Because of time constraints, the current situation does not allow for a detailed investigation using the normal procedures. Instead, a proxy method of assessing the rate of decomposition will be used. This involves the measurement of the non-decomposed litter remaining on the surface of the ground at the end of the summer in relation to annual leaf fall. Some assumptions made in this method are that the sites being examined are not significantly different from each other in any way other than the amount of accumulated metals in the soil. Tree species, soil moisture, soil type, etc. at the different study sites are all as similar as possible.

It is necessary to assess the amount and kind of leaf litter that is produced at each site to ensure that any differences being measured are not due to differences in litter production rates. Typically, the amount of litter production is measured using litter traps of fixed known area on a year-round basis. These traps can collect flower parts, seeds or fruits, twigs, and bark, the volume of which is not insignificant. The most conspicuous part of the litter fall; however, is in the form of leaves in the autumn. It is suggested that a measure of leaf fall might be obtained by collecting the fresh leaf litter from the ground surface shortly after it has fallen. While less complete than using formal litter traps, this approach should at least provide a measure that will demonstrate that litter fall volume is at least comparable at equivalent sites.

2. OBJECTIVES

The primary objectives of the litter accumulation/decomposition study are:

1. To collect leaf litter accumulated at the soil surface horizon.

2. To establish any relationship between leaf litter quantities and levels of CoCs in the soil.

3. To provide receptor and ecological process data for the ERA.

3. GENERAL APPROACH

Kilty Springs Environmental was retained by Jacques Whitford Environment Limited (JWEL) on behalf of Inco Limited to undertake the work for collecting and evaluating the data. Field examinations of representative woodlots in the area have shown them to consist mainly of hardwood species tolerant of wet conditions. This includes silver and red maples and/or their hybrid, red, white and bur oak, white elm, and white and green ash. The drier sites have a higher frequency of sugar maple, hickory and beech. Comparable woodlots can be found in each of the three chosen CoCs deposition zones (see below) and on the two general soil types (clay and organic) within the study area.

Samples of undecomposed leaf litter were collected from each of the selected woodlots to provide estimates of the amounts of standing litter. From the geographic pattern of amounts of litter (i.e. comparing sites with known levels of CoCs with control sites) the effect of CoCs on rates of litter decomposition might be assessed. The assumption is that greater amounts of litter are indicative of lower rates of decomposition. It is assumed that because the woodlots to be compared are of similar composition and structure, soil type, hydrology, and climate, any differences in leaf litter accumulation may be attributed to CoCs levels in the soil.

CoCs levels were confirmed through the collection of soil samples at each study site. In addition, site characteristics will be described by recording the kinds and amounts of vegetation within the study plots.

The final component of the study was the collection of fresh litter to determine the amounts of fresh organic material being added annually to the organic matter on the ground surface. This information can be used as a confirmation that the amounts of organic material being added to the soil ecosystem is within the normal range and that any differences which were discovered in the amounts of standing litter was not due to differences in amount of litter entering the system.

4. SAMPLE COLLECTION LOCATIONS

Samples were collected from 21 woodot locations within the Study Area (as defined by MOE soil nickel concentrations east of the Inco Refinery (Kuja et al. 2000a, Kuja et al. 2000b) and at control sites west or east of the City of Port Colborne (Table 1). For specific woodlot locations, please see the sample location maps (Figures 1-1 and 1-2) at the back of this Volume. Sampling was undertaken in areas representing two different soil types, clay and organic, that exist in the area and in three different levels of CoCs concentrations (high 500-3000 ppm Ni, moderate 500-200 ppm Ni, Control <50 ppm Ni) as determined by MOE mapping (Kuja et al. 2000a, Kuja et al. 2000b). These zones were taken as only a guide as mapping of soil CoCs levels is subject to a number of limitations. Actual CoCs levels were determined for each specific study area and interpretation of the leaf litter data was based on the metal levels actually measured in the soils.

Table 1: Numbers of Sample Collection Woodlots

Study Area	Soil Type	
	Organic	**Clay**
Heavy – over 2000 ppm Ni	4	3
Moderate – 200-2000 ppm Ni	NA	5
Low/ Control – less than 50 ppm Ni	4	5

Consideration was also given to including sandy soils as one of the soil type parameters. No sand dune forest of appropriate size was available in the moderate deposition zone due to habitat destruction resulting from residential and recreational developments. The extent of the area where potentially suitable forest cover was present was very limited and the opportunity for inclusion of local control sites was marginal. Owing to the restricted areas of sand dune communities in general and the significant disturbance of the remaining sites, inclusion of sandy soils in the studies was not considered further. Wherever possible, the woodlot sites were co-located with other sampling programs established for the ERA study.

5. COLLECTION METHODS

5.1 LITTER SAMPLE COLLECTION

5.1.1 Standing Litter Assessment

5.1.1.1 Sample Collection

Samples of old or standing organic litter were collected at the twenty-one selected woodlots sites throughout the later summer (last week in July through first week of September 2001). Fifteen samples were obtained from each woodlot site following the protocol described below based on general procedures for quadrat studies outlined by Bonham (1989) and Chapman (1976).

A starting point was selected in a representative part of the woodlot as far as practical up to 100 meters away from the edge of the woodlot. This point was subsequently used as the southwest corner of a sampling grid consisting of fifteen sample points. The grid was arranged in three rows of five sample points with the longer axis oriented north-south. Spacing between sample points and rows was 5 meters (See Figure 1). A flag was placed at each target sample point to facilitate locating that point during the sample collection process.

At each sample point, samples of litter were collected from the 'open' area nearest the flag. Open areas used were of sufficient area to avoid the immediate and direct influence of trees trunks, tree roots, shrubs, large fallen branches or poison ivy. If more than one suitable and equivalent open area was present around the flag, the point nearest the north and east of the flag was selected for sample collection. In most cases, the sampled area was within 0.5 meters of the flag.

The sampling process consisted of laying a sheet of plywood 50-cm square, over the ground surface to be sampled. A knife was used to cut through the litter around the perimeter of the board. When twigs were too large to cut with the knife, they were severed with pruners. Litter outside the board was then scraped away so that when the board was lifted, the underlying litter was still in place. The litter comprising the LF layer soil horizon was then scraped together and placed in labeled polyethylene bags. In a number of locations, the underlying soil had developed deep cracks due to drying of the soil. With practice, it was possible to minimize the amount of litter that fell into these cracks. The collected samples were placed in labeled plastic bags and then brought to the laboratory for sorting.

5.1.1.2 Sample Sorting

Samples of litter were sorted individually by hand. Three classes of litter were distinguished including woody litter (twigs and bark), fruits (including maple and ash samaras, acorn, hickory nuts, beech mast, etc.); and mixed leaf litter. The latter included leaf materials, stems of herbaceous plants and vines, fine woody particles and other small organic fragments, Any green plants, recently fallen litter (2001 foliage), living plant roots, or lumps of soil were removed and discarded. Notes were made of any notable organisms, including snails, present in the samples. As a standard procedure, any twigs or wood over 2-cm diameter were excluded as these could individually contributed a large portion of the total litter weight and introduced unnecessary variability into the data set. Because branches of this size were originally excluded in the field or avoided from the sampled area in a few cases, there were relatively few of these found in the samples. A single large elm at Site 8 had lost a significant portion of its current-year's foliage, in part due to the late date of sample collection (September 13 2001). These leaves were not included in the weighed samples. Following sorting, samples were placed in labeled plastic bags and then submitted to the laboratory for dry weight determination.

5.1.2 Fresh Litter Assessment

5.1.2.1 Sample Collection

At five of the established woodlots best representing the average tree cover for each of the five soil/contamination study zones, fresh leaf litter which had fallen in the fall of 2001 was collected. The plots sites included in this sampling were Plots 2, 10, 13, 17, and 21. Three sample points within the established 15-point grid area were selected (Figure 1). At each sample point, a 1-meter square frame was laid on the ground. A knife was used to cut through the leaf deposit at the edge of the frame. All fresh foliage within the frame was collected and placed in labeled polyethylene bags and brought to the laboratory. At the time of collection (November 2, 2001), the amount of foliage still present on the trees in the woodlot was estimated. The collected samples were placed in labeled plastic bags and then brought to the laboratory for sorting.

5.1.2.2 Sample Sorting

Samples were hand sorted and any material exclusive of the current-year foliage was discarded. The remaining leaves were sorted into any distinguishable species with an additional category for species that were of uncertain identity (mainly part leaves) or were miscellaneous materials such as grass or ferns. Following sorting samples were placed in labeled plastic bags and then submitted to the laboratory for dry weight determination.

5.2 Sample Site Description

5.2.1 Vegetation Cover

The character of the vegetation cover on the sample plot was described under four general parameters - namely tree cover, shrub cover, herbaceous vegetation, and moss cover (see Appendix 1). The percentage of the area in a circular area within 2 meters of the sampled point covered by the specified vegetation was estimated to the nearest 5%. Tree species and shrub species overhanging the specified circular area were listed but no attempt was made to evaluate the relative amounts of each. Frequently, the canopies of individual trees over-lapped and it was difficult to distinguish between the individual trees involved, especially when the crowns were high. For herbaceous cover, species were listed individually and the cover estimated in 5% intervals or in 1% intervals when less than 5%. Mosses made only small contributions to the cover and were not evaluated further.

5.2.2 Woody Species Inventory

At each sample site, detailed assessment of woody vegetation was undertaken (see Appendix 2). All tree and shrubs 2 meters tall or greater were tallied and the Diameter at Breast Height (DBH) was measured. Some trees just outside the plot boundary but having a significant impact within the plot (i.e., at least 20% of canopy overhanging the plot) were also evaluated. Such trees were not included in the plot stand calculations but the information was available for interpretation of the results. The stem diameter measurements were made using a standard DBH tape or with electronic calipers in the case of shrubs. The health condition of each tree or shrub was also evaluated on a scale of 1 to 10 (1 = healthy, 10 = dead; See Appendix 1 for details). The percentage of dead branches in the crown was estimated to the nearest 5% but those branches that were considered to have died as part of the normal self pruning process were not included. Plot summaries for the kind, size, basal area, and health were prepared.

5.2.3 Ecological Land Classification

Using the information obtained from the biological inventories, soil maps and general on-site observations, each of the portions of the woodlots where the samples were collected were classified using the Ecological Land Classification (ELC) system established by the Ontario Ministry of Natural Resources (Lee et al. 1998). Descriptions were made to the nearest closest forest type described in the ELC classification.

5.3 Soil Sampling

Samples of soil were collected in duplicate from the 0-5 cm depth at each sample location on September 4 and 5, 2001. Soil was sampled at one woodlot on September 13, 2001. Each sample consisted of approximately 30 cores distributed uniformly across the sample plot. The cores taken by a stainless steel Oakfield® soil corer were combined in a plastic bag, crushed and thoroughly mixed. A portion of the mixed sample was transferred to a labeled jar that was submitted for laboratory analysis. Soil sampling followed the soil sampling protocols developed by JWEL (JWEL, 2001a; JWEL, 2001b) for the Community Based Risk Assessment (CBRA).

6. FIELD AND LABORATORY PREPARATION OF SAMPLES

Following collection and sorting, the standing litter and fresh samples collected required no special treatment with respect to preservation. Sorted samples were placed into labeled plastic bags and closed immediately to minimize loss of moisture and kept cool, approximately 5^{o}C until analysis.

Soil samples were collected into polyethylene bags, thoroughly mixed, and sub-samples placed into sample bottles. The sample bottles were forwarded to the laboratory for analysis as soon as possible after collection to minimize moisture loss prior to moisture determination (i.e., next day). The remaining sample (i.e., part of sample that is not placed in sample bottle) was retained for later use in the event that additional tests are required.

7. FIELD COLLECTION SCHEDULE

To achieve the most appropriate measurements that might demonstrate the maximum difference between collections sites, the field sampling was undertaken within weeks. Collection of standing litter occurred between July 26 and September 13 2001, with most samples collected between August 8 and September 4 2001. Fresh litter samples were collected in one day on November 2 2001. Soil samples were collected September 4 and 5, 2001.

8. LABORATORY ANALYSIS

Following the collection and sorting of the litter samples, samples were delivered to the laboratory for dry weight determination. Weights for each total sample was determined gravimetry in the laboratory following oven drying to a constant weight at 38 ^{o}C. The nature of this particular part of the investigation did not lend itself to control or blank samples. However, all dried samples of litter and soil will be retained (should the need arise for chemical composition determination) in the dried state in cool storage until the CBRA process is completed.

Soil samples were analyzed, Inductively Coupled Plasma (ICP) for 17 metals, arsenic and selenium. The procedures used for the soil analysis are detailed in a separate protocol prepared by JWEL (JWEL, 2001b) for the CBRA.

9. TREATMENT OF DATA

The procedures used for the statistical analysis of the data are detailed in a separate report prepared by JWEL (2002) for the ERA.

10. QUALITY ASSURANCE/QUALITY CONTROL

All testing was carried out in conformance to strict quality control/quality assurance methods, conforming to Jacques Whitford's QA/QC protocols and under the company's ISO 9001 registration. Representatives of the Public Liaison Committee's (PLC's) consultant were allowed to monitor field collection of specimens and could take a fraction of duplicate samples (20%) for analyses. Laboratory QA/QC is presented in a separate chemical analysis protocol developed by JWEL (JWEL, 2001b) for the CBRA.

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APPENDIX 1

Kilty Springs Environmental

Project Code				Year				Project No.#				Data Sheet No.				
		K	S	2	0	0	1	0	0	5	7					

Project Name	**Leaf Litter Accumulation Study - Port Colborne** - Litter Samples
Location	
Site Codes	County: **Niagara**; Project CodeProject Project Station: 2 0 0 5 7 0 0 _ _; Unit: 0 _ _; Time: _ _ _ _
	NAD: 8 3; Zone: 1 7; Easting: ; Northing: ; Error:
Weather	Temp: ; Precip: ; Wind: ; General Weather:

Sample Point	Wet Wt g.	Dominant Trees			%	Dominant Shrubs			%	Sample No*
Litter Depth Cm	Organic Depth cm	Moss Cover %	Herb Cover %	Herb A	%	Herb B	%	Herb C	%	
Comment								Verification Sample (Y/N)		
Sample Point	Wet Wt g.	Dominant Trees			%	Dominant Shrubs			%	Sample No*
Litter Depth Cm	Organic Depth cm	Moss Cover %	Herb Cover %	Herb A	%	Herb B	%	Herb C	%	
Comment								Verification Sample (Y/N)		
Sample Point	Wet Wt g.	Dominant Trees			%	Dominant Shrubs			%	Sample No*
Litter Depth Cm	Organic Depth cm	Moss Cover %	Herb Cover %	Herb A	%	Herb B	%	Herb C	%	
Comment								Verification Sample (Y/N)		
Sample Point	Wet Wt g.	Dominant Trees			%	Dominant Shrubs			%	Sample No*
Litter Depth Cm	Organic Depth cm	Moss Cover %	Herb Cover %	Herb A	%	Herb B	%	Herb C	%	
Comment								Verification Sample (Y/N)		

Form #	Signature	Date
KS008		

* Sample numbers refer to Leaf Litter, Woody Litter and Other in sequence

APPENDIX 2

Kilty Springs Environmental

Project Code	Year	Project No.#	Data Sheet No.
K S	2 0 0 1	0 0 5 7	

Project Name	**Leaf Litter Accumulation Study - Port Colborne** - Major Vegetation
Location	
Site Codes	County: **Niagara**; Project Code: 2 0 0 5 7; Project Station: 0 0; Unit: 0; Time:
	NAD: 8 3; Zone: 1 7; Easting: ; Northing: ; Error:
Weather	Temp: ; Precip: ; Wind: ; General Weather:

Trees

Rep	Unit	Species Code	Species Name	DBH cm	Crown Condition	% Dead Branches	Comment
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Shrubs

Rep	Unit	Species Code	Species Name	DBH cm	Crown Condition	% Dead Branches	Comment
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Form # KSE007	Signature	Date

Kilty Springs Environmental

Project Code	Year	Project No.#	Data Sheet No.
K S	2 0 0 1	0 0 5 7	

Project Name	**Leaf Litter Accumulation Study - Port Colborne** - Fresh Litter Samples
Location	

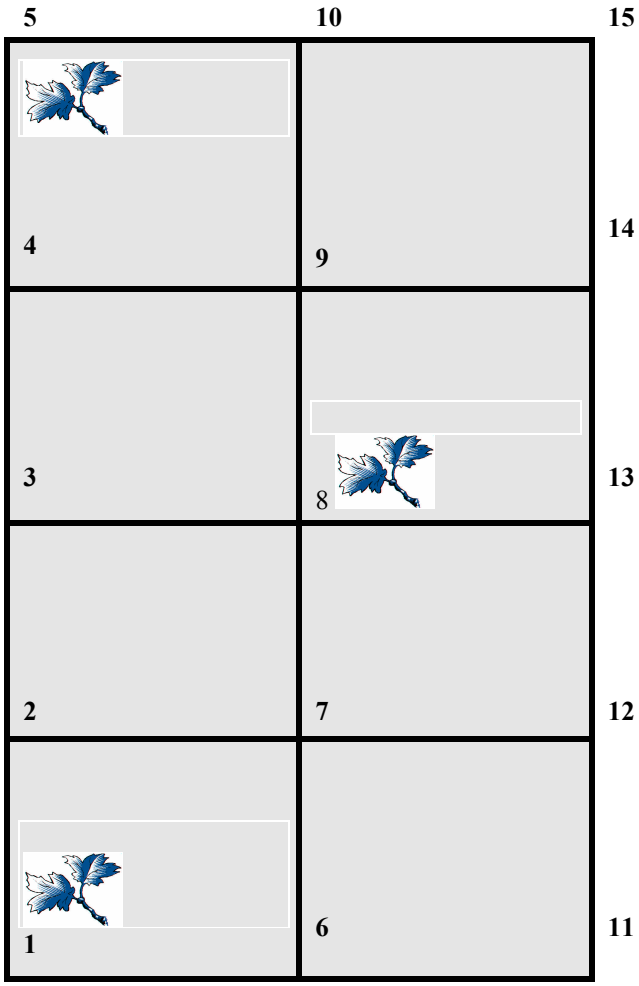
Site Codes

County	Project Code	Project Station	Unit	Time
Niagara	2 0 0 5 7	0 0	0	

NAD	Zone	Easting	Northing	Error
8 3	1 7			

Weather

Temp	Precip	Wind	General Weather



Form #	Signature	Date
KS013		

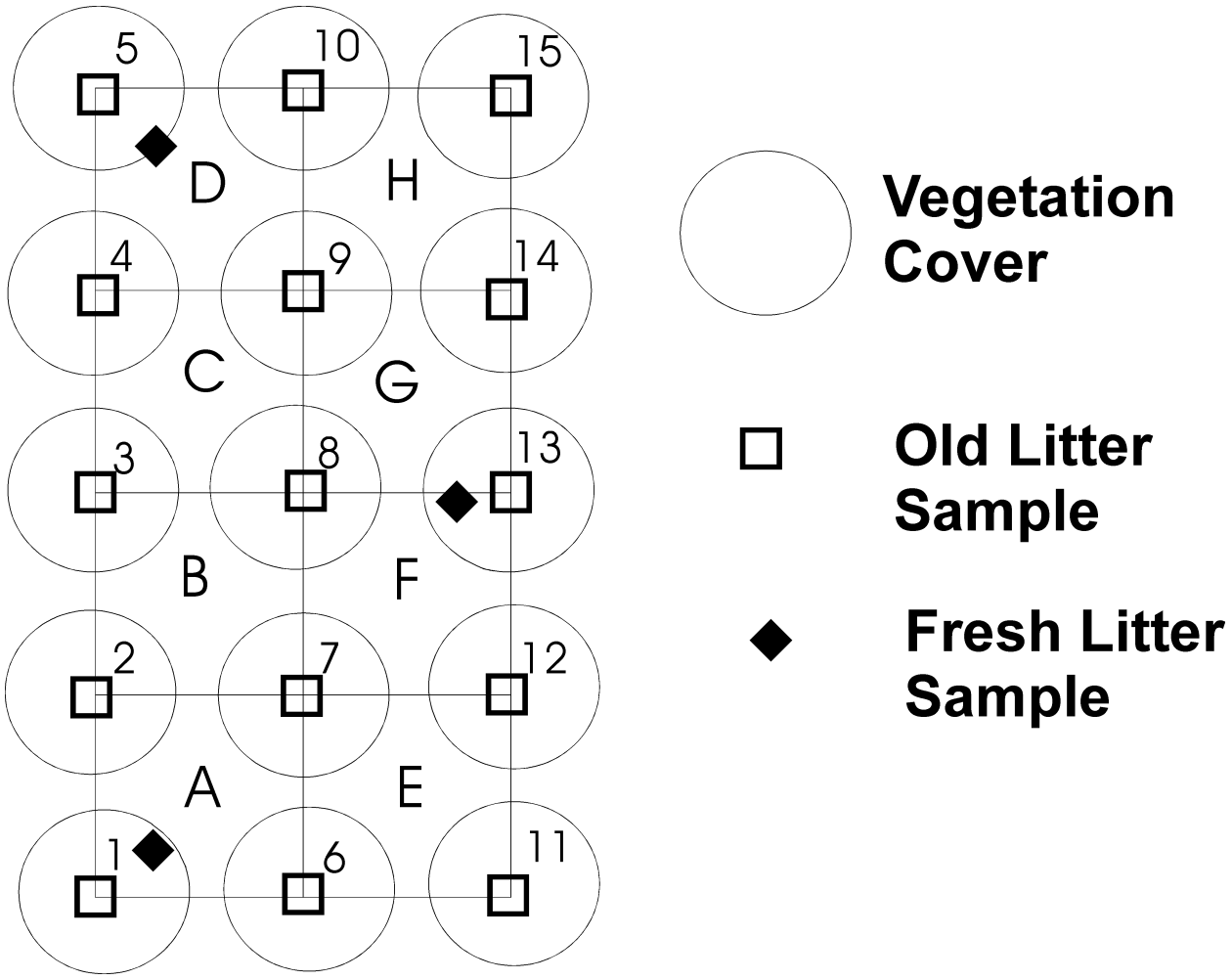


Figure 1. Layout of sampling points utilized for leaf litter assessment at woodlots in the Port Colborne, Ontario, 2001

Insect Collection and Analysis Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

Insects are a major part of terrestrial ecosystems and are food for approximately 60% of the birds occupying the Port Colborne Study Area. Insects may accumulate Chemicals of Concern (CoCs) in their tissues, but no values are available for use in the Ecological Risk Assessment (ERA). Therefore, representative collections of insects were made from the Study Area (as defined by soil nickel concentrations, MOE, 2000a; MOE, 2000b) and reference sites so that they could be analysed for CoCs.

A variety of methods are used to collect insects (Sutherland 1996). Because of the very different nature of vegetation in woodlots and grass fields, three methods have been selected for the purpose of this study to collect representative insects.

2. OBJECTIVES

The primary objectives of the insect collection program was:

1. To collect insects from fields and woodlots located east of the Inco Port Colborne Refinery and at reference sites west of Port Colborne.

2. To determine levels of CoCs in body tissues specific insect groups as well as composite samples of insects.

3. To determine if CoCs levels in insect tissues are correlated to CoCs soil concentrations.

4. To provide food web data for bird receptors for the ERA.

3. INSECT COLLECTION LOCATIONS

The collection of insects was undertaken at various locations within the Study Area (as defined by soil nickel concentrations, MOE, 2000a; MOE, 2000b) and a reference site located west of the City of Port Colborne. In the Study Area samples were collected from fields and woodlots located in two general areas, (i) Primary study area with reported high soil nickel between 500-3000 ppm (MOE, 2000a; MOE, 2000b) and (ii) Secondary study area with reported moderate soil nickel between 200-500 ppm (MOE, 2000a; MOE, 2000b). Where possible, the selection of woodlots for the collection of insects was the same as those woodlots which had been used for the collection of other data including maple sap, maple leaves and leaf litter assessment. For specific sample site locations, please see the sample location maps (Figures 1-1 and 1-2) at the back of this Volume.

4. COLLECTION METHODS

4.1 Sweep Net

For each field or woodlot, insects were collected along a single 100m long transect. This method used walking at a slow steady pace along the transect and sweeping a net through the vegetation using alternate backhand and forehand strokes. At the end of the transect the insects, and any vegetation captured in the net, were transferred to a numbered plastic bag and placed in a cooler. Samples for a sweep transect were collected when the vegetation was dry. Samples were not collected when the vegetation was wet (early morning dew), or during or after rain events.

When using a sweep net in woodlots, the net was swept against all herbaceous and shrubby vegetation along the transect to a height of about two metres. At the end of the transect the insects, and any vegetation captured in the net, were transferred to a numbered plastic bag and placed in a cooler. However, larger woody pieces of vegetation were removed before putting the sample in bags.

Beating

For each woodlot, insects were also collected from shrubs by beating. A white sheet is placed under the shrubs and they were then beaten vigorously with a stick. Fallen insects were then shaken to the centre of the sheet and transferred to a numbered plastic bag which were then labelled and placed in a cooler.

For this study, two beatings and one sweep transect was undertaken in each woodlot.

Hand Collection

Tent caterpillars were collected from their tents, by cutting the supporting branch and stripping the tent and associated caterpillars into a numbered plastic bag and placed in a cooler. Following field collection, all insect samples were transferred from the cooler and were frozen at approximately –5°C until sorting and analysis.

Soil Samples

Soil samples (surface 0-5 cm) were collected at each insect sampling location. For forest sample sites, soils were collected during the collection of data for maple sap/leaves and during the leaf litter study. For field sample sites, following the collection of the insects, the transect was walked and soil plugs were collected at random spacing. Following collection the soil plugs were mixed to provide a composite sample for chemical analysis. For the collection of soils, collection methods followed the soil sampling protocols developed by JWEL for the Community Based Risk Assessment (CBRA) (JWEL, 2001a; JWEL, 2001b).

5. POST FIELD AND LABORATORY PREPARATION OF SPECIMENS

For each insect sample, the frozen insects were sorted according to their order. Grasshoppers (Orthoptera) were separated from each of the field samples and placed in separate pre-weighed vials. For the woodlots, Spiders (Arachnida) were separated from the sample and placed in separate pre-weighed vials. Grasshoppers, spiders, and caterpillars were separated from the insect samples to provide the most uniform insect samples (biomass) for analysis.

For the remaining insects in each sample, the insects were sorted to order and each order were placed in separate pre-weighed vials. Following the sorting of each insect sample, the sub-samples represented by insect orders were weighed. Sub-samples were then recombined to produce a composite sample. Composite samples were stored in a freezer (approximately –5°C) prior to tissue analysis.

6. FIELD COLLECTION SCHEDULE

Insects were collected through the summer months from mid July until the end of August 2001.

7. INSECT CHEMICAL ANALYSIS

Frozen samples were delivered to the laboratory for analysis. Samples were analyzed by Inductively Coupled Plasma (ICP) for 17 metals, Arsenic and Selenium at an Canadian Association for Environmental Analytical Laboratories (CAEAL)-accredited laboratory and appropriate controls and blanks0 used. Details for laboratory analysis procedures for tissue sample analysis are presented in a separate chemical analysis protocol (JWEL, 2002a).). Details for laboratory analysis of soil samples is detailed in a separte protocol developed by JWEL for the CBRA (JWEL, 2001c).

8. TREATMENT OF DATA

The procedures used for the statistical analysis of the data is detailed in a separate report prepared for the ERA (JWEL 2002b).

8.1 Quality Assurance/ Quality Control

All testing was carried out in conformance to strict quality control/quality assurance methods, conforming to Jacques Whitford's QA/QC protocols and under the company's ISO 9001 registration. Representatives of the Public Liaison Committee's (PLC's) consultant were allowed to monitor field collection of specimens and could take a fraction of duplicate samples (20%) for analyses. Laboratory QA/QC is presented in a separate chemical analysis protocol (JWEL, 2002b).

9. REFERENCES

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Wild Grape Collection and Analysis Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

During the collection of various samples for the Ecological Risk Assessment (ERA) for the Port Colborne Community Based Risk Assessment (CBRA), it was noted that Wild Grapes (*Vitis* cf *riparia*) were a common species in many woodlots of the study area. Wild grapes have a shallow rooting system that is exposed to soil Chemicals of Concern (CoCs) at both the soil surface and the B-horizon. In addition, wild grapes yield an abundant source of fruit once well established. As a result it was concluded that wild grapes are probably well utilized by many mammals, birds and insects that occur in the Port Colborne area and that meaningful data could be used in various risk calculations from the sampling of wild grapes.

2. OBJECTIVES

The objectives of the wild grape collection program were to:

1. Collect wild grapes from three to four vines from three woodlots in each of the three locations in the study area (Primary, Secondary and Reference as detailed below).

2. Determine levels of CoCs in the soils of woodlots where grapes were sampled.

3. Determine if CoCs levels in grape tissues are correlated to CoCs soil concentrations.

4. Provide receptor data for the ERA.

3. SAMPLE COLLECTION LOCATIONS

Wild grapes were collected from locations within the Study Area (as defined by soil nickel concentrations, MOE, 2000a; MOE, 2000b) and at reference sites east and west of the City of Port Colborne. Samples were collected from woodlots from three areas: (i) Three woodlots in the Primary Study Area with reported high soil Nickel between 500-3000 ppm (MOE, 2000a; MOE, 2000b), (ii) Four woodlots in the Secondary Study Area with reported moderate soil Nickel between 200-500 ppm

(MOE, 2000a; MOE, 2000b), and (iii) Three woodlots representing reference areas located east and west of the City of Port Colborne. The terms Primary and Secondary areas have been defined previously (JWEL, 2001a). For the specific location of sample sites, please refer to the sample locations maps (Figure 1-1 and 1-2) a the back of this volume.

When possible, woodlots selected for the collection of wild grapes were those woodlots which had been used for the collection of leaf litter data or maple sap. By sampling wild grapes, sap data and leaf litter data from the same woodlot, a more complete and correlated assessement of CoCs in the enivroment can be made.

4. COLLECTION METHODS

Grapes were sampled along the perimeter of woodlots using a knife to remove the cluster. Samples were placed in appropriately labeled zip-lock plastic bags and stored on ice.

Collection of surface soil (0-5 cm) samples for the woodlot area where grapes were sampled was undertaken during the leaf litter assessment study. These soils were analyzed for CoC concentrations to allow for direct comparison between soil and grape tissue concentrations. Soil sampling and analysis was undertaken as outlined in protocols developed by JWEL for the CBRA (JWEL, 2001b; JWEL, 2001c).

5. FIELD AND LABORATORY PREPARATION OF SPECIMENS

Within five days grapes were separated from the stem, rinsed three times thoroughly with distilled water using a pre-rinsed colander, and placed in appropriately labeled glass jars. Grapes were washed to remove any potential surface contamination with soil and dust. All sample jars were then stored in a freezer and later submitted by JWEL to the laboratory (PSC) for chemical analysis.

6. FIELD COLLECTION SCHEDULE

Wild grapes were collected during September 2001 to ensure maturity of the grape.

7. CHEMICAL ANALYSIS

Collected, grape and soil samples were analyzed for CoCs by an Canadian Association for Environmental Analytical Laboratories (CAEAL)-accredited laboratory and appropriate spiked blanks were used. Grape and soil samples were analyzed for Inductively Coupled Plasma (ICP) metals (17), Arsenic and Selenium. Sample treatment and analysis protocols are detailed in a separate protocol developed by JWEL for the CBRA (JWEL, 2001c).

8. TREATMENT OF DATA

The procedures used for the statistical analysis of the data is detailed in a separate report prepared for the ERA (JWEL, 2002).

9. QUALITY ASSURANCE/QUALITY CONTROL

For QA/QC purposes, a portion of grapes was given to the Public Liaison Committee's (PLC's) consultant from 20% of grape samples collected by JWEL. All sample collection was carried out in conformance to strict quality control/quality assurance methods, conforming to Jacques Whitford's QA/QC protocols (JWEL, 2001b; JWEL, 2001c) and under the company's ISO 9001 registration. Representatives of the PLC's consultant were allowed to monitor field collection of specimens and to collect a fraction of duplicate samples (20%) for analyses.

10. REFERENCES

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Red Maple Leaf Collection and Analysis Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

Initial site characterisation for the Ecological Risk Assessment (ERA) for the Port Colborne Community Based Risk Assessment (CBRA) was undertaken in year 2000 (JWEL, 2001a). These investigations identified that Red Maple (*Acer rubrum* L.), Silver Maple (*A. saccharinum*) and their hybrid Freeman's Maple (*Acer rubrum X Acer saccharinum*) are common species in the woodlots in the study area. Of these species, the hybrid Freeman's Maple is the most common species in the Port Colborne area. Because of the difficulty of distinguishing these taxa, they will hereafter be referred to collectively as soft maple.

Soft Maples have a shallow rooting system that is exposed to soil Chemicals of Concern (CoCs) at both the soil surface and the B-horizon. Studies of have also shown that soft maples are susceptible to nickel toxicity symptoms (Temple and Bisessar 1981). In addition, because of the commonness of soft maple, it is probably well utilized by many mammals, birds and insects that occur in the Port Colborne area. Therefore, soft maple represents a potentially important pathway for bioaccumulation of CoCs in woodland food webs. In addition, sampling and analysis of maple tree leaves has been undertaken in the Port Colborne area for the past two decades (MOE, 1994) providing an opportunity for an assessment of change over time with respect to CoCs uptake.

2. OBJECTIVES

The objectives of the maple leaf collection program are:

1. To collect leaves from three trees in each of five woodlots, for three areas (Primary, Secondary and Reference).

2. To determine levels of CoCs in soils of woodlots where trees are sampled.

3. To determine if CoCs levels in leaf tissues are correlated to CoCs soil concentrations.

4. To conduct a visual leaf injury assessment of collected maple leaves to determine if tissue and soil CoCs are correlated with observed leaf injury.

5. To provide receptor data for the ERA.

3. SAMPLE COLLECTION LOCATIONS

Leaves were collected from locations within the Study Area (as defined by soil nickel concentrations, MOE, 2000a; MOE, 2000b) and at reference sites east and west of the City of Port Colborne. Samples were collected from woodlots from three areas: (i) Five woodlots in the Primary Study Area with reported high soil Nickel between 500-3000 ppm (MOE, 2000a; MOE, 2000b), (ii) Five woodlots in the Secondary Study Area with reported moderate soil Nickel between 200-500 ppm (MOE, 2000a; MOE, 2000b), and (iii) Five woodlots representing reference areas located east and west of the City of Port Colborne. The terms Primary and Secondary areas have been defined previously (JWEL, 20001a).). For specific sample site locations please refer to Figures 1-1 and 1-2 at the back of this Volume.

Three trees were sampled from each woodlot, and where possible, one from the windward side, one from the middle and one from the leeward side.

When possible, woodlots selected for the collection of maple leaves were those trees and/or areas in the woodlots which had been used for the collection of leaf litter data or maple sap. By sampling both tree leaf data, sap data and leaf litter data from the same woodlot, a more complete and correlated assessement of CoCs in the enivroment can be made.

4. COLLECTION METHODS

For each woodlot, leaves were collected from three (3) soft maple trees from the windward edge (downwind of the Inco Refinery); the middle of the woodlot; and at the downwind edge of the woodlot. Investigations by the MOE (MOE, 2000c) have identified that woodlots located downwind of the Inco Refinery have soil metal levels that show a “snowfence/rain shadow” effect.

From each tree, leaves (blade and petiole) were collected from the terminating branches as near as possible to the mid-crown of a mature tree. To achieve this, branches were cut with the aid of a 30-foot tree pruning pole and a 6-foot “A” frame aluminum ladder. During the collection, leaves were not allowed to touch the ground in order to prevent soil CoCs contamination of the leaf samples. For each tree sampled, twelve leaves were randomly collected, three leaves from each of the four sides of the tree. For each of the four branches cut, 6 to 10 of the terminal leaves were collected. Once the leaves had been removed from a branch, a random selection of three leaves was taken by one of the collectors holding the leaf blades and the other collector picking the petioles of three leaves. This yielded a total of

twelve leaves per tree that are randomly selected. The twelve leaves were then placed in a plastic bag and pooled to represent one composite sample for each tree.

Following the selection of the twelve leaves, a visible injury assessment was undertaken for each leaf. Each leaf was visually assessed for necrosis, chlorosis, flecking and stippling and assigned a foliar injury rating the MOE rating key (MOE, 1993a; MOE, 1993b)(Refer to Visual Injury Leaf Assessment Data Sheet at the end of this protocol). In addition, the presence and or absence of galls on a leaf was recorded separately. During the visual assessment, all leaf discoloration, spots, scabs, etc., were assessed. However, for areas of a leaf that could clearly be identified as damage due to insect feeding (cut holes, leaf edges) this physical area was not assessed as part of the percentage of leaf with damage. To ensure standardization in the assessment of leaf injury, the same person conducted the injury assessment for all leaves sampled.

Where possible, trees were sampled from those trees from which sap was collected in the spring of 2001 in order to compare CoCs in the leaf tissue to that of the sap. In addition, where possible, woodlots and sample locations where selected in the same general locations as those for the leaf litter and earthworm assessment programs being undertaken for the study. In this way, data from leaf sampling in the woodlots may be correlated to findings of the leaf litter study and earthworm study. Soils samples were collected from the woodlots in the sample locations to determine soil CoCs concentrations.

Collection of surface soil (0-5 cm) samples for the woodlot area where trees were sampled was undertaken during the leaf litter assessment study. These soils were analyzed for CoCs concentrations to allow for direct comparison between soil and leaf tissue concentrations. Soil sampling and analysis was undertaken as outlined in protocols developed by JWEL for the CBRA (JWEL, 2001b; JWEL, 2001c).

5. FIELD AND LABORATORY PREPARATION OF SPECIMENS

Following collection and injury assessment the leaf samples were stored in a labeled Zip Lock bag and placed on ice in a cooler until delivered for analysis. Prior to submission to the laboratory, the leaves were washed with distilled water to remove potential surface contamination with soil and dust. All samples were submitted to Philips Analytical Services Inc. (PSC) within 48 hours following collection.

6. FIELD COLLECTION SCHEDULE

Leaf samples were collected during September, 2000, to ensure full maturity of the leaf.

7. CHEMICAL ANALYSIS

Collected, leaf and soil samples were analyzed for CoCs by an Canadian Association for Environmental Analytical Laboratories (CAEAL)-accredited laboratory and appropriate spiked blanks were used. Leaf and soil samples were analyzed for Inductively Coupled Plasma (ICP) metals (17), Arsenic and Selenium. Sample treatment and analysis protocols are detailed in a separate protocol developed by JWEL for the CBRA (JWEL, 2001c).

8. TREATMENT OF DATA

The procedures used for the statistical analysis of the data is detailed in a separate report prepared for the ERA (JWEL, 2002).

9. QUALITY ASSURANCE/QUALITY CONTROL

All sample collection was carried out in conformance to strict quality control/quality assurance methods, conforming to Jacques Whitford's QA/QC protocols (JWEL, 2001b; JWEL, 2001c) and under the company's ISO 9001 registration. Representatives of the PLC's consultant were allowed to monitor field collection of specimens and to collect a fraction of duplicate samples (20%) for analyses.

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Port Colborne CBRA-ERA

Maple Leaf Collection Data Sheet

Site Number _____

Date _____

Tree Number _____

Tree Characteristics_____

_____

_____

_____

_____

Leaf Injury Rating

Side of Tree	North			South			East			West		
Gals												
Leaf Injury Rating												

Composite Sample Lab ID Code_____

Meadow Vole Collection Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

The Meadow Vole (*Microtus pennsylvanicus*) occurs in grasslands and old field habitats and its diet consists almost entirely of plant materials such as grasses and forbs (Adler 1985, Linzey and Cranford 1984; Ostfeld 1985; Ostfeld and Canham 1993; Peles and Barrett 1996). They avoid forests and woodlots, where the deer mouse *Peromyscus* spp is the most common rodent. Meadow voles feed largely on grasses and sedges during the summer, and in the fall and winter consume roots, seeds, fungi, insects and bark (Banfield 1974, Kurta 1994). The home range of meadow voles is between 0.08 and 0.23 acre and varies with season and population density (Banfield 1974). The population fluctuates with high levels occurring every three to five years (Boonstra and Hoyle 1986). Meadow Voles are an important part of food webs and are a food source for a variety of predators including red fox, ermine, various snakes, many raptors and other species such as gulls, shrikes and herons (Banfield 1974).

The Meadow Vole was identified as a Valued Ecosystem Component (VEC), or receptor, which would be used for tracing Chemicals of Concern (CoCs) through terrestrial ecosystems. The Meadow Vole was selected as the best mammal receptor species for the field program based on the following factors:

- *Microtus pennsylvanicus* are abundant in fields and meadows within the area;
- they occupy small home ranges (less than 0.3 ha, Kurta 1994);
- as a herbivore, they are a major source of food for many predators and are possibly a good candidate for CoCs bioaccumulation;
- they can occur at relatively high densities;
- they are common in the study area; and,
- studies regarding heavy metal effects and bioaccumulation for Meadow Voles are available in the literature.

Meadow Voles breed between mid-April and mid-September and do not hibernate. As with many small mammals, their numbers are usually lowest in the spring and highest in the early autumn. Densities of this species can also fluctuate dramatically in cycles lasting several years. In southern Ontario, Boonstra and Hoyle (1986) found that the cycle lasts approximately three to five years. Depending on the quality of the habitat, time of year and period in a population cycle recorded densities in Ohio range from 2.3 individuals/ha to 550 individuals/ha (Hall *et al*. 1991). Meadow Voles grow rapidly being

approximately 2.1 g at birth. Young females mature rapidly and mate at approximately 25 days, bearing young at 45 days when they are about half grown and weigh approximately 20 gm. Young males mature at about 45 days and weigh 20 – 25 g (Banfield 1974). Nickel concentrations in small mammals are typically highest in the kidneys, liver, gastrointestinal tract and on the skin and fur (CEPA, 1994).

2. OBJECTIVES

The primary objectives of the Vole field collection program was:

1. To collect adult Meadow Voles from fields located east of the Inco Port Colborne Refinery and at reference areas located to the west of Port Colborne.

2. To determine levels of CoCs in liver tissues of adults.

3. To determine if CoCs levels in tissues are correlated to concentrations of CoCs in soil.

4. To provide whole body CoCs concentrations for food chain receptor data for the ERA.

It is important to note that it was not the objective of this sampling program to provide detailed population data for the comparison of vole populations between areas. The primary objective of the sampling program was to determine average CoCs concentrations (CoCs/gram: whole body and tissue) for voles for the three study areas (Primary, Secondary and Control – see below).

3. COLLECTION METHODS

Collection of Voles

Meadow Voles were "live trapped" in each study area as well as at reference areas located west of Port Colborne. A Wildlife Collectors Permit was obtained from the Ontario Ministry of Natural Resources. Voles were collected from the following study areas: (i) Primary Study Area with reported soil nickel between 500-3000 ppm (MOE, 2000a; MOE, 2000b), and (ii) Reference Areas located west of Port Colborne. The term Primary Study Area was defined for the ERA previously (JWEL, 2001a). For sample site locations please refer to Figures 1-1 and 1-2 at the back of this Volume.

Trapping was undertaken in the fall (September-October 2001). Approximately 100 Sherman live traps (23 x 7.5 x 9 cm) were set each night along transects in suitable habitats. The traps were baited with a mixture of peanut butter and rolled oats, placed in suitable locations and marked with flagging tape. Traps were set during the afternoon and checked each morning.

Animals that were not to be collected (by catch) were released after identification. To euthanise captured voles, the individual was removed from the trap and placed in a sealed plastic bag into which a cotton ball soaked in chloroform had been placed. After five minutes, the bags were opened and the animals placed into labeled plastic bags and transferred to a cooler with ice.

Collection of Soil Samples

Soil samples (surface 0-5 cm) were collected at each vole sampling location. For some sample areas, soils were collected during the collection of data for insect collection program undertaken for the ERA. For field sample areas, a transect was walked and soil plugs were collected at random spacing. Following collection the soil plugs were mixed to provide a composite sample for chemical analysis. For the collection of soils, collection methods followed the soil sampling protocols developed by Jacques Whitford Environment Limited (JWEL) for the Community Based Risk Assessment (CBRA) (JWEL, 2001b; JWEL, 2001c).

4. PREPARATION OF SPECIMENS

Following the collection of the day's samples, specimens were dissected to remove the liver. Prior to dissection, each animal was weighed and sexed. Whole animals were weighed using an electronic scale to the nearest 0.01g (Acculab VI-200). Following dissection, the tissue of the whole liver was placed into individual pre-weighted plastic vials to which a label was added. The liver tissue and remaining body for each specimen was then placed in one bag which was frozen (approximately –5°C) until delivered for laboratory analysis.

Safety precautions during the trapping and dissection included wearing appropriate clothes and protective gloves to avoid surface skin scratches. Bags containing chloroform were left opened and left standing for a period to eliminate exposure to harmful gases. Sample bags were not reused during the trapping process and all bags and used cloths were placed in a sealed container prior to destruction. After trapping and dissection, equipment (i.e., traps, dissection tools) was washed in a hypochlorite solution.

These precautions were undertaken to reduce the chances of exposure to Hanta virus. Hanta virus is a virus that is known to be carried by deer mice (Peromyscus sp.), other rodents and some birds. The main human hazard of exposure to the virus is entering enclosed, unventilated spaces that are rodent-infested. Infection occurs by inhalation of dusts and aerosols arising from rodent fecal matter, urine or saliva or being bitten by rodents (CDC, 1993). Infection risk is low, especially in Ontario, but the disease can be fatal when transmitted to humans.

5. FIELD COLLECTION SCHEDULE

Vole populations in Ontario are typically at their highest in the fall. Collection was therefore scheduled to l be undertaken during September and October to reduce the trapping effort and to ensure the collection of an adequate sample size. The goal of the program was to collect five specimens from each sampling area.

6. CHEMICAL ANALYSIS

Following the collection of specimens and tissue, frozen samples were delivered to the laboratory for analysis. For each specimen, tissue analysis was undertaken for the liver and remaining body. Tissue and soil samples were analyzed for ICP metals (17), Arsenic and Selenium by an CAEAL-accredited laboratory and appropriate controls and spike blanks were used. Details for laboratory analysis procedures for tissue sample analysis are presented in a separate chemical analysis protocol (JWEL, 2002a). Details for laboratory analysis of soil samples is detailed in a separte protocol developed by JWEL for the CBRA (JWEL, 2001c).

7. TREATMENT OF DATA

The procedures used for the statistical analysis of the data is detailed in a separate report prepared for the ERA (JWEL 2002b).

8. QUALITY ASSURANCE/QUALITY CONTROL

All testing was carried out in conformance to strict quality control/quality assurance methods, conforming to Jacques Whitford's QA/QC protocols and under the company's ISO 9001 registration. Representatives of the Public Liaison Committee's (PLC's) consultant were allowed to monitor field collection of specimens and could take a fraction of duplicate samples (20%) for analyses. Laboratory QA/QC is presented in a separate chemical analysis protocol (JWEL, 2002a).

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Woodlot Health Assessment Study Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

As part of the Ecological Risk Assessment (ERA), potential long term effects of Chemicals of Concern (CoCs) on woodlot health for forests located east of the Inco Port Colborne Refinery were identified as requiring investigation. For the ERA, the assessment of woodlot health, defined as tree species representation, age class distribution and the health of trees, was undertaken by Trees Unlimited Forestry Consultant. Trees Unlimited has extensive knowledge and experience in hardwood forest management, inventory collection and analysis, and multiple-use management. Trees Unlimited was retained by Jacques Whitford Environment Limited (JWEL) on behalf of Inco Limited to undertake the work for collecting and evaluating a woodlot health study as part of the Community Based Risk Assessment (CBRA) in the Port Colborne area. The study was undertaken in accordance with existing provincially recognized forest resource inventory studies carried out in southern Ontario.

2. OBJECTIVES

The primary objectives of the woodlot health assessment study were:

1. To collect woodlot inventory data for woodlots located east of the Port Colborne Inco Refinery in the areas where soil levels of Nickel are above Ministry of Environment guidelines and from reference sites located east and west of Port Colborne.

2. To establish any relationship between a number of woodlot data sets (e.g. species composition, woodlot rank) and levels of CoCs in the soil.

3. To provide qualitative and quantitative data for a woodland community receptor and ecological process data for the ERA.

3. STUDY APPROACH

The study was conducted in four stages:

Stage 1: Identification of Study Area, Preliminary Assessment & Development of Terms

Stage 2: Research and Consultation

Stage 3: Inventory & Woodlot Health Data Sheet

Stage 4: Woodlot Health Assessment Summary

Stage 1: Identification of Study Area, Preliminary Assessment & Development of Terms

For the assessment of woodlot health, the study investigated all woodlots located east and north of the Inco Port Colborne Refinery. The study area woodlots are all found within the area in which soil nickel levels are >200 ppm (as defined by MOE, 2000a; MOE, 2000b). Reference woodlots were woodlots located outside of the elevated nickel soil levels (<200 ppm Ni). For the purpose of the woodlot heath study, the general study area is identified as Lots 14-25, Concessions 1-3, in the City of Port Colborne, totaling 20 woodlots or 600 acres (245 ha). The Reference study area included ecologically similar woodlots outside of the general study area that could be used for comparison purposes, totaling 6 woodlots or 150 acres (60 ha) (Appendix A and B).

All woodlots within the study area were reviewed and included in the study provided access to the property was not denied and the woodlot was larger than 2 ha (5 ac) and not severely disturbed (i.e., through mowing).

A preliminary field assessment of the study area was undertaken to confirm the number of woodlots, locate property and compartment boundaries, determine Ecological Land Classification (ELC) for each compartment, record any obvious evidence and impacts of past forest management, and meet with landowners (Appendix C). An historical assessment was also undertaken using aerial photographs from as early as 1934 from the Brock University Aerial Photo Library, Inco archives and the Regional Municipality of Niagara. The aerial photographs were used to determine historical changes, delineate acreages, stand type, and access. Regional assessment maps from either the City of Port Colborne or the Regional Assessment Office were used to delineate property ownership.

Stage 2: Research and Consultation

Various government and private reports such as *Soil Contamination in Port Colborne Woodlots: 2000* and *Phytotoxicology Technical Memorandum: Inco, Port Colborne (1999),* and recognized assessment evaluation systems were consulted and reviewed with regards to impacts of CoCs on forest health, appropriate evaluation system(s), rare and endangered species list for the study area, physiography, woodlot management history, and property ownership. These sources included the following:

- Atlas of Rare Vascular Plants of Ontario
- Brock University
- Committee on the Status of Endangered Wildlife in Canada
- Federation of Ontario Naturalists
- Property owners
- Nature Conservancy of Canada
- Natural Heritage Information Centre
- Niagara Peninsula Conservation Authority
- Ontario Ministry of Natural Resources
- Regional Municipality of Niagara
- University of Toronto Library

Trees Unlimited's library of woodlot management plans, inventories and site visits were also reviewed for historical data, ownership and comments.

Stage 3: Inventory & Woodlot Health Data Sheet

A woodlot or forest is a plant community predominantly composed of trees and other woody vegetation, growing more or less closely together (OMNR, 2000). A woodlot may contain one or more compartments where the groups of trees are different enough in composition (species), age, and structure to be distinguishable from each other. Past management activities and ownership often dictate different compartments. To allow for proper data analysis and comparison, compartments were identified and inventoried individually.

A compartment or stand is an aggregation of trees occupying a specific area and uniform enough in ELC site type, composition (species), age, structure, and property boundaries to be distinguishable from the

forest on adjoining lands. All woodlots under investigation were assessed to the following inventory and analysis. Each compartment was also classified using the ELC classification (Lee *et al.* 1998) to the Ecosite level.

Prism cruise inventory guidelines (OMNR, 1990) and Ecological Land Classification for Southern Ontario field sampling methods (Lee *et al.* 1998) were used to conduct the forest health inventories. However, where required sampling methods were modified to achieve the intended purpose of this study. A crew of two persons including a forester and resource technician conducted all the inventories.

Aerial photographs and preliminary field inspection notes were used to determine woodlot and compartment boundaries. Regional Assessment Maps were used to identify the civic property address and ownership name(s). A digital planimeter and a mapping at the scale of 1:5,000 were used to calculate woodlot and compartment size. The forested area belonging to individual landowners was also calculated.

Aerial photographs, Regional Assessment Maps and preliminary field inspection notes were used to layout the cruise lines and plot centres. Cruise lines and plot centres were systematically selected at regular intervals along the cruise line in multiples of 10 metres, striving for a minimum of 80 metres between plot centres. Plot centres were located throughout the compartment in an attempt to increase the probability that any heterogeneity in compartment characteristics were accounted for in the sample. The plot intensity was approximately one plot per hectare with a minimum of two plots sampled per compartment. The starting point and plot centres were marked with a painted orange band on the closest tree to facilitate any audit or subsequent cruises.

Property boundaries, woodlot and compartment sizes, cruise lines and plot centres within the compartment boundaries, and landowner names were marked on copies of the aerial photographs for field use and ease of transferring information to a single map.

Reference woodlots and compartments were selected based on similar ecological land classification, stand structure, management history, acreage and use of the woodlot in the other scientific studies that are part of the ERA.

For a consistent collection of data, a Forest Health Data Sheet (Appendix D) was completed for each compartment that included the following parameters.

A. SITE INFORMATION:

Woodlot Number: Each woodlot was given a number. Any previously recognized names were also recorded (i.e., Woodlot 2 - Weaver Road).

Compartment Number: Each compartment was assigned a letter to distinguish it from other compartments in the woodlot. Compartment boundaries were delineated by stand structure, ownership and/or access.

Compartment Size: Acreage was measured using a digital planimeter on 1:5,000 1994 aerial photography.

Drainage, Topography, Aspect, UTM Co-ordinates, Aerial & Digital Photo information was recorded from either field inspection or relevant reference materials.

B. SITE DATA:

Plot Locations used in the field inventory, as determined from aerial photographs, was recorded on the aerial photos and in the field to allow for auditing purposes and future re-assessments.

Maximum Tree DBH by Plot Tally was the tally of the diameter at breast height (DBH) in centimeters of the largest tree found in each plot.

Tree Tally: Trees were tallied by species, diameter class (in cm taken at DBH), and quality. Tree species short forms followedntario Ministry of Natural Resources (OMNR) Alpha code protocols (OMNR, 1990). All species had a specific alpha code except red maple (*Acer rubrum*) and silver maple (*A. saccharinum*) and their hybrids which was pooled under the code Ms (Maple, soft). Individual tree quality was defined by two classes. Acceptable Growing Stock (AGS) are trees suitable for retention in the compartment for at least one cutting cycle (15 to 25 years). They are trees of commercial species and of such form and quality as to be saleable for sawlog products at some future date. Unacceptable Growing Stock (UGS) are trees that have a high risk of dying and are expected to decline over the next cutting cycle. They include trees that are of poor form and/or low quality (OMNR, 2000). A tree tally also provided an estimate of basal area (BA) by diameter class, quality and total in m^2/ha.

Compartment Tree Age was determined by increment coring two trees in the dominant crown position located in the centre of each compartment and averaging the results. Preferred species for coring include species that generally have solid heartwood such as white ash and oak species. A visual count of growth rings was undertaken with a hand lens to determine age.

Wildlife Trees were tallied within each plot using the prism. A cavity tree is defined as a living or dead standing tree that has a hole in the trunk of the tree that might be used by a species of vertebrate wildlife. A snag is defined as a standing, dead tree or standing section of the stem of a tree broken off at a height of three metres or more. Downed woody debris (DWD) is defined as sound and rotting logs that provide habitat for plant, animals and insects and a source of nutrients for soil development. The minimum diameter of DWD would be 8 cm as determined through the prism. All three types of wildlife tree will be measured in m^2/ha.

Tree Regeneration was recorded by species and percent cover within a five-metre radius of the plot centre. Percent cover codes from 1 to 4 represent four classes of percent cover. Cover Code 1 represents 0% - 10% cover, Cover Code 2 represents 10% - 25% cover, Cover Code 3 represents 25% - 60% cover, Cover Code 4 represents greater than 60% cover.

Shrubs and Vines were recorded by species within a five-metre radius of the plot centre.

Management / Disturbance was determined using a modified table from Section 8 of the Data acquisition Form from the Ecological Land Classification for Southern Ontario. Within the table, two parameters, Type and Intensity, and Extent of anthropogenic and natural disturbances are identified. Four classes of Type and Intensity, and Extent of Disturbance are used. Classes of Type and Intensity of the Disturbances are, in most cases:

0) None;
1) Light;
2) Moderate; or
3) Heavy.

Classes of Extent of Disturbance are;

0) None;
1) Local;
2) Widespread; or
3) Extensive.

In cases where Type and Intensity of Disturbance do not conform to the four main classes, a more detailed description of the type of disturbance is given. The parameters that don't conform are:

- Alien species;
- Access trails;
- Intensity of logging; and
- Gaps.

An additional Type & Intensity of Distances is the Time since loging, which is partioned into four "age" classes:

0) greater than 30 years since logging;
1) between 15 - 30 years since logging;
2) between 5 - 15 years since logging; and
3) between 0 -5 years since logging.

With the exception of the parameter Time since Logging, the numerical code for Type and Intensity of Disturbance and Extent of Disturbance, are multiplied together giving a score for each disturbance regime. All scores are tallied for an overall Management/Disturbance score within each compartment.

General Comments of a qualitative nature will be made for each compartment. General health comments on Roots, Tree Trunks, Crown, Diseases, Overall Tree Health and Regeneration will be made. Canopy closure as a percent will be included in the comments on the crown. Average Tree Height will be included in comments of Tree Health.

Stage 4: Woodlot Health Assessment Summary

A Woodlot Health Assessment Summary (Appendix E) by compartment was completed using the Woodlot Health Data Sheet and other resources. The Woodlot Health Assessment Summary included the following information.

A. SITE INFORMATION

Woodlot number, Compartment number and Size will be recorded. The address including Township, Lot and Concession number(s) were also recorded.

B. OWNERSHIP INFORMATION

The individual Landowner's Name(s), the Size, in acres, of the forested compartment on the individual landowner's property, the civic address, including Lot and Concession number, the Mailing Address and the Home Telephone number are included in this section.

C. SITE DESCRIPTION

1) Physiography/Hydrology

Landform(s), Soil Type(s), Soil Texture(s), Topography, Aspect, and Drainage found in the woodlot compartment are outlined in this section

2) Vegetation Analysis

i) Trees and Shrubs

Composition of the dominant tree species to a minimum of 10%, number of species found, average age of the tree species, stocking which is the tree cover expressed as a percent, mean and maximum DBH in centimeters, total Basal Area (BA) expressed in square metres per hectare broken down into the BA of AGS and the BA of UGS, composition of regeneration of tree species to a minimum of ten percent, the number of shrub and vine species, the Ecological Land Classification (ELC); including ELC Community Class, ELC Community series, Ecosite and Vegetation Type, Wetland, Wetland Class, Area of Natural and Scientific Interest (ANSI); Earth Science (ES) or Landform Science (LS), Carolinian Canada Site (CCS), and Environmentally Significant Area (ESA) are recognized in this table.

ii) Flora and Fauna

Significant species of flora and fauna that are found in the compartment along with their designation as Regionally Rare (RR), Provincially Rare (RP), Nationally Rare (RN), Threatened (T) or Endangered (E) and a Score which gives one point for RR, two for RP, three for RN, five for Threatened and 10 for Endangered species.

3) Management History

The management history section will be a text description of the past disturbances in the compartment inferred from the observations taken during the field inventory and recorded from OMNR Forest Management History files.

4. FIELD COLLECTION SCHEDULE

The collection of field data was undertaken during the period from October 2001 to April 2002. The locations of the woodlots sampled in provided in Appendix A and B.

5. ANALYSIS

Following the collection of the field data similar ELC classified compartments were pooled together, summarized by compartment and by parameter. Through mapping, tables, graphs and/or text the results will be presented and conclusions made regarding the state of woodlots and potential past and present effects on woodlots which could provide explanation of the data, including potential effect from soil CoCs.

6. QUALITY ASSURANCE/QUALITY CONTROL

All field surveys and analysis was carried out in conformance to strict quality control/quality assurance methods, conforming to Jacques Whitford's QA/QC protocols and under the company's ISO 9001 registration. Representatives of the Public Liaison Committee's (PLC's) consultant were allowed to monitor field collection of data.

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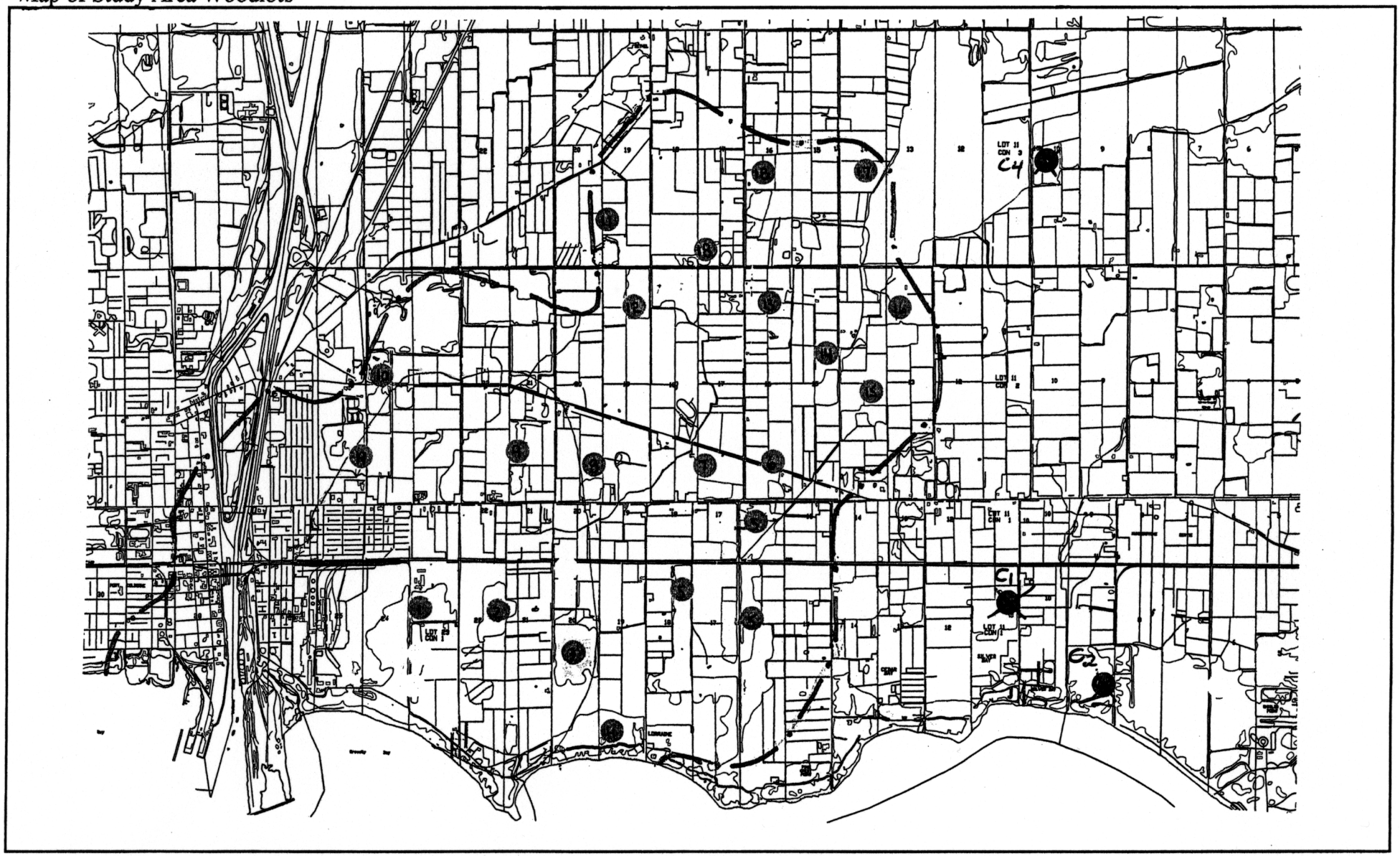
APPENDIX A

LOCATION WOODLOTS ASSESSED IN THE STUDY AREA

Appendix A

Map of Study Area Woodlots



Note: Size and location of woodlots are approximate

- Study Area Woodlots

- Control Woodlots

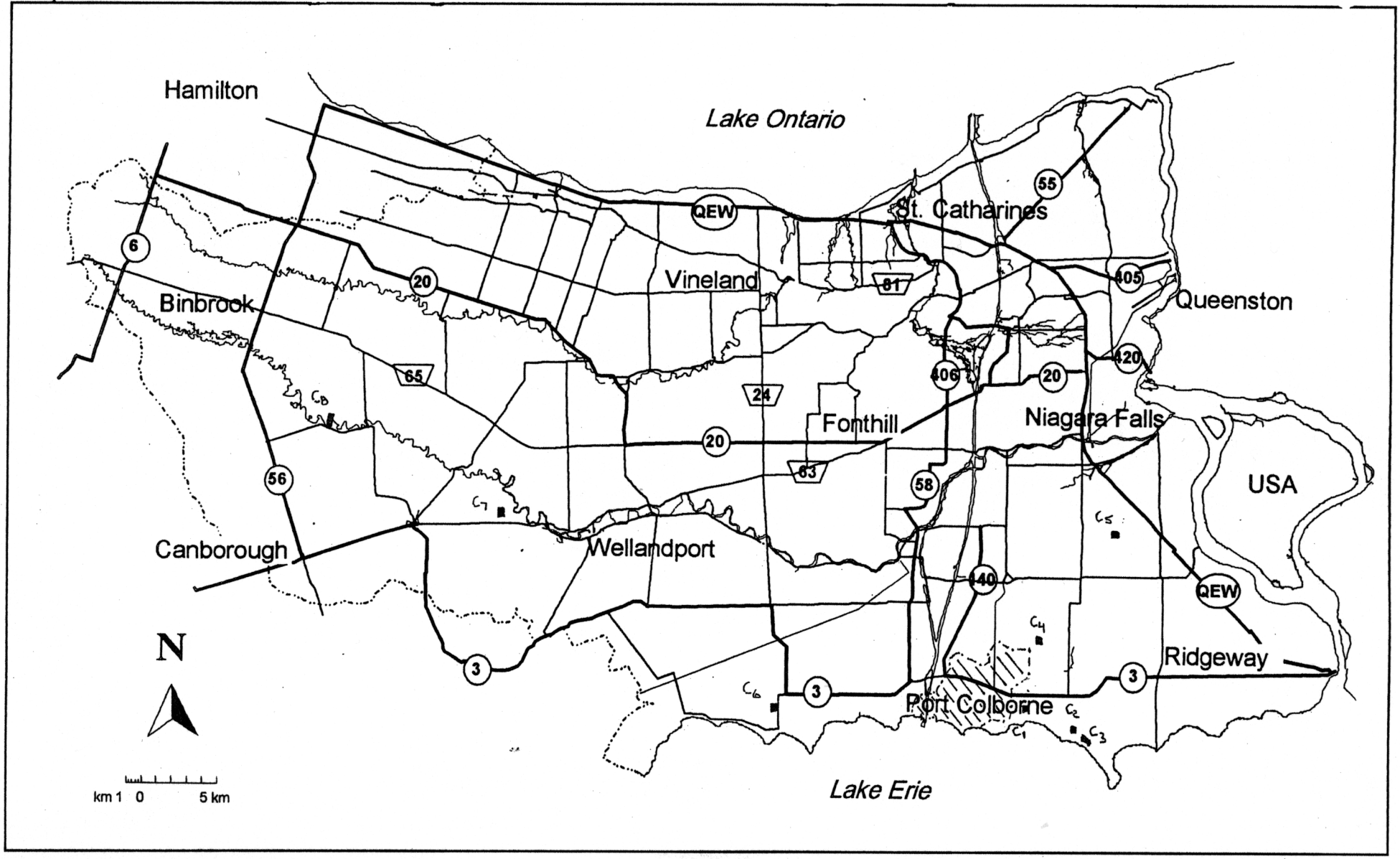
- Study Area Boundary

APPENDIX B

LOCATION WOODLOTS ASSESSED IN THE REFERENCE AREA

Appendix B

Map of Control Woodlots



Note: Size and location of woodlots are approximate

■ C1 - Control Woodlots

▨ - Study Area

APPENDIX C

STUDY AREA WOODLOTS BY NUMBER, LOCATION, OWNERSHIP
And
ECOLOGICAL LAND CLASSIFICATION

Appendix C

Study Area Woodlots by Number, Location, Ownership & Ecological Land Classfication

ID	Woodlot	Compartmen	Location	Acreage (ac	Ownership	Ecological Land Classification
1	01	A	Con. 1 Lot 24 - North	23.0	INCO	SWD4 - with Ms
2	01	B	Con. 1 Lot 24 - South	17.5	INCO	SWD4 - with Ms
3	01	C	Con. 1 Lot 24 - North and South of INCO Rd	29.4	INCO	FOD2
4	01	D	Con. 1 Lot 23 - North	17.1	INCO	FOD6 - with Or
5	01	E	Con. 1 Lot 23 - Middle	3.3	INCO	FOD7 - with Cb
6	01	F	Con. 1 Lot 23 - South	4.0	INCO	?
7	02	A	Con. 1 Lot 22	22.9	Verdonk, Inco	FOD7
8	03	A	Con. 1 Lot 20	6.0	Van Kralingen	FOD7
9	04	A	Con. 1 Lot 19	17.7	Bankert, VanAntwerp, Potter	FOD7 - with He
10	05	A	Con. 1 Lot 18 - North	21.4	Robinson, Harrieth, Never, Foster, Frey	FOD9
11	05	B	Con. 1 Lot 18 - South plus Lot 17	37.2	Burger, Keatley, Czinege, Weaver, Stevens et al	FOD7 - with Ms
12	05	C	Con. 1 Lot 17	15.8	Flickinger	SWD3
13	05	D	Con. 1 Lot 17	22.8	Flickinger	FOD7 - with Ms
14	05	E	Con. 1 Lot 16, 17	116.2	Hatch, Toepp, Snelling, Nigh, Bliek	FOD7 - with Hi
15	05	F	Con. 1 Lot 16	28.6	Toepp, Lavoie, Yalowica, ?	FOD7 - with Ms, Or
16	06	A	Con. 2 Lot 16	4.6	Guay	FOD9 - with Aw
17	07	A	Con. 2 Lot 17	12.0	Young	FOD9
18	08	A	Con. 2 Lot 20	9.6	Kramer	FOD9 - with Aw
19	09	A	Con. 2 Lot 21	6.5	Kramer	FOD9 or FOD2
20	10	A	Con. 2 Lot 24	16.8	Augustine	?
21	11	A	Con. 3 Lot 19, 20	12.1	Orsetto, Giacomi, ?	FOD2
22	12	A	Con. 2 Lot 18	24.0	Fehrman	FOD7 - with Ms, Or
23	12	B	Con. 2 Lot 19	18.8	Fehrman	FOD7 - with Ms, Or
24	13	A	Con. 2 Lot 16	13.5	?	FOD9 - with Aw
25	14	A	Con. 2 Lot 15	13.0	Trepanier, Mazzuto	FOD9 - with Op
26	14	B	Con. 2 Lot 14	2.9	Cook, Gibson	FOD7
27	15	A	Con. 2 Lot 14	14.8	The Mill Open Air Market Ltd.	?
28	16	A	Con. 2 Lot 13	13.2	Golf Course ?	FOD2
29	17	A	Con. 3 Lot 14	9.4	Smith	SWD3
30	17	B	Con. 3 Lot 14	11.9	Smith	FOD7
31	17	C	Con. 3 Lot 14	12.6	Smith	FOD2 or FOD9
32	18	A	Con. 3 Lot 16	10.3	Noonan, Vanderlan	FOD2

Appendix C - continued

Control Woodlots by Number, Location, Ownership & Ecological Land Classfication

ID	Woodlot	Compartment	Location	Acreage (ac)	Ownership	Ecological Land Classification
1	C1	A	Con. 1 Lot 11 Port Colborn	10.9	Favaro	FOD9 or FOD7 with Or, Hi
2	C2	A	Con. 1 Lot 6,7 Port Colbor	31.6	Sherkston Shores	FOD7 with He, By
3	C2	B	Con. 1 Lot 6,7 Port Colbor	21.6	Sherkston Shores	FOD9
4	C3	A	Con. A Lot 34, 35 Fort Erie	44.2	Marcy	FOD7
5	C3	B	Con. A Lot 34, 35 Fort Erie	71.4	Marcy	FOD2 with Tp
6	C4	A	Con. 3 Lot 10 Port Colborn	?	Storm	FOD7 with Cb
7	C5	A	Con. 6 Lot 4 Niagra Falls	?	NPCA	SWD4
8	C6	A	Con. 1 Lot 20 Wainfleet	17.5	Niagara Region	FOD2
9	C7	A	Con. 1 Lot 9 Canboro	38.2	VanderHoeven	FOD2
10	C8	A	Con. 1 Lot 23 West Lincoln	35.0	Oliver	FOD9

APPENDIX D

WOODLOT HEALTH DATA SHEET

Appendix D

WOODLOT HEALTH DATA SHEET	Assessor(s): P. Robertson K. Vitols	☐ Date(s) Inventoried: ☐

1. SITE INFORMATION

dlot No.:	Compartment:	Size (ac):	Twp.:	Lot(s): Conc(s).:
Drainage:		Topography:		Aspect:
UTM Coord.:		Aerial Photo:	Digital:	Other:

2. SITE DATA:

1) Plot Locations (plot interval in meters):

Start at Offset m @ ° P Head m @ ° P P	Start at Offset m @ ° P Head m @ ° P
Offset m @ ° P Head m @ ° P Offset m @ ° P	Offset m @ ° P Head m @ ° P Offset m @ ° P
Head m @ ° P Offset m @ ° P Head m @ ° P	Head m @ ° P Offset m @ ° P Head m @ ° P
Offset m @ ° P Head m @ ° P Offset m @ ° P	Offset m @ ° P Head m @ ° P Offset m @ ° P
Head m @ ° P Offset m @ ° P Head m @ ° P	Head m @ ° P Offset m @ ° P Head m @ ° P

2) Plot Tally / Max. DBH (cm):

Plot #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
DBH																									

3) Tree Tally (prism factor 2 m ² / ha):

Class (cm)	Saplings (4-10)		Poles (12-28)		Sawlogs (30-36)		Sawlogs (38-48)		Sawlogs (50-60)		Oversized (> 60)		Total (all)	
Spp.	AGS	UGS	AGS	UGS	AGS	UGS	AGS	UGS	AGS	UGS	AGS	UGS	AGS	UGS
1.														
2.														
3.														
5.														
6.														
7.														
8.														
9.														
10.														
11.														
12.														
13.														
14.														
15.														
16.														
17.														
18.														
19.														
Total trees														
BA (m ² / ha)														
BA (m ² / ha)														

4) Compartment Tree Age

Spp. Age:	Spp. Age:	Spp. Age:	Spp. Age:	Average:

5 Wildlife Trees (prism factor 2 m ² / ha):

ity: Total: . . m ²/ha:	Snag: Total: m ²/ha	Downed Woody Debris : Total m ²/ha:	Total m ²/ha:

6) Tree Regeneration (Cover Codes - (1 : 0 % - 10 %) , (2 : 10 % - 25 %) , (3 : 25 % - 60 %) , (4 : > 60 %)):

Species / Value	1	2	3	4	Pts	Species	1	2	3	4	Pts	Species	1	2	3	4	Pts
1.						6.						11.					
2.						7.						12.					
3.						8.						13.					
4.						9.						14.					
5.						10.						15.					

7) Shrubs / Vines (5 m radius):

Species	Species	Species	Species	Species
1.	6.	11.	16.	21.
2.	7.	12.	17.	22.
3.	8.	13.	18.	23.
4.	9.	14.	19.	24.
5.	10.	15.	20.	25.

8) Management / Disturbance:

Disturbance/Extent	0	1	2	3	Score	Disturbance/Extent	0	1	2	3	Score
Livestock (Grazing)	None	Light	Moderate	Heavy		Gaps in Forest Canopy	None	Small	Intermediate	Large	
Extent of Livestock	None	Local	Widespread	Extensive		Extent of Gaps	None	Local	Widespread	Extensive	
Alien Species	None	Occasional	Abundant	Dominant		Disease/Death of Trees	None	Light	Moderate	Heavy	
Extent of Alien Species	None	Local	Widespread	Extensive		Extent of Disease/Death	None	Local	Widespread	Extensive	
Recreational Use	None	Light	Moderate	Heavy		Wind Throw (Blow Down)	None	Light	Moderate	Heavy	
Extent of Recreational Use	None	Local	Widespread	Extensive		Extent of Wind Throw	None	Local	Widespread	Extensive	
Access Trails	None	Faint Trails	Well-Marked	Tracks or Roads		Browse (eg. Deer)	None	Light	Moderate	Heavy	
Extent of Trails	None	Local	Widespread	Extensive		Extent of Browse	None	Local	Widespread	Extensive	
Dumping (Rubbish)	None	Light	Moderate	Heavy		Flooding	None	Light	Moderate	Heavy	
Extent of Dumping	None	Local	Widespread	Extensive		Extent of Flooding	None	Local	Widespread	Extensive	
Earth Displacement	None	Light	Moderate	Heavy		Ice Damage	None	Light	Moderate	Heavy	
Extent of Displacement	None	Local	Widespread	Extensive		Extent of Ice Damage	None	Local	Widespread	Extensive	
Time since Logging	> 30 Yrs.	15-30 Yrs.	5-15 Yrs.	0-5 Yrs.		Other:	None	Light	Moderate	Heavy	
Intensity of Logging	None	Fuel Wood	Selective	Diameter Limit		Extent of ...	None	Local	Widespread	Extensive	
Extent of Logging	None	Local	Widespread	Extensive						Intensity Score	

9: General Comments

Root:	Trunk:	Crown:
		Canopy Closure (%):
Diseases:	Tree Health	Regeneration:
	Tree Heights (m): Average Tree Height (m):	

10 Notes :

11 Map :

APPENDIX E

WOODLOT HEALTH ASSESSMENT SUMMARY SHEET

Appendix E

WOODLOT HEALTH ASSESSMENT SUMMARY	Assessor (s): P. Robertson K. Vitols	☐ Date (s) Inventoried: ☐ Date (s) Assessed:

H) SITE INFORMATION

dlot No.:	Compartment #:	Size (ac):	Twp.	Lot(s):	Conc(s).:

I) OWNERSHIP INFORMATION

Landowner Name (s):	Size (ac.)	Property Address: (Lot and Conc.)	Mailing Address:	Home Telephone

J) SITE DESCRIPTION

1) Physiography/Hydrology:

Landform (s):	Soil Type (s):	Soil Texture (s):	Topography:	Aspect:	Drainage:

ELC:	Wetlands: Class:	ANSI: ES ☐ LS ☐	CCS: ☐	ESA: ☐

2) Vegetation Analysis:

i) Trees / Shrubs:

Comp. (10%+):	# of Spp.:	Age (Yr):
Stocking (%):	DBH (cm): Mean: Max:	BA (m²/ha): AGS: UGS: Total:
gen.: Comp. (10%+):	Shrubs: # of Spp.:	

ii) Flora & Fauna (Significant Species):

Species	RR	RP	RN	T	E	Src	Species	RR	RP	RN	T	E	Src	Species	RR	RP	RN	T	E	Src

D. SITE SCORING / RANKING:

1) Quality:

Stocking (%) : RS:	AGS (%) : RS:	Ht. (m): Mean: RS:	Ht. (m): Max: RS:
DBH (cm): Mean: RS:	DBH (cm): Max: RS:	Wildlife Trees (m²/ha): RS:	Mean: RS: Rank:

2) Vegetative Diversity:

# Tree Spp.: # Shrub / Vine Spp.	Total:	RS: Rank:

3) Significant Species:

Significance (Pts): RR (1) RP (2) RN (3) T (5) E (10)	Total:	RS: Rank:

4) Presence of Management/Disturbance:

Total:	RS: Rank:

5) Size of Compartment:

Total:	RS: Rank:

6) Amount of Forest Edge

Total:	Edge/Area m/ha	RS: Rank:

7) Forest Fragmentation:

Total:	RS: Rank:

E. RANKING SUMMARY:

Criterion	1	2	3	4	5	6	7	TOTAL
RS								
Rank								

F. MANAGEMENT HISTORY:

Corn Crop Sampling Protocol
Ecological Risk Assessment
Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

In the summer of 2001 as part of the Ecological Risk Assessment (ERA) - Crops Phytotoxicity Study various crops were grown in field trails on clay soils just east of the Inco refinery in Port Colborne (Jacques Whitford 2001). As part of the ERA–Natural Environment, Chemical of Concern (CoC) values of corn kernels harvested from the field trial crop were to be used to determine dose exposure for risk assessments for deer and raccoons within the study area. In August of 2002, the Public Liaison Committee suggested that "natural" locally grown corn crops be sampled within the study and reference area in Port Colborne. The rational for undertaking this corn collection program was to verify if CoC values in the field trial corn crops were in fact representative of corn crops eaten by deer and raccoons in the study area.

2. OBJECTIVES

The following is a list of objectives that will be fulfilled during the proposed corn sampling detailed in this protocol:

1. To determine the level of CoCs in kernels of corn.
2. Determine corresponding soil CoC values which have been obtained from the base of the source plant.
3. To determine the level of CoCs in basal leaves of the source plant.

3. METHODOLOGY

In late August of 2002 an initial survey was done within the study and reference area. Findings of this survey determined that a total of two fields/plots could be sampled in each of the secondary (small plots) and reference areas (large fields) on clay soils. No corn samples were collected from the primary study area since the only landowner with corn fields in this area would not participate in the study. No cornfields were found growing on organic soils.

To obtain a representative data set the following samples were obtained from cornfields.

Sample Matrix	Secondary Area (Two Plots)	Reference Area (Two Fields)
Corn Cobs	6[1]	6[1]
Soil Samples (Composites)	6[2]	6[2]
Basal Leaves	6[3]	6[3]

Notes:
1- one cob of corn was taken from three different plants in each of two fields
2- one composite soil sample was taken from the base of each source plant as mentioned below
3- basal leaves were taken from each source plant in accordance with (Jacques Whitford 2001)

For sample site locations, please see the sample location maps (Figures 1-1 and 1-2) at the back of this Volume.

3.1 Plant Sampling

Three cobs were sampled from three different plants in each field along a transect that was arbitrarily chosen. All plants sampled were at least 10 meters apart. Only mature cobs were selected. Where size of the field permitted, corn plants were sampled from at least 10 meters from the edge of the crop so as to eliminate the possibility of edge effects. Individual cobs of corn were placed in appropriately labeled plastic bags.

Basal leaves were taken from each plant mentioned above in order to compliment toxicological field studies described in Jacques Whitford 2001. Basal leaves were placed in appropriately labeled plastic bags.

3.2 Soil Sampling

Samples of soil were collected from the 0-5 cm depth around the base of each source plant mentioned above. Each sample consisted of approximately 10 cores distributed uniformly within the rooting zone of each plant. Cores for each sample were taken by a stainless steel Oakfield® soil corer and were combined into an appropriately labeled glass sample jar. All soil samples were submitted for laboratory analysis.

3.3 Sample Preparation and Analysis

Cobs of corn were husked within seven hours of being collected. Cobs were then rinsed with distilled water, and then placed in appropriately labeled paper bags to dry. Basal leaves were rinsed within seven hours of being collected and placed in appropriately labeled paper bags to dry.

Whole cobs of corn and basal leaves were sent to Philip Analytical Services Inc. (PSC) for chemical analysis in person by a member of Jacques Whitford and the Public Liaison Committee's (PLC's) consultant. All vegetation and soil samples were chemically analyzed using arsenic hydride generation and inductively coupled plasma atomic emission spectrometry (ICPMS) following procedures outlined in Jacques Whitford 2001.

4. QUALITY ASSURANCE/QUALITY CONTROL

All testing and collection of samples was carried out in conformance to strict quality control/quality assurance methods, conforming to Jacques Whitford's QA/QC protocols under the company's ISO 9001 registration. Representatives of the PLC's consultant were allowed to monitor all field collection of the samples.

5. CITED REFERENCES

Jacques Whitford Environment Limited. 2001. Port Colborne Ecological Risk Assessment Year 2001 Field Trials on the effects of CoC impacted soils on plant toxicity at the clay 2 field test site. Field trials protocol #1. Prepared for Inco Limited 30 May, 2001.

An Approach to Data Analysis and Interpretation Natural Environment Ecological Risk Assessment Port Colborne Community Based Risk Assessment

Final Draft – November 2002

1. INTRODUCTION

Inco Limited (Inco) has committed itself to the Community of Port Colborne (represented by the Public Liaison Committee, PLC), the City of Port Colborne (The City) and the Ontario Ministry of the Environment (MOE) to conduct a Community Based Risk Assessment (CBRA). The CBRA is being conducted for chemicals of concern (CoCs) in the Port Colborne area that are elevated as a result of historical emissions from Inco's Refinery. The ecological risk assessment (ERA) is one component of the CBRA process. The components of the process as defined in the Technical Scope of Work (TSOW; JWEL 2000) include:

- an evaluation to confirm that all relevant CoCs are considered,
- a quantitative human health risk assessment,
- a quantitative ecological risk assessment, and
- an evaluation of all applicable remediation options.

Due to the size and scope of the ERA study for the CBRA, the ERA has been undertaken under two separate component studies, ERA-Natural Environment, and ERA-Crop Greenhouse and Field Phytotoxicity Studies. This protocol details how data analysis and interpretation will be undertaken for the ERA-Natural Environment. A data interpretation protocol for the crop phytotoxicity studies is detailed under a separate cover.

1.1 Scope of this Ecological Risk Assessment

This ERA focuses on the terrestrial environment in the Port Colborne area, as defined by JWEL (2001a). For the ERA, current impacts to the natural environment resulting from historical emissions of CoCs from the Inco Refinery in Port Colborne will be assessed.

In most cases, site-specific ecological risk assessments are based on accepted and approved models, equations and published values, with little or no field data collection for the identified receptors. However, many detailed field data have been collected from Port Colborne's natural environment for inclusion in this risk assessment to allow the ERA to be relevant to Port Colborne. Additionally, several controlled experiments were performed for the ERA, including earthworm dose-response tests and

greenhouse maple germination/seedling growths experiments, to further detail and assess the effects CoCs are having on the local biota.

Following these data collection and applied research efforts, the interpretation of risk for the ERA follows both traditional hazard and risk calculations and other statistical methods used to analyse the response to CoC concentrations. These various assessment methods are integrated to establish community-specific soil CoC concentrations that are safe for specific receptors on certain soils and in certain habitats.

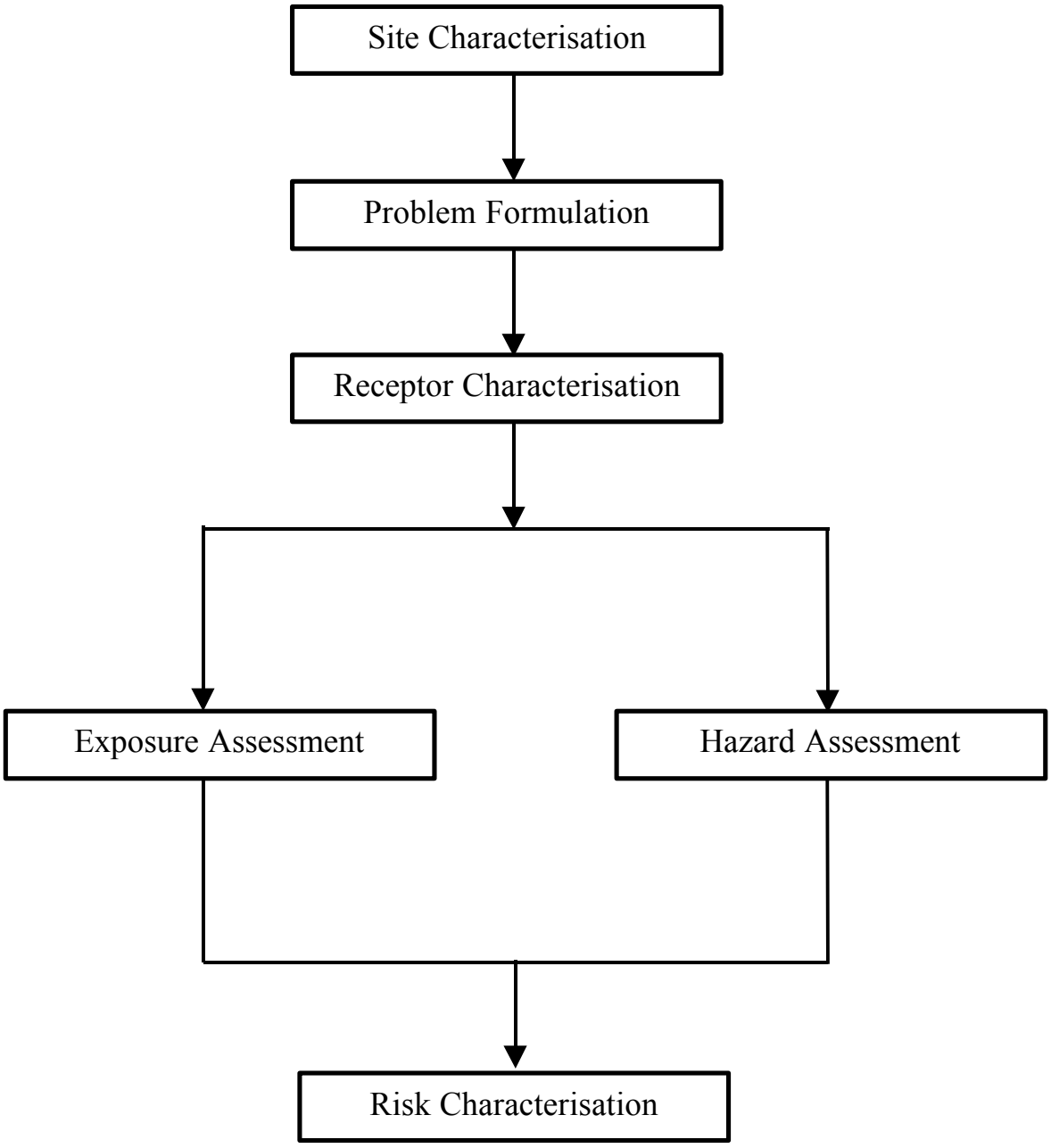
1.2 General Methods of the Ecological Risk Assessment

The ERA is being developed following provincial (MOE 1997) and federal risk assessment guidance documents (CCME 1996, 1997), and includes the following general phases, as presented in Figure 1:

- Site Characterisation;
- Problem Formulation;
- Receptor Characterisation;
- Exposure Assessment;
- Hazard Assessment; and,
- Risk Characterisation.

This risk assessment incorporates site-specific biophysical data and predictive modelling, and is thus considered a detailed quantitative ecological risk assessment. The purpose of this document is to provide details of the methods and approach for interpretation of data collected for undertaking the quantitative assessment for the ERA. In addition to the quantitative assessments, the ERA describes and discusses many qualitative environmental features of the study area, which is touched upon in the following sections.

Figure 1. Framework of Ecological Risk Assessment.



The primary objective of the ERA-Natural Environment is to develop the weight of evidence that emissions of CoCs from the Refinery are having effects and will continue to present undue risk to the natural environment of Port Colborne. The natural environment we define as populations of wild animals and plants in the Port Colborne area. Where there is undue risk, the ERA has the follow-up objective of estimating CoC concentrations that produce "safe" or more acceptable levels of risk to populations of wild animals and plants.

For more detail on the identification of the four CoCs, characteristics of the site, detailed methodology of chemical analyses, field data collection and other aspects of the ERA and CBRA, we refer the reader to the many other JWEL documents listed in the reference list at the end of this document.

2. OBJECTIVES OF THIS PROTOCOL

The objectives of this protocol is to provide descriptions of:

- Data collected as part of the ERA;
- The statistical methods employed as part of the ERA;
- The types of qualitative and quantitative analysis performed as part of the ERA; and,
- How results of the analyses are integrated to arrive at conclusions.

3. SITE-SPECIFIC DATA COLLECTION

To allow for an ERA that is relevant to the specific community, a large amount of biophysical data was collected from within the Port Colborne area (hereafter, "study area"; see JWEL 2001a for a discussion of the study area's boundaries). These data specific to Port Colborne (hereafter, "site-specific") pertain to media in the area (e.g., soil, water, and air) and to biological materials (e.g., animal and plant tissues). Site-specific data collected as part of the ERA also include what we consider ecological variables (e.g., animal abundance, species richness). The following subsections discuss what data were collected and how they may be used as part of the ERA.

3.1 Media

Media examined in the study area include soils, air, water and sediment taken from inland waterbodies (see JWEL 2001q,r and JWEL 2002d). Chemical analyses were performed on these media, as outlined in JWEL (2002a). These data are used to:

- Assess the distribution of CoC concentrations in the study area;
- Examine relationships between tissue CoC concentrations and media CoC concentrations;
- Examine relationships between media CoC concentrations and ecological variables; and,
- Calculate potential exposure of selected receptors to CoCs in the natural environment.

Where several values were obtained for a site through field duplicate samples and laboratory replicate analyses, means are calculated for each relevant site and used in statistical analyses. More details on the sampling of media are provided in the respective data collection protocols and in Tables 1-3.

Table 1. Soil and sediment sampling program used for the ERA-Natural Environment. (Note: for the purpose of exposure assessment only soil CoC values from sampling efforts at the 0-5 cm depth, taken from within the natural environment of the study area, are used for estimating CoC exposure of receptors.)

Sampling Program	Stations Sampled	Soil Depth (cm)	Uptake Pathway	Soil/Sediment Sampling
Leaf Litter Study	21	0-5	- Woodlot Insects - Woodlot earthworms - Maple Leaves - Caterpillars - Wild grapes	- 30 soil cores from each station
Maple Sap Study	17	0-5 5-15	- Maple Sap - Maple Leaves - Woodlot Insects	- 8-10 soil cores from around each tree
Field Insect Sampling	15	0-5	- Field Insects - Meadow Voles - Field earthworms	- 8-10 soil cores from each transect
Bio-monitoring Study	8	0-15	- Goldenrods	- 60-80 soil cores from each station
Frog Collection (sediment)	15	0-5	- Frogs - Tadpoles	- 8-10 sediment cores from each pond
Maple Keys	2	0-5	- Maple Cotyledons	- 8-10 soil cores from around each tree

Table 2. Summary of Surface Water Sampling Scheme for the ERA-Natural Environment. (Note: only values from sampling efforts within the natural environment of the study area are used for estimating CoC exposure of receptors.)

# Stations Sampled	Sampling Period	Collection Methods	Travel Blanks	QA/QC
37	May – September 2001	Surface water was sampled from a central portion in the water column with every effort made not to disturb sediment. The pH and temperature were determined with a Fisher Scientific portable Accumet pH meter (model AP61).	Exposed to ambient air during sampling	20% additional samples (field duplicates) taken by JWEL and PLC's consultant

Table 3. Sampling Scheme for Air Quality Data Collection. (Note: only values for TSP taken from sites sampled in the natural environment within the study area are used for estimating CoC exposure of receptors.)

Study type	Date Sampled	Samplers in Primary/ Secondary Area	Samplers in Reference Area	QA/QC
Farming Activities	October	- 1 PM 10 - 1 PM 2.5 - 1 TSP	0	A PLC's consultant was present to monitor collection methods. Glass and quartz filters (blanks) were exposed to ambient air during field collection of used filters.
Ambient Air Sampling	August-September	- 2 PM 10 - 5 PM 2.5 - 2 TSP	- 1 PM 10 - 1 PM 2.5 - 1 TSP	

PM 10 = Suspended Particulate Matter (SPM) <10 microns, PM2.5 = SPM<2.5, TSP = Total SPM

Following laboratory analysis of various environmental media (i.e., soils, water, and tissue), QA/QC of each data set regarding CoCs concentrations will be undertaken. As a first step, the data will be reviewed to identify any immediate outlying results. As a means of determining the reproducibility or variability related to analytical procedures, the sample homogeneity or sampling, JWEL will calculate the percentage differences between analyzed values for the original and duplicate or replicate samples (JWEL 2001p).

Following QA/QC for each data set, data are summarised into a table in the following format (e.g., CoC concentrations in vole liver tissue):

Table 4. Example table presenting how data will be summarised. Note that the values in the table do not represent real values determined for the Port Colborne CBRA.

Calculation	Primary Study Area				Secondary Study Area				Reference Study Area			
	Ni	Cu	Co	As	Ni	Cu	Co	As	Ni	Cu	Co	As
Mean	200.00	90.97	23.820	11.7	175.90	96.23	32.360	9.4	58.10	72.10	13.940	9.6
SD	100.29	30.34	4.981	5.8	116.21	59.00	24.828	5.0	54.41	66.43	19.560	8.6
N	3				3				2			

For various statistical and risk calculations, where the data set indicates a "nd" (non-detect), half the detection limit for specific parameters (CoCs) analysed are substituted. Although data will be summarised in tables such as Table 4 for the reader's benefit, discussion of how data will be used is found in following sections.

3.2 Biological Materials

Details of how biological materials were collected and analysed are provided in JWEL protocols. These materials include the following tissues:

- Maple (sap, leaves, seeds; JWEL 2001l,m,o);
- Wild grape (fruit; JWEL 2002c);
- Corn (seeds; JWEL in prep.);
- Soy* (seeds, whole plant; JWEL in prep.);
- Oats* (seeds, whole plant; JWEL in prep.);
- Goldenrod* (whole plant; JWEL in prep.);
- Earthworms (whole animal with and without stomach contents; JWEL 2001g, JWEL 2002b);
- Terrestrial arthropods (whole animal; JWEL 2001j);
- Frogs (adult: gastrointestinal {GI} tract, liver, carcass; tadpole: GI tract, carcass; JWEL 2001i); and,
- Meadow Vole (livers, carcass; JWEL 2001n).

(*Data was provided by the ERA-Crop Studies)

These data will be used in examinations of the relationships between tissue CoC concentrations and media CoC concentrations, and to calculate potential exposure of selected receptors to CoCs in the natural environment. Further details regarding the sampling and chemical analysis of media is provided in Table 5.

3.3 Measured Ecological Variables

Details of how ecological variables were collected are provided in JWEL protocols listed in Section 8. These variables were obtained through field surveys (e.g., earthworm abundance and species richness in the natural environment) and through experimentation in controlled settings (e.g., earthworm toxicology laboratory studies). They include:

- Frog call survey (relative abundance and species richness in the natural environment; JWEL 2001e);
- Earthworm relative abundance, age class, mass and species richness in natural environment (2001g);
- Leaf litter dry weight in the natural environment (JWEL 2001k);
- Maple leaf health in the natural environment (JWEL 2001o);
- Earthworm survivorship, reproduction and mass in a controlled environment (JWEL 2001g); and,
- Maple germination and seedling health in a controlled environment (JWEL 2001m).

These measured ecological variables will be used in statistical analyses to examine the observed response of Port Colborne's flora and fauna to CoCs present or potentially present in the natural environment. Further details on data collection are provided in the respective protocols and in Table 5.

Further ecological and biophysical data were collected for the Port Colborne area to qualitatively assess the natural environment and the potential impact of CoCs on the fauna and flora. These qualitative data include:

- Breeding birds;
- Mammal species;
- Reptile and amphibian species;
- Tree and shrub species;
- Ecological Land Classification of natural vegetation areas;
- Woodlots;
- Soil types (clay-organic); and,
- Significant natural areas.

For species data, qualitative analysis is undertaken by comparing species occurrence in the study area to existing databases and reference documents that detail what species should/could be expected to occur.

Table 5. JWEL biological data sampling framework.

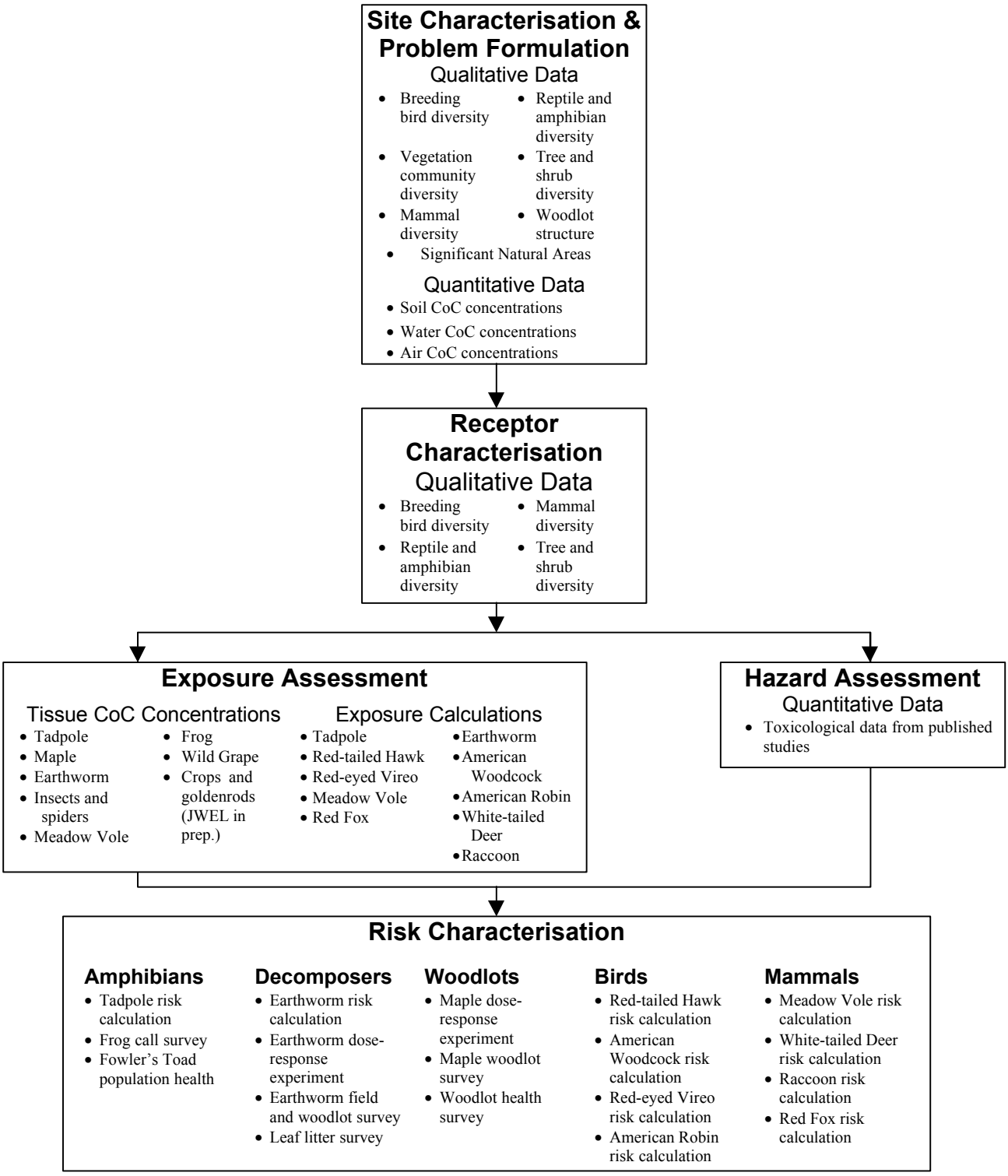
Receptor	Sample Period	Reference		Primary		Secondary	
		# Stations	# Replicates/ Station	# Stations	# Replicates/ Station	# Stations	# Replicates/ Station
Frog Call Survey	May-June 2001	8	4[1]	9	4[1]	12	4[1]
Adult Frogs GI-liver-Carcass	June-August 2001	5	5[2]	5	5[2]	5	5[2]
Tadpoles GI-Carcass	June 2001	2	1	3	1	3	1
Earthworms Whole worm	September 2001	5	3	5	3	5	3
Earthworms Whole worm	June 2002	10	1	13	1	7	1
Earthworms Purged	June 2002	4	1	5	1	2	1
Wild Grapes	September 2001	3	1	3	1	4	1
Soft Maple							
Leaves	August 2001	5	3	5	3	5	3
Sap	March 2001	1	1	3	1	3	1
Leaf Litter	July-October 2001	9	15	7	15	5	15
Meadow Vole	September 2001	3	4	2	5	1	1
Arthropods	July-August 2001	10	1	10	1	10	1
Caterpillar	August 2001	1	1	1	5	0	0
Goldenrods	August 2001	6	4	6	4	6	4

[1] each station was visited four times during the sample period

[2] number of specimens retained from each station

These many types and sets of data will help to develop a clear picture of the effects of CoCs on the natural environment. Data will be used during certain steps of the ERA (Figure 2), with statistical analyses performed to test the significance of patterns or trends in biological data as they relate to CoC concentrations in the environment. These statistical analyses are introduced in Section 4, but Figure 2 shows where collected data will be used in the ERA process.

Figure 2. Diagram showing how data and analysis will be incorporated into steps of the ERA.



4. STATISTICAL METHODS

Statistical analysis is a means of analyzing data and searching for, and quantifying, patterns that one would otherwise miss. For certain components of this ERA (Exposure Assessment and Risk Characterization), statistical analysis is a valuable tool in assessing the relationship between biological responses (tissue CoC concentrations and ecological responses, such as worm abundance) and CoC concentrations in the surrounding media. Where these statistical methods are applied is outlined below, but here we briefly discuss the main method we use for evaluating data patterns, the Generalized Linear Model. Other methods for assessing risk, including qualitative examination of Port Colborne's Natural Environment and estimations of CoC exposure for certain receptors are discussed in Sections 5 and 6.

The Generalized Linear Model (*glm*) is a generalization of the linear regression model such that non-linear and linear relationships with the biological response are tested. In its simplest form, a linear model specifies the linear relationship between a dependent (response) variable Y and a set of predictor variables Xs

$$Y = (b_0 + b_1X_1 + b_2X_2 + \ldots + b_iX_i) + e \qquad (1)$$

where, b_0 is the intercept;
b_i are the regression coefficients for the respective predictor variables (X_i); and,
e is the error term.

One can use *glm* to model both linear and non-linear relationships between a response and predictors by specifying the *link function* (McCullagh and Neldar 1989).

Equation 1 can be altered slightly to create Equation 2 illustrating the *glm*:

$$Y = g(b_0 + b_1X_1 + b_2X_2 + \ldots + b_iX_i) + e \qquad (2)$$

where, g is the link function (and other terms are the same as in Eq. 1).

The power of glm is that it can incorporate both continuous (e.g., mass, CoC concentration) and categorical predictor variables (e.g., soil type, habitat type), and allows the dependent variable to have a non-normal distribution (e.g., gamma, Poisson, binomial, etc.). For further detail on the use and applications of *glm*, refer to McCullagh and Neldar (1989).

Generalized linear models estimate the coefficients for the predictor effects and predictor interactions on the response variable. The likelihood that each coefficient could be obtained in the absence of a true predictor effect is also provided in the model's output and used to evaluate whether it is likely the predictor effect actually exists. These probability estimates will be used along with professional judgement to determine the significance of observed models. Generally, probabilities ≅ 5% or less are used to trigger a check to ensure model assumptions are met and conclusions are valid. The statistical software package S-Plus will be used to perform these analyses, and reference is made to both McCullagh and Neldar (1989) and MathSoft (1998) for appropriate procedures.

For the Exposure Assessment, response variables will be tissue CoC concentrations fit against habitat (woodlot/field), soil type (clay/organic) and the relevant media CoC concentrations (e.g., nickel for nickel). For example, earthworm tissue copper concentrations will be fit against habitat, soil type and soil copper concentrations. Also, first-order interactions between the predictors will be fit against each response to determine, for example, if the relationship between tissue and soil nickel concentrations are influenced by soil type. For any of the responses, it may be that CoC uptake differs between soil types. For the Risk Characterisation, ecological responses, such as earthworm abundance, will be fit against relevant predictors (e.g., habitat, soil type, distance from presumed source, soil CoC concentrations) and their first-order interactions. For each response, four models will be ran, each identical except for the media CoC concentration fit as a predictor. For example, earthworm abundance will be fit against modifying variables (e.g., soil type, habitat) and soil nickel concentration, soil copper concentration, soil arsenic concentration and soil cobalt concentration separately.

Mathematical transformations (e.g., log transformations) of the data may be employed in some circumstances to improve the fit of a model or to fulfil statistical assumptions. The type of *glm* used will depend on the distribution of the response, and may be a Gaussian model, a binomial model or a Poisson model. Quasi-likelihood models may be used to compensate for under- and over-dispersed data (McCullagh and Neldar 1989). Quasi-likelihood estimation allows one to estimate regression relationships without fully knowing the error distribution of the response variable (McCullagh and Neldar 1989). The likelihood need not be specified and fewer assumptions are made in estimation and inference. However, there is a great deal of flexibility in fitting *glm*, and JWEL will take an adaptive approach in fitting models. All methodology associated with the fitting of *glm* will be fully explained in the final report.

5. DATA ANALYSIS AND EVALUATION

For this ERA, many components are evaluated to examine the potential risk of CoCs to the natural environment of Port Colborne. Five main groups of data are analysed as part of the ERA, answering the following questions:

- How does the study area (Primary and Secondary areas - JWEL 2001a) qualitatively compare to other areas?

- What relationship exists between CoC concentrations in certain animal and plant tissue and the surrounding media?
- What relationship exists between CoC soil concentrations and observed ecological responses in a natural setting?
- What relationship exists between soil CoC concentrations and ecological responses in a controlled setting?
- Based on site-specific data and values in published literature, what is the potential exposure of certain receptors to CoCs, and do the CoC concentrations present in the study area place the receptors at potential risk?

Any gaps in the data or limitations in either the available data or analyses will be identified and fully discussed in the final report, as will the adequacy of the data for drawing conclusions. Any comparisons with data collected from reference areas will be discussed in the final report, including the spatial and temporal boundaries of reference areas.

5.1 Study Area Comparison

This is an important step in assessing the potential risk of CoCs to the natural environment of an area. For this ERA, several comparisons will be performed. They include comparisons of the species richness and incidence of rare species for breeding birds, mammals, reptiles and amphibians and tree and shrubs. The study area is coarsely evaluated based on these comparisons to determine how the species richness compares to other areas with comparable diversity of natural habitat in the same general area, and if certain species should be present in the study area but are not (and the contrary). Species richness comparisons will use results of JWEL fieldwork alone, compared to what other efforts have recorded in the area and elsewhere in the region. For the purpose of this comparison for birds, the Ontario Breeding Bird Atlas will be used to compare breeding bird richness at the following mapsquares: 17NH95, 17PH27, 17PH35, 17PH45 and 17PH65. Methods of comparison will be fully described in the final report.

Comparisons will be made with respect to forest health. Woodlots within the study area were surveyed by a professional forester, and the surveys consisted of both quantitative measurements and subjective appraisal of other features. The result will be a ranking of woodlots that will be used to compare overall health of the woodlots in the study area with those of the reference area. Non-parametric tests, such as the Wilcoxon Rank-Sum Test or the Kruskal Wallis Test, may be used to evaluate differences in forest health ranking between the Study Area and reference areas.

5.2 Relationships between Tissue and Media CoC Concentrations

An examination of the relationship between tissue CoC concentrations and those of the relevant surrounding media is valuable in understanding if CoCs are moving through the biological components of the environment. Statistical analyses, using *glm*, will be undertaken to look at such relationships in earthworms, adult frogs, tadpoles, Meadow Voles, maple leaves and arthropods (insects and spiders). As discussed in Section 4, these *glm*s will be structured with the CoC concentration of the tissue set as the response and CoC concentration of the relevant medium as a predictor (e.g., water and sediment for tadpoles, soil for earthworms). Soil type and habitat type, if appropriate, will be included as modifiers. Identified limitations of the analyses and specific methodologies will be detailed in the final report.

5.3 Relationships between Ecological Responses and Media CoC Concentrations

Measured ecological responses of earthworms, frogs, maples and leaf litter will be examined in relation to soil CoC concentrations measured or predicted in the study area. As discussed in Section 4, this will be done using *glm*, with the measured ecological response fit against measured soil CoC concentrations (for earthworms, maples and leaf litter), or predicted categorical soil nickel concentrations based on the distribution of this CoC in the study area (for calling adult frogs). Other variables will be set as modifiers, such as soil type, habitat type, and distance from the presumed source. The responses included in these statistical analyses are presented in Table 6. For responses sampled during different years (i.e., earthworms in 2001 and 2002), analyses will be done for each year separately. Identified limitations of the analyses and specific methodologies will be detailed in the final report.

Table 6. Ecological responses statistically analysed to examine the relationships between the responses and soil CoC concentrations.

Frogs	**Earthworms**	**Maples**
➢ American Toad relative abundance ➢ Spring Peeper relative abundance ➢ Western Chorus Frog relative abundance ➢ Northern Leopard Frog incidence (presence/absence) ➢ Species richness	➢ Species richness ➢ Total biomass ➢ Total frequency ➢ *Aporrectodea tuberculata* frequency and biomass ➢ *Lumbricus rubellus* frequency and biomass ➢ *Lumbricus terrestris* incidence (presence/absence)	➢ Frequency of leaf relative health classes **Leaf Litter** ➢ Dry weight of leaf litter ➢ Dry weight of fruit litter

5.4 Dose-Response Experiments

Two dose-response experiments were performed to examine the response of earthworms and maple seeds to a gradient of CoC concentrations. The structures of these experiments are discussed in detail in JWEL (2001g,m).

For the earthworm experiment, regression analyses and Analysis of Variance (ANOVA, a statistical test within the *glm* group) will be employed to examine at what CoC concentration an effect is present, using number of juveniles, biomass of juveniles, adult survivorship and numbers of hatched and unhatched cocoons as responses. The results of 14-d earthworm acute mortality tests are normally entered manually into data spreadsheets, graphed, and analyzed using the Probit, the Moving Average, and the Trimmed Spearman-Karber methods of analysis (Stephen 1989). The results of the chronic earthworm tests (both with the clay and organic-contaminated soils) will be analyzed by applying linear or non-linear regression procedures to the earthworm reproductive data after the data are entered into electronic spreadsheets. The analyses use a linear or four nonlinear regression models (i.e., logistic, gompertz, exponential and logistic with hormesis) that re-parameterizes to include the EC_{20} and the associated 95% confidence limits. The residuals will be examined for homogeneity of variance among treatments. When data show heteroscedasticity among treatments, data will be weighted with the inverse of the variance of each treatment (Myers 1986; Stephenson *et al*. 2000). ANOVA procedures will be applied to the data and a two-tailed Dunnett's test will be used to compare each treatment mean to the mean of the control treatment. The Dunnett's pairwise comparison test will be used to determine the NOEC (no observable effect concentration) and LOEC (lowest observable effect concentration) values (SPSS 1997). Thirty-five-day adult survival data will be analyzed using ANOVA procedures followed by a two-tailed Dunnett's and a Fisher's protected Least Significant Difference pairwise comparison test. All analyses will be performed by an independent consultant (ESG International) and checked by JWEL. ESG performs analyses using SYSTAT 7.0.1 (SPSS 1997). A more detailed description of the statistical procedures used to analyze earthworm test data can be found in either Environment Canada (1998) or Stephenson *et al*. (2000).

The maple experiment had fewer treatments so critical dose will not be determined. However, *glm* will be used to determine the significance of a relationship between the response and the CoC concentrations. The following measured variables are set as responses in separate *glm*s: germination success (count out of five), seedling height, number of leaves and frequency of relative leaf health. Soil type will be set as a modifier and soil CoC concentrations are predictors.

5.5 Calculated Potential Exposure and Risk

Calculations of potential average daily dose and comparisons with toxicity reference values to assess risk will be done for ten receptors. These receptors include an amphibian (frog tadpoles), an invertebrate (earthworms), four birds (Red-tailed Hawk *Buteo jamaicensis*, American Woodcock *Scolopax minor*, Red-eyed Vireo *Vireo olivaceus*, and American Robin *Turdus migratorius*), and four mammals

(Meadow Vole *Microtus pennsylvanicus*, White-tailed Deer *Odocoileus virginianus*, Red Fox *Vulpes vulpes*, and Raccoon *Procyon lotor*).

5.5.1 General Exposure Calculations

Other than tadpoles and earthworms, exposure to CoCs will be calculated following CCME (1997). For the assessment for a receptor, the exposure to each of the four CoCs will be calculated independently. The potential average daily dose (ADD_{pot}) will be generally considered equivalent to the combined doses from consumption of water, soil, through diet, and inhalation, so that:

$$ADD_{pot} = ADD_{water} + ADD_{diet} + ADD_{soil} + ADD_{air} \qquad (3)$$

Dermal exposure to burrowing mammals (i.e., voles) will not be evaluated in the assessment due to the lack of accepted models available for dermal uptake in wildlife. Further, the fur on burrowing mammals precludes direct contact of soil to skin, and the major exposure pathway is through ingestion of soils during grooming.

The average daily dose from consuming water will be calculated using the following:

$$ADD_{water} = (C \bullet FR) \bullet NIR \qquad (4)$$

where,

ADD_{water}	=	Potential average daily dose from water (mg/kg-d);
C	=	CoC concentration in the i^{th} water source (mg/kg);
FR	=	Fraction of total water ingested from the contaminated water source (unitless); and
NIR	=	Normalized water ingestion rate (fraction of body weight consumed as water per unit time, g/g d).

Note that this equation differs from CCME (1997), as it is missing the summation sign. Since we are assuming 100% exposure to contaminated sites geographically (but see Section 5.5.3), the summation is not necessary. This is true for Equations 6 and 7 also.

The average daily dose from the diet will be calculated using the following:

$$ADD_{diet} = \sum (C_k \bullet FR \bullet DF_k \bullet NIR) \qquad (5)$$

where, ADD_{diet} = Potential average daily dose (mg/kg-d);
C_k = Average CoC concentration in the k^{th} type of food (mg/kg);
FR = Fraction of total diet ingested from the contaminated food source (unitless);
DF_k = Fraction of total diet accounted for by the k^{th} food group (unitless); and
NIR = Normalised ingestion rate of food on a weight-to-weight basis (g/g d).

The average daily dose from consumption of soil will be calculated using the following:

$$ADD_{soil} = \frac{(C \bullet FS \bullet IR \bullet FR)}{BW} \qquad (6)$$

where, ADD_{soil} = Potential average daily dose (mg/kg-d);
C = CoC concentration in soil in the contaminated foraging area (mg/kg dw);
FS = Fraction of soil in diet (g/g dw);
IR = Food ingestion rate on a dry weight basis (kg/d);
FR = Fraction of total soil intake from the contaminated foraging area (unitless); and
BW = Average body weight (kg) of an adult of the species.

The average daily dose from inhalation will be calculated using the following:

$$ADD_{air} = \frac{(C \bullet FR) \bullet IR}{BW} \qquad (7)$$

where, ADD_{air} = Potential average daily dose (mg/kg-d);
C = CoC concentration in air within the study area (mg/m^3);
FR = Fraction of inhaled air within contaminated area (unitless);
IR = Inhalation rate (m^3/d); and
BW = Average body weight (kg) of an adult of the species.

For the most part, there are site-specific field data on concentrations of CoCs in diet items of all receptors in the food chain models except Red-tailed Hawk and Red Fox. As part of their diets, hawks and foxes are expected to consume primarily Meadow Voles, as well as other small mammals and birds. For the exposure assessment, field data for CoCs in voles is used to represent small mammals. No field data were collected for American Robins, American Woodcock and Red-Eyed Vireo. Therefore, it is necessary to estimate tissue concentrations of American Woodcocks, Red-Eyed Vireo and American Robins. The CoCs in birds will be estimated by multiplying the ADDs for water, diet, soil and air by appropriate transfer factors (USEPA 1999):

$$C_{bird} = (ADD_{water} \bullet TF_{water-bird}) + (ADD_{soil} \bullet TF_{soil-bird}) + (ADD_{prey} \bullet TF_{prey-bird}) + (ADD_{veg} \bullet TF_{veg}) + (ADD_{air} \bullet TF_{air}) \quad (8)$$

where, C_{bird} = CoC concentration in the bird's tissue;
TF = Transfer factor (see below);
ADD_{water} = Average daily dose to the bird accounted for by water consumption;
ADD_{soil} = Average daily dose to the bird accounted for by soil ingestion;
ADD_{prey} = Average daily dose to the bird accounted for by animal prey items;
ADD_{veg} = Average daily dose to the bird accounted for by terrestrial vegetation; and
ADD_{air} = Average daily dose to the bird accounted for by inhalation.

For fowl in particular, air-to-flesh transfer factors are not readily available for the majority of inorganic chemicals. In the absence of suitable air-to-flesh transfer factors for bird or mammal species, ingestion transfer factors are used as a conservative approximation. This assumes that all of the CoCs in all air inhaled eventually enter the digestive tract and are available for absorption there as part of the whole body dose. This is consistent with assumptions made in the human health risk assessment. The TFs that are used in these calculations are presented in Table 7.

Table 7. Transfer factors used to estimate body CoC concentrations in American Woodcock, American Robin and Red-eyed Vireo for the four CoCs.

CoC	TF (d/kg)	Source
Nickel	0.001	for poultry; Napier 1988
Copper	0.5	for poultry; IAEA 1994
Cobalt	2	for poultry; IAEA 1994
Arsenic	0.83	for poultry; Napier 1988

5.5.2 Parameter Estimates

Necessary for the dose equations detailed above are specific parameter values, which include such factors as exposure duration and ingestion rate. Discussion of the parameter estimates to be used for this study is detailed below.

For most receptors for which risk is calculated (7 out of 10), exposure to the affected area is assumed to be 100%, due to published home range size and residency. The three species where exposure to the affected area is assumed to be less than 100% are migratory birds, namely American Robin, American Woodcock and Red-eyed Vireo. Although their breeding territories are much smaller than the size of the affected area, they do not occupy their breeding ranges for a full twelve months of the year, instead spending several months during the winter in areas south of Ontario. Their exposure is adjusted according to the proportion of the year they are assumed to occupy their breeding range in southern

Ontario. Although Red-tailed Hawks do migrate, a population persists in southern Ontario throughout the winter and we take the conservative approach in estimating risk to resident Red-tailed Hawks.

Home range sizes are taken from US EPA (1993) or other applicable sources for Canadian (particularly southern Ontario) populations (e.g., Environment Canada 1989a,b, 1993) and compared with the size of the affected area. Movement behaviour and residency is based on published accounts relevant to southern Ontario populations (e.g., Banfield 1977, Preston and Beane 1993, Keppie and Whiting 1994, Kroodsma and Verner 1997, Sallabanks and James 1999).

Other parameters include body weight, ingestion rates of air, water and food, and diet composition. Where possible, empirical values will be used for the individual species, following US EPA (1993) and studies cited therein. In the absence of empirical values, estimates will be calculated using equations presented by US EPA (1993), Sample *et al.* (1996) and Sample *et al.* (1997). To estimate diet composition, relevant available literature will be used as guidance. In certain cases where CoC concentrations of food items are unknown and can not be estimated (such as crayfish as part of a Raccoon's diet), then either a suitable food item where the CoC concentration is known will be used as a surrogate, or the food item will be excluded and the percent composition recalculated. Specific references and explanations of parameter values derivation will be presented in the ERA report.

5.5.3 Employed CoC Concentrations

Site-specific CoC concentrations will be used to calculate the exposure a receptor receives from food items and surrounding media when occupying the affected area. As a conservative approach, an Upper Confidence Limit for the Mean (UCLM), which is the confidence limit above the mean set with an $\alpha = 0.05$ for this study, will be calculated for each set of data. For the dose equations, UCLMs will be used to represent the CoC concentrations for the relevant media. Review of our data indicate calculated UCLMs are conservative estimates of CoC concentrations in the Study Area (i.e., close to the highest values found in the Study Area). If there is a strong reason to depart from this practice of using UCLMs, based on a review of the data, rationale and methods of the departure will be detailed in the report.

Only data from non-urban sample sites within the primary and secondary study areas, and sites within ~ 2 km to the east of the secondary study area will be pooled to calculate the UCLM for each data set. Since we are dealing with the impact of receptors found in non-urban areas, we excluded data values from samples taken in built-up areas west of Reuter Road. Conversely, based on a preliminary review of JWEL soil chemical analyses, the distribution of soils with Nickel concentrations greater than 200 mg/kg may extend to the east of the secondary study area; inclusion of sample values within 2 km to the east of the secondary study area would allow for inclusion of the extent of contamination.

To aid in interpreting risk in different components of the landscape, and to focus any future remediation efforts, exposure assessment and risk characterisation will be undertaken for five scenarios: overall study area, woodlots on organic soils, woodlots on clay soils, fields on organic soils and fields on clay soils. Soil and food chemical data representing each of these four scenarios will be pooled into subsets, and a UCLM for soil and each food item (e.g., earthworms) will be calculated for each scenario, where

possible. CoC values for food items not found in all scenarios (e.g., corn, meadow voles) will be calculated from data pooled across the Study Area only. Along a similar vein, three receptors will not undertake all five scenarios: only an overall exposure will be calculated for tadpoles, since exposure is assumed to be equal to water CoC concentrations, and is calculated for the overall Study Area; only an overall exposure will be calculated for White-tailed Deer, since these large animals have home ranges that extend across all habitats over the whole Study Area and have a diet consisting of food items found in fields (e.g., corn, oats) and food found in woodlots (e.g., maple leaves); and exposure for Meadow Voles will be calculated for field habitat only, both on clay and organic soils, since this species is restricted to field habitat. Table 8 lists what scenarios will be performed for each receptor.

Table 8. Scenarios undertaken in calculating CoC exposure for each receptor.

Receptor	Overall	Woodlot - Clay	Woodlot - Organic	Field - Clay	Field - Organic
Tadpole	x				
Earthworm	x	x	x	x	x
Meadow Vole				x	x
White-tailed Deer	x				
Red Fox	x	x	x	x	x
Raccoon	x	x	x	x	x
American Woodcock	x	x	x	x	x
American Robin	x	x	x	x	x
Red-eyed Vireo	x	x	x	x	x
Red-tailed Hawk	x	x	x	x	x

Regard will be given to the results of the *in vivo* bioavailability study (JWEL 2002e), and will be used to revise potential exposure of mammal receptors through soil.

5.5.4 Toxicity Reference Values (TRVs)

The objective of the hazard assessment phase of the project is to describe the relationship between the CoCs and receptor assessment endpoints (CCME 1997). Ultimately, hazard assessment determines a safe level of CoCs that has been demonstrated to have no significant biological effects. The “safe” level is referred to as the Toxicity Reference Value (TRV) for the purposes of the risk characterisation phase. Literature reviews have been conducted to establish safe levels for each of the four CoCs (Ni, Cu, Co, As). The ERA report will provide a detailed account of the selection of TRVs for the assessment.

TRVs for each CoC separately will be used in this assessment. Combined effects or interactions of CoCs with respect to toxicity will be addressed only where field data and toxicity testing have been collected. In the field, observations are based on the presence of all CoCs and thus address combined affects. Similarly in greenhouse testing for maple growth and toxicity testing of earthworms used actual Port

Colborne soils containing all of the CoCs and thus account for potential combined affects. In these cases, we may not well understand which CoC or what combination of CoCs drives potential toxic affects.

Since the way in which combined affects of the CoCs, if any, may impact on toxicity is not well understood, a detailed numerical analysis of additive, synergistic or other interactions is not feasible (CCME 1997). Available literature on such potential interactions will be reviewed and this will be discussed as a source of uncertainty.

5.5.5 Assessment Endpoints

Literature reviews for each of the four CoCs have been conducted to establish TRVs that are protective of ecological receptors. Assessment endpoints may be based on individual or population responses. Individual responses may include survival, growth, reproduction, behaviour or histopathology (e.g., lesions, tumors, etc.). Population responses may include changes in size of a population through a combination of birth, death, emigration and immigration. Individual responses may also change the overall population characteristics.

The selection of appropriate endpoints is guided by the protection goals for different land use categories. Agricultural, residential and park land use categories require a sustainable level of ecological functioning in order to sustain activities associated with those land uses. The activities associated with industrial/commercial land uses do not require a sustainable level of ecological functioning (CCME 1998).

CCME and US EPA recommend the selection of ecological endpoints based on the goals and level of protection of the environment sought. In the current assessment, a sustainable level of ecological functioning is selected as the most appropriate level of protection and thus the assessment goal.

Based on this, the lowest observable adverse effects level (LOAEL) is selected as the assessment endpoint for individuals for mammals and birds. Since severe affects such as increased mortality are not expected at the LOAEL, this assessment endpoint will achieve the assessment goal. For rapidly reproducing species such as amphibians and invertebrates the US EPA (1997) report that at a 40% mortality effect level, a population is likely to be unable to sustain itself. In order for a species to survive, up to a 20% population effects level of a less severe nature (i.e., EC_{20}) was determined to be an appropriate assessment endpoint to maintain the assessment goal of a sustainable level of ecological functioning.

The 20% effects level has been applied in numerous assessments and criteria for quickly reproducing species such as plants, microbes, earthworms, and fish (e.g., US chronic National Ambient Water Quality Criteria (NAWQC), as cited in Suter and Tsao 1996, Cameco Corp. 1994, SENES 2001). Because effects to less than 20% of the population are difficult to measure, the 20% effects level has been referenced as a No Observable Effects Concentration (NOEC) in plants, soil and litter invertebrates

and heterotrophic processes (Efroymson *et al.* 1997a,b). For slower reproducing species with less dense populations such as larger mammals a 20% decrease in population may not be acceptable. For these types of populations, a Lowest Observed Adverse Effects Level (LOAEL) is considered a more appropriate endpoint. Where suitable LOAELs were not available, NOAELs were selected. The selected LOAEL based TRVs represent threshold levels of adverse effects based on the methodology of selection outlined by Sample *et al.* (1996). The rationale and selection for each endpoint chosen from the literature is detailed in the sections that follow.

For significant effects such as mortality of 50% of the test population, these are converted to TRVs by approximating the concentration that would affect 20% of the population assuming a linear dose-response relationship. The relationship (see Efroymson *et al.* 1997a,b) for estimating the TRV from a 50% mortality concentration (i.e., LC_{50}) is as follows:

$$TRV = LC_{50} \times \frac{2}{5} \qquad (9)$$

where LC_{50} = Lowest concentration at which 50% mortality is observed.
TRV = Toxicity Reference Value or EC_{20} (concentration at which less severe effect to 20% of the population is estimated)

For mammals, the methods of Sample *et. al.* (1996) is used to estimate LOAELs as follows:

$$LOAEL_w = LOAEL_t \left(\frac{BW_t}{BW_w} \right)^{1/4} \qquad (10)$$

where, $LOAEL_w$ = lowest observable adverse effects level for wildlife species
LOAELt = lowest observable adverse effects level for test species
BWw = body weight for wildlife species
BWt = body weight for test species

A similar adjustment for body weight was not made for birds. Because of metabolic differences, similarity of species was considered in selecting test species. TRVs are based on survival and reproduction endpoints. TRVs are also generally based on either Lowest Observed Adverse Effects Levels (LOAELs), or are concentrations that may affect 20% of the population (e.g., 20% mortality). There are no data demonstrating the toxicity of metals to adult amphibians through ingestion or contact. Potential effects on adult frogs are, therefore, not assessed. Synergistic effects reported in the literature are discussed with regard to their relevance to the Port Colborne area.

5.5.6 Risk Characterisation

For each receptor and assessment endpoint using numerical methods, the Quotient Method (USEPA 1987) is used to determine the likelihood that ecological effects to animal and plant populations may or have occurred, using the following equation:

$$Risk = \sum \frac{EEC}{TRV} \qquad (11)$$

where EEC is the expected environmental concentration; and,
TRV is the toxicity reference value.

Based on a review of the literature, no clear additive, less than additive or greater than additive effects between the four CoCs has been identified. Therefore for the ERA the risk characterisation for each receptor is undertaken separately for each of the four CoCs.

Where significant ecological risk are identified, it is necessary to determine safe concentrations of CoCs in soils that do not pose risk. For the quantitative risk calculations, exposure, or dose parameters are modified to determine CoC concentrations at which there are acceptable risks to receptors. It is anticipated at this time that the exposure due to the ingestion of soil will be the primary determinate for most receptors, although differences in uptake of prey in different habitats or soil types, as determined by the statistical analyses discussed in Section 5.2, will have to be incorporated into the back-calculation.

Appendix A provides a purely hypothetical example of how exposure and risk will be calculated for one of the ERA's receptors, the Red-eyed Vireo.

6. INTEGRATION

In total, there are 16 receptors for which specific risk assessments will be conducted through either risk calculations, toxicity tests, and statistical comparative assessment. In addition, during field investigations, qualitative assessments of the study area's fauna and flora are also undertaken. It is anticipated that there will be variation in sensitivity of receptors to CoCs in the vicinity of the Inco refinery in Port Colborne. Conclusions about safe environmental CoC concentrations is based on a weight-of-evidence approach that synthesizes risks associated with various CoCs concentration scenarios (field vs. woodlot; organic vs. clay soil) for each receptor and assessment endpoint.

For the determination of "safe soil values" for each of the CoCs, results of the quantitative risk characterisation are considered the most conservative test for the ERA. Should no risk be identified for a receptor in any part of the study area (field/woodlot habitat, clay/organic soil), then the highest recorded soil values could be considered as safe for that receptor. If a specific receptor is calculated to be at risk at some CoC soil value, then this result is reviewed against field observations and, if applicable, laboratory experimental results (see Figure 3 for a schematic example). For the purpose of the ERA, for a specific receptor, field data and/or laboratory results are given equal or greater weight in determining a safe soil value over that determined through back-calculations of the potential risk. In addition, although some receptor assessment endpoints can be anticipated to be more sensitive than others, greater consideration *may* be given to more holistic endpoints. Figure 4 provides a an example on how the integration of various data sets would be used, if required, to determine safe environmental soil value for woodlot based on the health of a ecological community.

In some cases, the potential exists for the quantitative risk assessment to be less conservative than the field observations or toxicity testing using Port Colborne soils since these latter two cases account for the potential of interactions with the CoCs to alter toxicity. Generally, field data will be considered the most reliable and toxicity testing with actual Port Colborne soils will be considered more reliable than literature based calculations. Because of difficulties in interpreting field data and toxicity data where the interactions and impacts of the specific CoCs are not well understood, review of all of the available data together may be required in order to develop a better understanding of potential risks.

Where field or laboratory data are not fully used to directly question the conservative calculated risk value (due to uncertainties or limitation of the field/laboratory data), then the calculated value may be used, or an adjusted conservative value determined (see Figure 5). In all cases, the integration steps are discussed in detail in the ERA.

Figure 3

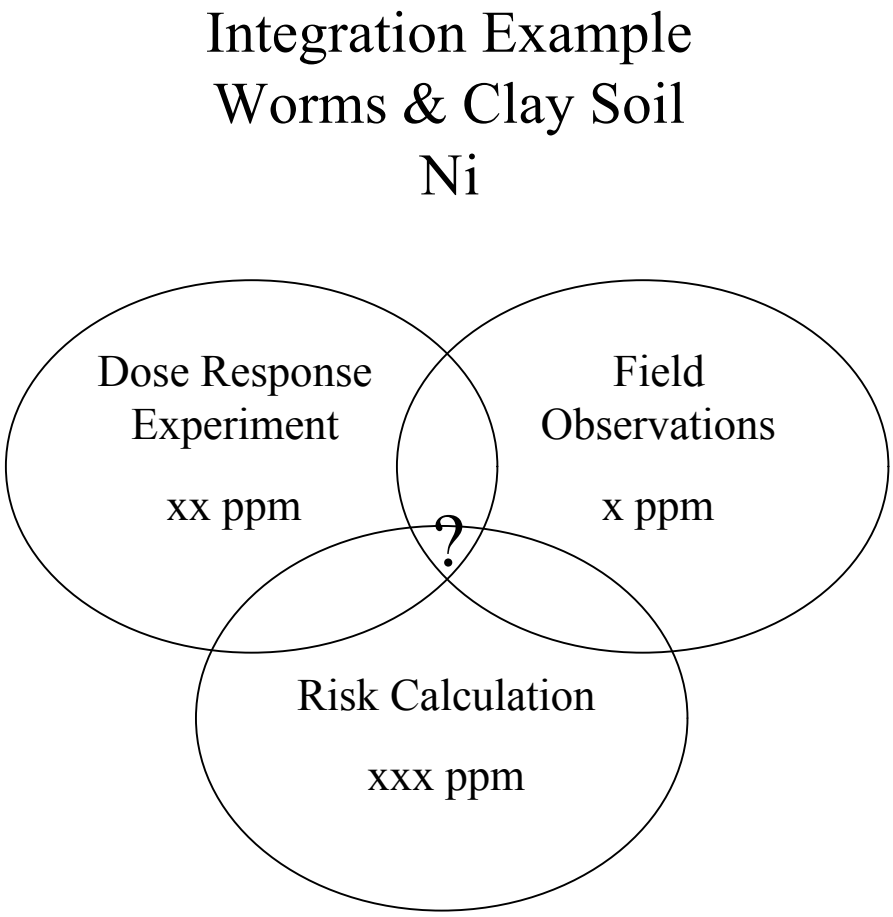


Figure 4

Woodlots
Ni

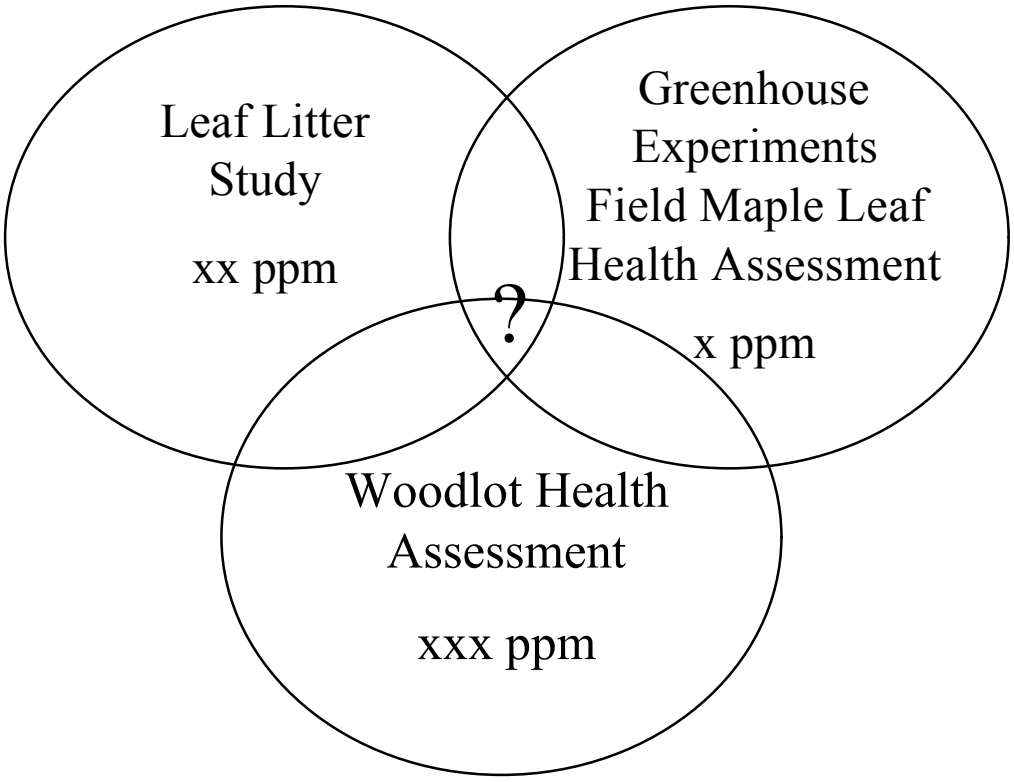
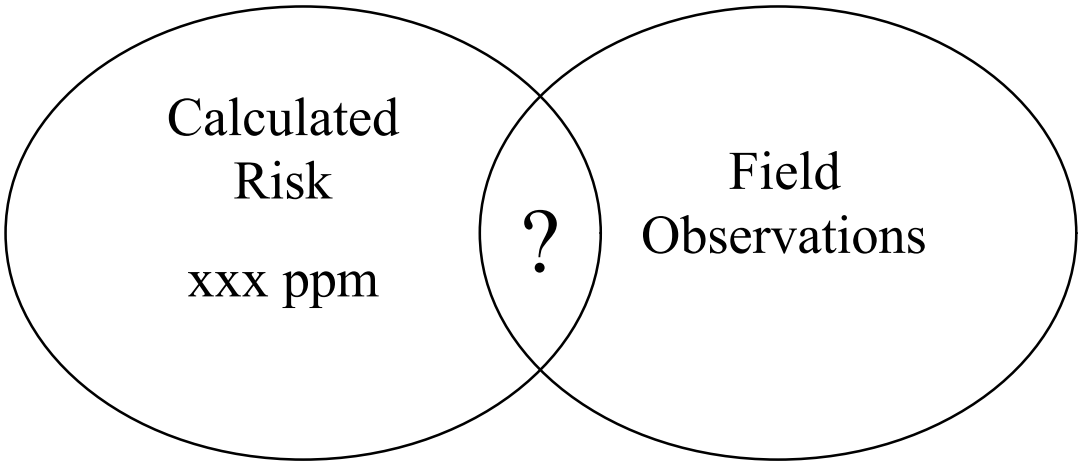


Figure 5

Integration Example
White Tailed Deer
Ni



7. CLOSURE

This document has been prepared to provide a general outline to the approach JWEL are using in statistically analysing data and calculating potential exposure of receptors in the ERA - Natural Environment for the Port Colborne CBRA. Although the general approach for the risk assessment detailed here is followed, as the analyses of data progress the need to undertake additional methods and approaches for completing the ERA may be identified and detailed fully in the final report.

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APPENDIX A

Calculating Exposure and Risk for Red-eyed Vireo

Hypothetical Example

This example is intended to provide some clarification regarding how exposure and risk will be calculated for Red-eyed Vireo and other receptors. Values were arbitrarily chosen and are not intended to represent any real situation in the Port Colborne area.

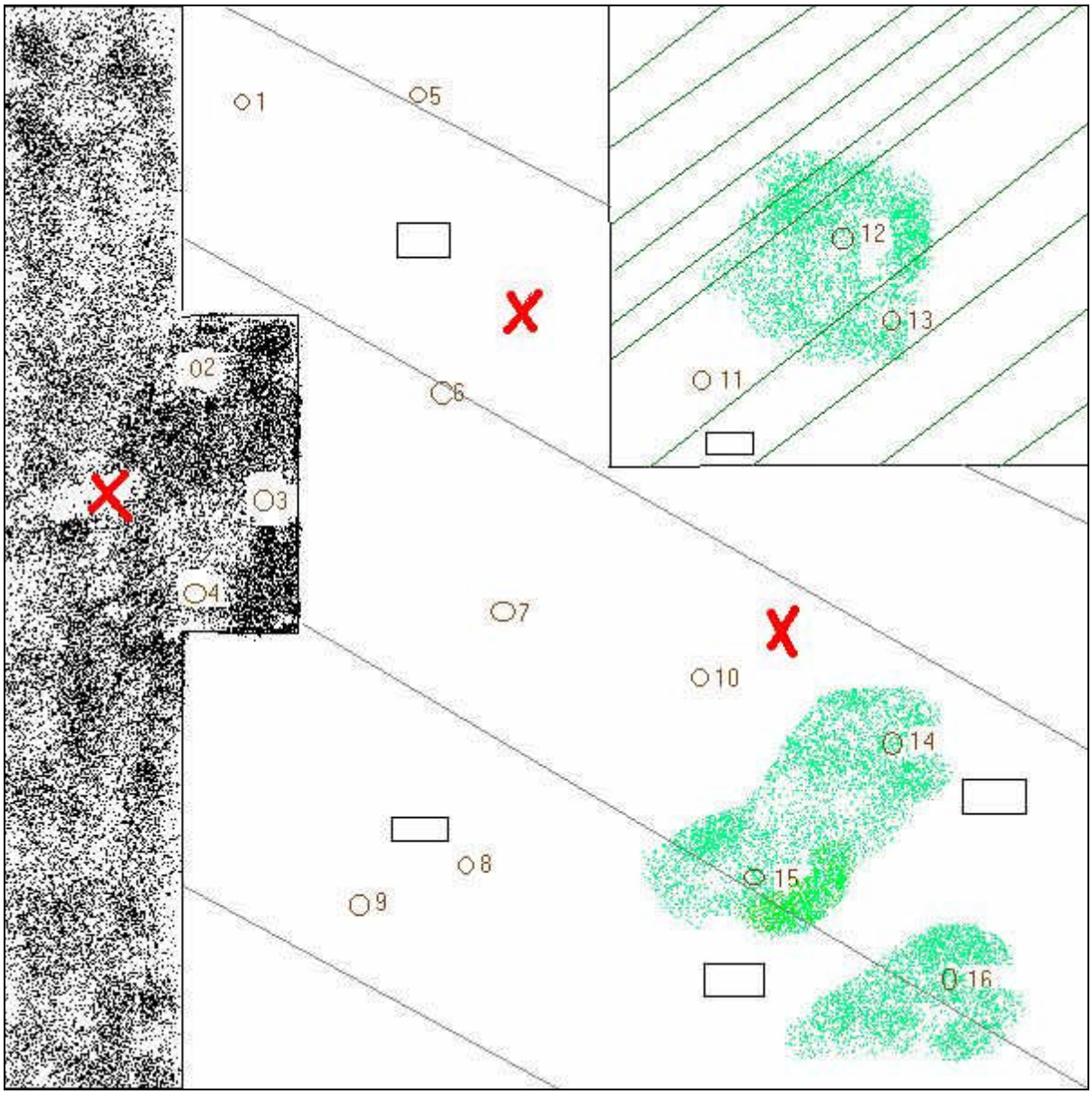
Characterising the Receptor

Based on literature values for Red-eyed Vireo, parameters were given the following values (hypothetical only):

FR	Fraction of total water ingested from the contaminated water source, total food ingested from the contaminated area and total air intake from contaminated area	0.33	assumed on basis of a small home range relative to the size of the contaminated area and information on migration periods, indicating Red-eyed Vireos spend approximately 4/12 of the year in Ontario
NIR_{water}	Normalized water ingestion rate (fraction of body weight consumed as water per unit time, g/g d)	0.21	
DF_k	Fraction of total diet accounted for by the k^{th} food group	Spiders and insects (100%)	
NIR_{food}	Normalised ingestion rate of food on a weight-to-weight basis (g/g d)	0.845	
FS	Fraction of soil in diet (g/g dw)	0.02	
BW	Average body weight of an adult Red-eyed Vireo (kg)	0.0203	
IR_{air}	Inhalation Rate (m^3/d)	0.1	
IR_{food}	Food ingestion rate on a dry weight basis (kg/d)	0.0051	

Determining the CoC concentrations to which Red-eyed Vireos are exposed comes next. Refer to Figure A-1 for sample locations while reading the following explanation of steps.

Figure A-1. Hypothetical map of sample locations.



Dark Grey = Urban Block

Light Grey = Woodlot

Organic Soil

Clay Soil

Rectangle = Water Sample Station

O = Soil & Tissue Sample Station

X = Air Sample Station

For this example, exposure is calculated for field habitat on clay soils. The map shows seven sample stations for soil and insects on clay soils in field habitat outside of the urban area. Two sample stations for air are located outside of the urban area, and five water sample stations are located outside of the urban area also. The CoC concentrations for these samples of soil, water, air and insects are provided in Table A-1.

Table A-1. Hypothetical CoC concentrations from relevant samples in Figure A-1.

Soil (mg/kg)				Water (mg/l)				Air (mg/m^3)				Insects (mg/kg)			
Ni	Cu	Co	As	Ni	Cu	Co	As	Ni	Cu	Co	As	Ni	Cu	Co	As
150	15	40	0.1	0.12	0.008	0.002	0.001	0.00004	0.0001	0.00002	0.00001	1.5	3.5	0.02	0.1
120	110	43	0.1	0.1	0.009	0.005	0.002	0.00008	0.00008	0.00006	0.00004	1.6	5.4	0.03	0.1
180	252	41	0.1	0.08	0.015	0.004	0.001					0.9	8.2	0.01	0.1
243	80	41	0.2	0.16	0.014	0.003	0.001					0.5	3.4	0.01	0.1
320	96	36	0.3	0.05	0.012	0.001	0.002					0.6	2.1	0.01	0.1
246	42	45	0.1									0.8	5.6	0.01	0.1
231	260	56	0.2									0.6	6.8	0.01	0.1

Based on these data, UCLMs are calculated with the values as listed in Table A-2.

Table A-2. UCLMs calculated from data in Table A-1.

Parameter	Soil (mg/kg)				Water (mg/l)				Air (mg/m^3)				Insects (mg/kg)			
	Ni	Cu	Co	As	Ni	Cu	Co	As	Ni	Cu	Co	As	Ni	Cu	Co	As
UCLM	255	182	47	0.2	0.13	0.014	0.004	0.002	0.00009	0.00011	0.00007	0.00005	1.2	6.3	0.02	0.1

For air and water, all data from non-urban sites are pooled. For soil and insects, only data from fields on clay soil are pooled for this scenario.

With this information, exposure is now calculated for this scenario using the equations provided in Section 5.5.1.

Average daily dose of nickel due to air inhalation:

[(Air nickel concentration)*(FR)*(IR_{air})]/BW = [(0.00009)*(0.33)*(0.1)]/0.0203 = 0.00015 mg/kg-d

Average daily dose of nickel due to water consumption:

(Water nickel concentration)*(FR)*(NIR_{water}) = (0.13)*(0.33)*(0.21) = 0.0090 mg/kg-d

Average daily dose of nickel due to food consumption (only one food type, so no summation needed):

(Insect nickel concentration)*(FR)*(DF_{insect})*(NIR_{food}) = (1.2)*(0.33)*(1)*(0.845) = 0.335 mg/kg-d

Average daily dose of nickel due to soil ingestion:

[(Soil nickel concentration)*(FS)*(IR_{food})*(FR)]/BW = [(255)*(0.02)*(0.0051)*(0.33)]/(0.0203) = 0.423 mg/kg-d

Total average daily dose of nickel = 0.0002 + 0.0090 + 0.335 + 0.423 = **0.77 mg/kg-d**

For fields on clay soils in the hypothetical study area, as defined by the map in Figure A-1, the average daily dose of nickel for Red-eyed Vireo is 0.77 mg/kg-d.

With this knowledge of a Red-eyed Vireo's exposure to nickel in the study area, a comparison must be made to help determine if undue risk to the study area's Red-eyed Vireo population is present. A Toxicity Reference Value (TRV) for nickel based on an estimation of a No Observed Adverse Effect Level (NOAEL) for Mallard *Anas platyrhynchos* (as measured by body weight change), and corrected for body weight of Red-eyed Vireo, was estimated to be 110 mg/kg-d. Using the Quotient Method (Section 5.5.6), risk is calculated as:

Risk = 0.77/110 = **0.007**

Our conclusion is that the Red-eyed Vireo population in the hypothetical study area is presented with no undue risk from nickel in field habitat on clay soils. Similar procedures and assessment of risk will be done for each CoC and each scenario. If undue risk is believed to be present (i.e., the risk>1), then a soil concentration giving an acceptable level of risk (i.e., risk = 1) will be back-calculated and presented in the report.