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PORT COLBORNE COMMUNITY BASED RISK ASSESSMENT

ECOLOGICAL RISK ASSESSMENT – CROPS

ADDENDUM REPORT

SEPTEMBER, 2006

1.0 INTRODUCTION

As part of a Community Based Risk Assessment (CBRA) initiated by Inco Limited to address potential impacts resulting from historical emissions from a former nickel refinery in Port Colborne, Jacques Whitford Limited (Jacques Whitford) completed a risk assessment on agricultural crops for the Port Colborne area as part of the Ecological Risk Assessment (ERA) of the CBRA. Following completion of two years of fieldwork and analysis of data, Jacques Whitford prepared a draft Crops Report in April 2003 and then a subsequent revised draft Crops Report in July 2003. Following the CBRA process, copies of both draft reports were presented to the City of Port Colborne Public Liaison Committee (PLC) and the public for review and comment. These comments were reviewed by Jacques Whitford and, where revisions were required, these were incorporated into the Final Report.

In December 2004, Jacques Whitford tabled a final Crops Report for the CBRA. Following the CBRA process, a public review and comment period for the final report was identified, ending July 14, 2006. During this review period, Jacques Whitford received written comments from the Third Party Reviewer (Dr. M. McBride) and the PLC's Consultant (Watters Environmental Group Inc.). No written comment submissions were received from the public. Jacques Whitford prepared responses to these comments and these are included within this document. A Technical Sub-Committee (TSC) meeting was held on September 14, 2006 to review the comments and Jacques Whitford's response.

This addendum report has been prepared to document the CBRA public review process following the tabling of the Final December 2004 ERA-Crops Report and to address residual issues that were raised during the final review period. This addendum to the Final Report will be part of the formal submission of the ERA-Crops Report to the Ministry of the Environment (MOE).

This addendum report is presented as follows:

Tab 1: Jacques Whitford Addendum Report: "*Overview of Evidence for the Crops Risk Assessment (RA) – Addendum to December 2004 Crops Studies Report*", dated January 26, 2006. Attached appendices in this report include Jacques Whitford's Response Letters to Murray B. McBride's Commentary Letters of February 12, 2005 and September 26, 2005.

Tab 2: Jacques Whitford's Response Letter to Murray B. McBride's May 4, 2006 Comments on the Protectiveness of the Proposed Ni - PNEC values for Soils of Port Colborne. Response letter is dated September 1, 2006

Tab 3: Jacques Whitford's Response Letter to Watters Environmental July 13, 2006 Comments/Questions on the Jacques Whitford Crops Report. Response letter is dated September 12, 2006

Tab 4: Jacques Whitford's Response to Public Comments of Jacques Whitford Final Crops Report by deadline of July 14, 2006

Tab 5: Compilation of Public Notices, TCS and PLC meetings for documenting the public process for the CBRA

TAB 1

Jacques Whitford Addendum Report: *"Overview of Evidence for the Crops Risk Assessment (RA) – Addendum to December 2004 Crops Studies Report, January 26, 2006"* Attached appendices in this report include Jacques Whitford's Response Letters to Murray B. McBride's Commentary Letters of February 12, 2005 and September 26, 2005.

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REPORT

OVERVIEW OF EVIDENCE FOR THE CROPS RA - ADDENDUM TO DECEMBER 2004 CROP STUDIES REPORT

INCO LIMITED

PROJECT NO. ONT34657

PROJECT NO. ONT34657

REPORT TO	INCO LIMITED 145 King St. West, Suite 1500 Toronto, Ontario M5H 4B7
FOR	Overview Of Evidence For The Crops RA - Addendum to December 2004 Final Crop Studies Report
ON	Crop Studies, Part of CBRA Program, Port Colborne, On

January 26, 2006

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EXECUTIVE SUMMARY

The primary objective of the Crop Studies was to determine the concentrations of historically-deposited CoCs in soils that present an unacceptable risk (phytotoxicity) to crops grown in Port Colborne soils. To that end, dose response experiments were conducted in a greenhouse setting in Years 2000 and 2001. Year 2000 was preliminary in nature and a scoping study. With lessons learned from the Year 2000 greenhouse experiments, these were incorporated in the design of a more proper dose response experiment in Year 2001. One of the differences in design of the experiments between the two years is that unblended soils were used in Year 2000 and blended soils were used in Year 2001. Definitions of blended and unblended soils are provided in the report.

Toxicity thresholds of nickel EC_{25} (the effective concentration at which there is a 25% reduction in growth observed) and secondary thresholds consisting of the PNEC (predicted no-effects concentration or the maximum dose at which there is no significant decrease in response) were derived using the Year 2001 dose response relationships of oat grown on Port Colborne soil with varying levels of nickel and other chemicals of concern. The Year 2001 dose response experiments investigated oat grown on four soil types, including Welland Clay, Till Clay, Organic Muck and Sand and reliable EC_{25} and PNEC values were obtained from each of these experiments. The year 2000 dose response experiments did not provide sufficient information to derive reliable EC_{25} and PNEC values.

External peer reviewer comments of the December 2004 Final Crops Studies Report made on September 26, 2005 (Appendix C) had summarized five concerns, as noted below:

1) Usefulness of the Year 2000 data;
2) Use of oat as a single indicator species;
3) Use of plant biomass vs. economic yield;
4) Blending versus unblending and generation of EC_{25} values using unblended soils; and
5) Confidence intervals.

This report addresses each of the five concerns raised by the external peer reviewer and provides additional analyses, where possible, to the reader and members of the CBRA's Technical Sub-Committee so as to clarify evidence from the crops information and to bring closure to the Crops Risk Assessment (RA).

The common element in each of the reviewer's five concerns was the derivation of EC_{25} values using the GH Year 2001 data because these data were based on 'blended' soils. An inference was made by the reviewer that perhaps more realistic EC_{25} values could be obtained using the GH Year 2000 data which were based on 'unblended' soils. Another inference was made that perhaps soybean would be a more sensitive crop to study rather than oat.

Notwithstanding the uncertainties in the Year 2000 greenhouse data to establish reliable EC_{25} values as mentioned in this report, an attempt was made to back calculate or predict EC_{25} values in soil using the actual Year 2000 greenhouse dose response data for both oat and soybean on representative samples of Welland Clay and Organic Muck 'unblended' soils, and also, by using a literature value of 62.7 mg Ni/kg in tissue corresponding to an observed decrease of 25% in yield for crops grown on Port Colborne 'unblended' soils (Kukier and Chaney et al. (2004)). Welland Clay and Organic Muck soils constitute the major agricultural soil types in the Port Colborne area.

Predicted Ni EC_{25} values for the 'unblended' soils used in the GH 2000 oat dose response experiment showed no significant differences with those Ni EC_{25} values measured in 'blended' soils from the GH 2001 oat dose response experiment. Predicted values of soil Ni EC_{25} for soybean were similar, if not greater than those of oat. Clearly, the process of soil blending in Year 2001 did not bias the measured soil Ni EC_{25} values as reported in the Jacques Whitford December 2004 Final Crops Report. Thus the reported soil Ni EC_{25} values in the Final Crops Report remain valid.

In Jacques Whitford's opinion, all five of the external peer reviewer's concerns of September 26, 2005 have been satisfactory addressed. We believe that the perceived gap between Jacques Whitford's findings and interpretation as found in the December 2004 Final Crops Report and those made by the peer review in his letter of September 26, 2005 has been considerably narrowed, if not completely eliminated.

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OVERVIEW OF EVIDENCE FOR THE CROPS RA

ADDENDUM TO DECEMBER 2004 FINAL CROP STUDIES REPORT

1.0 INTRODUCTION

As part of the Port Colborne Community Based Risk Assessment (CBRA), Jacques Whitford Limited (Jacques Whitford) carried out crop phytotoxicity testing (hereafter, "Crop Studies") in 2000 and 2001. These Crop Studies included both Greenhouse Trials and parallel Field Trials near a metals refinery (hereafter "Refinery") owned by Inco Ltd. (hereafter "Inco") in Port Colborne, Ontario. The trials evaluated the performance of agricultural crops on soils representative of the main soil types found in the Port Colborne area (Kingston and Presant 1989, Jacques Whitford, 2003), which received particulate emissions from the Refinery with varying concentrations of the chemicals of concern (hereinafter referred to as CoCs under the CBRA).

The CoCs comprised arsenic, cobalt, copper and nickel. Of these elements, nickel was targeted as the primary CoC because of its much higher soil concentrations relative to, and defined ratios of, the other three CoCs to nickel.

External peer reviewer comments on previous drafts of the Crop Studies Report (June, 2003) have been incorporated in Binder 3, Volume V, Appendix A of the Final Crops Studies Report dated December 2004. External peer reviewer comments of the December 2004 Final Crops Studies Report were received on February 14, 2005 (Appendix A) and were addressed by Jacques Whitford in a letter dated July 26, 2005 (Appendix B). Follow-up comments by the external peer review comments in a letter dated September 26, 2005 (Appendix C) summarized five remaining concerns, as noted below:

1) Usefulness of the Year 2000 data;
2) Use of oat as a single indicator species;
3) Use of plant biomass vs. economic yield;
4) Blending versus unblending and generation of EC_{25} values using unblended soils; and
5) Confidence intervals.

Jacques Whitford responded to the external peer reviewer over his concerns in a letter dated October 12, 2005 (Appendix D). For the purpose of this report, each of the five concerns by the external peer reviewer are reviewed and discussed in Section 5.0 of this report and additional analyses, where possible, are provided to the reader and members of the CBRA's Technical Sub-Committee in order to clarify evidence from the crops information and to bring closure to the Crops Risk Assessment (RA).

1.1 Uniqueness of Port Colborne Soil

Port Colborne is the site where Inco's electrolytic nickel refinery operated from 1918 to 1995. Historical atmospheric release from this refinery and deposition of nickel-containing particles in the surrounding community resulted in elevated As, Co, Cu and Ni in soils. Nickel concentrations in soil above MOE generic soil quality guideline (SQGs) levels originating from the refinery were found to impact an area of approximately 30 km^2. The greatest deposition of CoC-particulates was within one km of the refinery. Deposition occurred on the surface of soils which in agricultural lands was mixed by tilling up to a depth of 15 cm. Four major soil groups were affected including a Heavy Clay (Welland soil series), a Shallow Till Clay (Alluvial soils series), an Organic Muck (Quarry soil series), and Sand (Beach – Scarp soil series). The Heavy Clay (hereinafter referred to as the Welland Clay), Shallow Till Clay (hereinafter referred to as the Till Clay)and Organic Muck soil groups are in current use for agricultural production; there are no agricultural areas with Sand soils in Port Colborne as they are only found in dunes along the shoreline of Lake Erie.

The Port Colborne soils have specific characteristics, such as an average pH of 6.2 in the clays and the Organic muck soils, and an average pH 6.9 in beach sands.

Scanning electron microscopy (SEM) by SGS Lakefield (SGS Lakefield, 2002) identified nickel-bearing particulates in each of the submitted soil samples. The predominant (90% and greater) nickel species identified were oxidic forms of nickel (Table 1). Less than 10% of the nickel was found in iron oxide/oxyhydroxide as trace nickel. The SEM did not identify any metallic nickel nor any sulphidic nickel species, but did identify nickel-particulates as being either liberated or as part of, or attached to, mineral aggregate grains and/or organic aggregate grains.

X-Ray Absorption Spectroscopy (XAS) analysis conducted on samples identified similar findings to that of the SEM in that the soils were primarily oxidic nickel, identified predominantly as nickel oxide (89% to 93%) and to a lesser extent, some nickel hydroxide (7% to 11%) (Trvidei, et al. 2002).

Table 1 Nickel speciation of metal elevated soils in Port Colborne by SEM

	Organic Muck	Sand	Till Clay	Welland Clay
Total Ni (mg/kg)	10,045	3,920	2,545	8,655
Percentage as Oxidic Ni[1]	99.6%	91.7%	99.1%	89.9%
Percentage as Iron oxide/oxy-hydroxide with trace Ni	0.4%	8.3%	0.9%	10.1%

[1]Oxidic Ni includes all forms of Ni oxide/hydroxide, Ni-Fe oxide/hydroxide and Ni-Fe-Cu oxide/hydroxide

The identified form of Ni in Port Colborne as nickel oxide and nickel hydroxide, both relatively insoluble in water, is an important context for the comparison of the results from this CBRA study to those of most other reported studies of Ni phytotoxicity (Davis and Beckett, 1978) that have used soluble nickel salts, such as nickel chloride. It should be noted that the MOE generic phytotoxicity values were derived from experiments using soluble nickel salts, a form of nickel not representative in soils from the Port Colborne area. Solubility product constants are equilibrium constants that refer to the product of the concentration of ions that are present in a saturated solution of an ionic compound. Most of the literature-reported solubility product constants are experimentally derived at 25 degrees Celsius; the lower the value, the lower is its solubility. The literature (Baes and Mesmer, 1976) value for the solubility product of nickel hydroxide is $10^{-17.2}$ which translates to an equilibrium concentration of nickel at saturation of solubility of 0.01 mg/L. A literature value could not be found for nickel oxide, likely because of its extremely low solubility. A solubility product for nickel chloride was not available in the literature as solubility products are only given for those ionic compounds of relatively-low solubility. In general, all metal chlorides are soluble. Mallinckrodt Baker, 2003 reports the solubility of its commercial product, nickel chloride at 2,540,000 mg/L, which if calculated, translates to a very high solubility product at $3 \times 10^{+4}$. Thus, there is a basis in solution chemistry to anticipate that the toxicity thresholds for plant growth in these Port Colborne soils containing nickel oxides and hydroxides would be higher than in nickel soluble-salt amended soils, in which the proportion of total Ni present as the free-ion (presumed to be the most bioavailable) is much greater.

1.2 Study Objective

The primary objective of the Crop Studies was to determine the concentrations of historically-deposited CoCs in soils that present an unacceptable risk (phytotoxicity) to crops grown in Port Colborne soils.

1.3 Study Design Requirements

In fulfilling the study objective, the study design requirements had to:

- Identify test crops that could be considered sensitive representatives of crops grown in Port Colborne and would, as well as, be able to be compared to previous scientific studies;
- Use standard methods of measuring phytotoxicity stress levels on plant growth from metal accumulation as described by national and international standards; and
- Design field and/or greenhouse experiments that measure the effects of CoCs on crops grown in real Port Colborne soils and are able to control other confounding factors such as changes in soil pH, over/under watering, over/under fertilization, etc.

The study design requirements led to the evaluation of four possible design options. These design options were:

- Option 1: Greenhouse experiments with crops grown on non-impacted Port Colborne soils spiked with soluble Ni- and other CoC- salts at increasing concentrations (similar to Davis and Beckett, 1978).
- Option 2: Field experiments with crops grown on sites in Port Colborne with impacted CoC soils at varying concentrations of CoCs and representative averages in values of soil chemistry and physical parameters. The major soil chemistry and physical parameters include pH, cation exchange capacity (CEC), organic carbon, clay content and oxides of Fe, Al, and Mn.
- Option 3: Greenhouse experiments with crops grown on representative soils collected from Port Colborne area for each of the four soil types, with a range of varying soil Ni concentrations and representative averages in values of soil chemistry and physical parameters.
- Option 4: Greenhouse experiments with crops grown on representative soils collected from Port Colborne and blended to maintain consistency of representative averages in values of soil chemistry and physical parameters irrespective of differences in CoC soil concentrations. The blending process involved mixing at various proportions a highly Ni-impacted soil type from Port Colborne with a background soil of the same soil type from Port Colborne, keeping representative averages in values of soil chemistry and physical parameters the same in both the impacted and background soil pairs.

Evaluation of Design Option 1.

Use of soluble Ni- and other CoC- salts (high bioavailability) in non-impacted Port Colborne soils would not properly represent the relatively-insoluble oxidic forms of nickel (low bioavailability) found in soils of the impacted area. Option 1 would lead to an over estimation of Ni uptake in plants and produce an incorrect assessment of Port Colborne soil CoCs' phytotoxicity to crops. Scientific literature overwhelmingly illustrates this when comparison of salt-amended *vs.* field-contaminated soils are made (Chaney et al., 2003). Option 1 was discarded particularly as the Ni speciation of the Port Colborne soils suggest that contamination was not soluble.

Evaluation of Design Option 2.

While soil Ni concentrations decrease with distance downwind from the refinery in a northeast direction along which field crops could have been potentially grown for this study, the soil types along this traverse also vary from Organic Muck with very high soil Ni concentrations close to the refinery, to Welland Clay with high to medium soil Ni concentrations further away, and then to Till Clay with low soil Ni concentrations at a further distance. Therefore the interaction of CoCs with crops grown on each of these three different and heterogenous soil types, with varying clay content and organic carbon content would not yield a proper dose-response relationship for each soil type. Because of the large variation of soil chemistries among the different soil types, dose-response curves obtained by using a range of nickel concentrations in different soil types would not measure the effect of CoCs only. Another impracticality of this option was that access to some key agricultural fields was restricted.

Design Option 2 would also require a control site which would have had to be at least 15 km away from the area of impact. Due to the way in which Port Colborne soils naturally occur across the agricultural areas and along the soil CoC concentration gradient, and also given that access was prohibited to key privately owned lands in the impact area, it was not possible to find and access field plots representing the necessary range of CoC concentrations required to develop scientifically defendable dose-response relationships from field data for each of the three agricultural soil types (ie. Welland Clay, Till Clay, Organic Muck) in the area. Thus, Option 2 was discarded.

Evaluation of Design Option 3.

Prior to implementing Option 3 during the Year 2000 Greenhouse Trials, concerns were raised internally within Jacques Whitford on the feasibility of collecting representative soil samples from the field with representative averages in values of soil chemistry and physical parameters **and** with varying soil Ni concentrations. Evaluation of findings of Option 3 confirmed the initial concerns, identifying deficiencies in the practical use of the experimental data. This is further discussed in Section 2. In particular, the findings of Option 3 in Year 2000 greenhouse work led the scientists at Jacques Whitford and their consulting scientists at the University of Guelph to conclude that there existed significant experimental deficiencies with Option 3 which prevented the results from being used to develop a reliable CoC dose-response relationship. These experimental deficiencies are detailed in Section 2.

Although not suitable for generation of valid dose-response curves (and phytotoxicity thresholds), the results from the year 2000 greenhouse study were used as bases for improving the experimental design in the follow-up 2001 greenhouse study.

Evaluation of Design Option 4.

Option 4 was considered and implemented during the Year 2001 Greenhouse Trials. Several aspects in the design of the trials and the interpretation of the generated dose-response curves for four soil types (Welland Clay, Till Clay, Organic Muck, Sand) are summarized below:

- Each soil type was blended/homogenized in various ratios depending on the level of CoCs concentration in the High Ni impacted soil to provide a range of soil Ni concentrations for each soil type, from control to high.
- A key factor affecting Ni phytoavailability, pH, was controlled by adjusting the soil pH of both the low nickel and high nickel soils to the Port Colborne average of pH 6.2 prior to blending. To increase or decrease soil pH, calcium carbonate or aluminum sulphate, respectively were added to the field soils.
- Subsamples of blended soils of the same CoC concentration were placed in greenhouse pots in quintriplicate, planted with seeds of oat in all four soil types and radish in Welland Clay only, and normal agricultural fertilizers were added to each pot.

- EC_{25} - 25 % decrease in Dry Weight (DW) was chosen as a point where the decrease in DW would be expected to be statistically significant relative to the variation that occurred in control soils; this level that would allow a scientifically valid conclusion about causality of DW reduction. This is a commonly used procedure for deriving environmental soil guidelines for soil contact for agricultural land uses by MOE (Ontario Ministry of the Environment), CCME (Canadian Council of Ministers of the Environment, 1999a), and OECD (Organization for Economic Co-operation and Development).
- PNEC (predicted no-effects concentration) values were also derived from upper confidence intervals of Weibull fits.

The results from the greenhouse 2001 study produced reliable dose-response curves to satisfy the study objective in the determination of the concentrations of historically-deposited CoCs in soils that present an unacceptable risk (phytotoxicity) to crops grown in Port Colborne soils. Further details are provided in Sections 4 and 5.

2.0 PHYTOTOXICITY EVALUATION - YEAR 2000 FINDINGS

2.1 Dose Response based on Greenhouse Findings

2.1.1 Design limiting factors:

Jacques Whitford and the University of Guelph scientists involved in the crops studies believe that the major confounding factors for the Year 2000 tests were soil chemical and physical properties, which varied widely among samples of each single soil type examined. Specifically:

- Counfounding factors. That is, there was no consistency in representative averages in values of soil chemistry and physical parameters with increasing soil Ni concentrations;
    - For example, pH varied in the Organic soil from pH 5.0 to 6.7 and in the Clay soil from pH 5.4 to 7.3.
    - For example, % organic carbon in the Clay soil varied from 3.8 to 9.0 and in the Organic soil from 23.4 to 33.
- Inadequate range in soil Ni concentrations for dose response;
- Human error and missing data in some of the tests;
- High analytical detection limits;
- Limited number of treatments and replicates resulted in large uncertainties. The results obtained in the testing of these soils led to a large variation within the population response; and
- Inadequate length of exposure (on average, 48 days) and thus insufficient growth duration to reach plant maturity and allow comparison of study data to relevant scientific literature. In hindsight, the exposure should have been about 70 days which is the minimum requirement for maturity. Hence, comparisons of the data obtained from this study with data from literature have to be made with caution, as different growth stages are involved.

These were the major design limitations for the Year 2000 dose-response study and no accurate scientifically conclusions in arriving at EC_{25} could be reached by interpreting the Year 2000 findings.

2.1.2 Additional stress factors:

- In Year 2000, greenhouse testing was done in a closed pot environment where the inside of each pot was artificially lined with a plastic bag. It was the opinion of Jacques Whitford at the time of the design of the Year 2000 experimental setup, that the lining inside the pots would prevent any soluble salts and CoCs from being washed out of the pots. However, as the experiments progressed, it became evident to Jacques Whitford that the use of a liner produced a growth limiting factor to the crops sown, as the created closed environment lowered the redox potential of the soils and created reducing conditions at the bottom of the pots. The lack of oxygen in the root zone created phytotoxic conditions and these reducing conditions are not normally found within active agricultural soils of Port Colborne. As the design of the Year 2000 experiment did not simulate the real redox conditions for the Port Colborne study site, the Year 2000 findings must be interpreted with caution. At the advice of the University of Guelph scientists, this design flaw was rectified by Jacques Whitford in the greenhouse experiments of 2001.

- Another important limitation of the Year 2000 Greenhouse experiments was the fertilizer requirement. Although based on soil fertility analyses and OMAF recommendations, the rates used were inadequate for pot experiments. It is general knowledge that higher rates of fertilizer must be applied in greenhouse pot studies (compared to field) in order to compensate for the limited amount of soil in each pot that is explored by roots to provide water and nutrients for the growing plants. This condition was not met in the Year 2000 Greenhouse experiments.
- The application of phosphorus to the tested soils in the Year 2000 as a dilute solution. This was inappropriate and a banded application should have been used.
- The volume of soil and size of the pots used in the Year 2000 were at best minimal. Roots were confined to a contaminated topsoil layer depth, a situation which does not occur in the field, and probably became root-bound in a very short time after seeding. It is well known that in pots the lengths of roots are decreased so that nutrients needed by plants are not absorbed as readily in small pots as in large pots or in the field. Nutrients such as phosphorus and potassium are depleted from the root-hair proximity. In pots, root length is substantially reduced compared to the field, and this effect is intensified the smaller the pot. If Ni phytotoxicity is affected by availability of a nutrient such as phosphate (toxicity increased by low or high phosphate supply), the use of small pots worsens the apparent phytotoxicity of Ni because cultures grown in pots reduce the phytoavailability of soil phosphorus (Chaney et al. 2003).

2.1.3 Phytotoxicity Symptoms

Typical phytotoxicity symptoms (perpendicular banding, chlorosis along the leaves) were NOT visually observed in any of the plants grown on soil with medium CoC concentration levels which for Clay and Sand soils were around 500 mg/kg Ni soil, and for Organic Muck soils, was about 1200 mg/kg Ni soil.

2.2 Field Findings

Field plots have been undertaken in the Port Colborne area since 2000 for this study. While field data were not sufficiently complete at varying soil Ni concentrations to have developed a direct dose-response relationship for test crops, the field observations and data did produce nonetheless very useful scientific information about CoC phytotoxicity to crops grown on Port Colborne soils as summarized in the following subsections.

2.2.1 Ni accumulation in plant tissue

One of the most relevant findings of the Year 2000 field testing was that plants growing on the Clay 1 (C1) Test Site which is a site that has a level of 600 mg/kg Ni in the soil, accumulated very low levels of nickel in the plant tissue, sometimes below the analytical limit of detection. Specifically oats accumulated on average 11 mg/kg Ni in the tissue, while soybean and radishes accumulated undetected levels below 3 mg/kg Ni.

Visual evidence of Ni phytotoxicity was not observed in crops on this field plot. These data, and observations from this plot, clearly indicate that phytotoxicity, at a minimum, does not occur at 600 mg/kg in clay soil, and also suggests that in the field, oat is more sensitive to the presence of nickel in the soil when compared to soybean and radishes.

When compared to crops grown on similar soil Ni concentration levels from the 2000 Greenhouse phytotoxicity findings, it was found that the **greenhouse testing overestimated the tissue Ni accumulation measured in the field**. Soybean grown in Welland Clay with 500 mg/kg soil Ni in the greenhouse had tissue Ni concentrations of 11 mg/kg. In comparison, soybean plants grown in Welland Clay in the field in the Year 2000 at the C1 Test Site with an average soil Ni concentration of about 600 mg/kg Ni had tissue concentrations of Ni that were less than 3 mg/kg.

The same difference was noted for oat, which in Year 2000, accumulated approximately 22 mg/kg tissue Ni on Welland clay with 500 mg/kg Ni in the greenhouse, whereas the same species grown in Welland Clay in the field at the C1 Test Site with 600 mg/kg soil Ni, accumulated half that concentration of Ni in the tissue.

This difference between field and greenhouse Year 2000 data is likely due to the rooting zone of field-grown plants which extends below the upper layer of soil where Ni concentrations are the highest. Thus, one would anticipate that the toxicity observed in the pot-grown soybean, and oat, in both Year 2000 and Year 2001 greenhouse studies, would be greater than that expected in the field-grown crops, relative to soil Ni concentration.

2.2.2 Phytotoxicity Symptoms

Phytotoxicity symptoms such as banding, chlorosis or stunted growth were not observed on the C1 Test Site (600 mg Ni/kg) or on the Organic Muck Test Site (3500 mg Ni/kg). The lack of phytotoxicity symptoms in any of the plant species tested at these specific sites indicated that farming can be conducted without risk to any crops given these conditions.

On the Clay 2 (C2) Test Site (approximately 6000 mg/kg Ni), site phytotoxicity symptoms such as chlorosis were observed only in plants that accumulated over 60 to 80 mg/kg Ni in the tissue. In comparison, the literature documents that phytotoxicity to crops should occur in the range of 40 to 80 mg/Kg Ni in plant tissue (Chaney et al. 2003).

2.2.3 Comparison to Previous Field Studies.

The Jacques Whitford Year 2000 Field Studies used the same experimental set-up (plots) as used by Dr. Chaney, a research agronomist with the U.S. Department of Agriculture. Chaney et al. (2001) found the accumulation of nickel in plants grown at the C1 Test Site was the lowest with an average of 5.9 mg/kg for oat plants, 8.1 mg/kg Ni for radish,13.3 mg/kg Ni for soybean and 2.5 mg/kg Ni for corn (all tissue concentration measured in diagnostic leafs). At the C2 Test Site, Chaney et al. (2001) found the oat plants accumulated 62.7 mg/kg Ni, while soybean accumulated 93.9 mg/kg Ni (Ni tissue measured in diagnostic leaves). Similar findings reported by Chaney et al. (2001) were found by Jacques Whitford (2004) as well.

2.3 Integration of Greenhouse and Field Findings

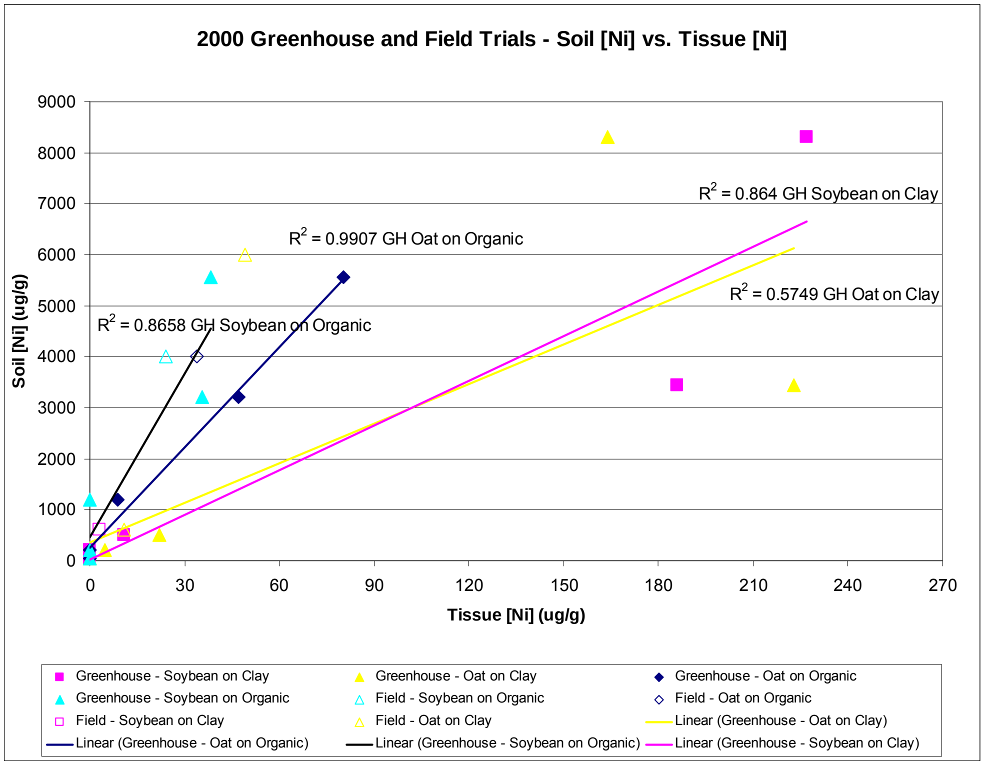
2.3.1 Dry Weight correlation with soil parameters

Variation in the dry weight grown on Clay was well explained by tissue Ni concentration in the December 2004 Crops Report (Volume 1, Part 4, section 3.1.2). The addition of soil pH and organic content explained 10 % of the variation.

2.3.2 Comparison between Field and Greenhouse Year 2000

As mentioned before, when the same plants were exposed in the greenhouse and in the field to the same soil Ni concentrations (about 600 mg/kg Ni), nickel accumulated in plant tissue to more than double the amount under greenhouse conditions than occurred in plants growing under field conditions (Figure 1). This can only be attributed to the rooting zone of field-grown plants extending below the upper layer of soil where Ni concentrations are the highest in the Port Colborne area, whereas in the greenhouse setting, the roots of the plant were exposed to a uniform concentration of Ni in soil throughout the pot and plant root zone.

Figure 1 Crop Studies Year 2000 show that plant tissue Ni concentration was higher in greenhouse studies compared to field studies.



In greenhouse tests, the addition of fertilizers needed to grow the test plants was done at the beginning of an experiment. These fertilizer salts can increase the ionic strength of the soil solution sufficiently to increase the solubility of soil Ni and increase the potential for phytotoxicity (Chaney et al., 2003). As a result, the toxicity observed in the pot-grown soybean, and oat, in greenhouse studies was more severe than observed in the field-grown crops, relative to soil Ni concentration.

2.3.3 Comparison with other scientific studies

There are numerous studies reported in the scientific literature showing that Ni phytotoxicity is not solely caused by the soil total nickel concentration; rather other specific soil properties play an equal if not more significant role. One of the most relevant examples of this, is the lack of phytotoxicity symptoms in plant growing in Ni-rich (serpentine) soils which contain nickel at levels varying from 500 to 10,000 mg /kg Ni soil. The scientific literature of metal phytotoxicity has been showing for decades that it takes 25 to 50 or more mg Ni/kg DW in the youngest fully open leaf before Ni reduces yields of many crops (Chaney et al., 2003 and references within). Specifically Kukier and Chaney (2004) determined phytotoxicity for a range of crop species, including corn, soybean, radishes and oat using similar soils from Port Colborne. This study determined that tissue Ni for oat at 25% reduction of shoot growth occurred at a Ni tissue level of 62.7 mg/kg. This toxicity threshold was derived from oats exposed to the same soil nickel concentrations (2930 mg/kg Ni), but at three different pH's (control, limed and calcareous).

3.0 INCORPORATION OF LESSONS LEARNED INTO DESIGN OF 2001 EXPERIMENTS

The results from the 2000 greenhouse and field study were discussed appropriately considering the limitations of the experimental design and data analysis. The learnings from the year 2000 work were reflected in the design of the Year 2001 experiments as follows:

Greenhouse:

- The experimental design in the Year 2001 tests sought to make the soil Ni level the single major variable; all the other soil properties were kept relatively constant. In doing this it was ensured that the observed response was a consequence of increasing the dose of soil Ni concentration and not of the changes in soil pH, organic matter or other soil chemical or physical parameter;
- Oat is usually considered the most characteristic plant indicator of nickel phytotoxicity based on research of Vergnano and Hunter (1952) which has been corroborated repeatedly over 50 years of further research (e.g. Anderson et al. 1973). In oat, iron deficiency is observed as interveinal chlorosis and the visible toxicity symptom specific to nickel phytotoxicity in oat is an alternating pattern of more chlorotic and less chlorotic bands across young leaves. The choice on oat was based on the uniqueness of the perpendicular banding of chlorosis severity along the leaves which makes the diagnosis of Ni phytotoxicity much more definitive than with any other species reported to date (Chaney et al., 2003);
- Sufficient replicates in that the number of replicates increased from three to five. This ensured that variability across the population would be small and that the level of confidence in the data would improve;
- Human error was reduced;
- Lower analytical detection limits (ex. from 1 to 0.01 mg/kg) were achieved;
- The use of open, un-lined soil pots ensured optimum growing conditions (oxic conditions);
- Large pots with (6.5 L) were used to reduce “pot effects”; and
- Fertilizer application rates were optimized.

Field:

- As phytotoxicity symptoms were not evident at the Organic Muck Test Site (3500 mg Ni/kg) and the C1 Test Site (600 mg Ni/kg) but only present at the C2 Test Site (6000 mg Ni/kg), the 2001 field design focused on the C2 Test Site and included the addition of a new clay site, Clay 3 (C3) Test Site with an average soil nickel concentration of approximately 3000 mg Ni/kg.
- Findings from the field experiments were correlated and ground proofed with an assessment of naturally-occurring flora in Port Colborne.
- An engineered field experiment on the Clay 3 site was also undertaken (see page 3-49 of the December 2004 Final Crops Report).

4.0 PHYTOTOXICITY EVALUATION - YEAR 2001 FINDINGS:

4.1 Dose Response based on Greenhouse Findings

4.1.1 EC_{25} and PNEC based on Plant Growth

The 2001 Greenhouse Study was designed as a dose-response experiment for oat grown in blends of each key Port Colborne soil type with varying nickel concentration. A Weibull function was fit to plant growth (measured by dry weight) and tissue nickel concentration data in order to identify toxicity thresholds. The Weibull function is a continuous mathematical function that provides estimates of key biological parameters, including toxicity thresholds and is well suited to dose-response modelling of plant-metal interactions (Taylor et al. 1991). For this investigation, the EC_{25} (the effective concentration at which there is a 25% reduction in growth observed) was the toxicity threshold of interest. Uncertainty about the function was represented by 5% and 95% confidence intervals.

For the purpose of comparison with the EC_{25}, a secondary threshold, the PNEC (predicted no-effects concentration) based on total soil Ni was also determined. By definition, PNEC is the maximum dose at which there is no significant decrease in response

Values of EC_{25} and PNEC generated from the Year 2001 dose-response data of oat grown on Welland Clay, Till Clay, Organic Muck and Sand are summarized in Table 2.

Table 2 Summary of Calculated EC_{25} and PNEC Nickel Values

Soil Type	EC_{25} (mg Ni/kg) in Soil	PNEC (mg Ni/kg) in Soil	EC_{25} (mg Ni/kg) in Oat Tissue
Sand	1350	750	71
Organic	>2400, 3400*	2350	46
Welland Clay	1880	1650	52
Till Clay	1950	1400	21

* derived from meta- analysis using both the 2001 and 2000 oat on Organic Muck soil data.

Jacques Whitford believes that the reported EC_{25}s and PNECs in Table 2 are very conservative and overstate the toxicity of Ni to crops in Port Colborne soils. It would have been ideal, if it had been possible, to have grown test crops on each key agricultural soil type having a range of CoC concentrations to produce scientifically defendable dose-response relationships from field data. As this was not possible, greenhouse experiments in year 2001 were undertaken to derive EC_{25}s and PNECs. Field observations in year 2000 (Section 2.2) clearly showed that greenhouse studies produce more conservative results (that is higher CoC bioavailability and phytotoxicity) than what actually occurs when crops are exposed to soil CoCs in the field and normal field cropping practice. Hence, the EC_{25}s and PNECs in Table 2 are overly conservative and thus considered safe for crops growing in Port Colborne soils.

4.1.2 Phytotoxicity Symptoms

In Sand soils: Plants grown in sand soils showed white banding perpendicular to leaf veins. This symptom was observed on the cotyledonary leaves in plants exposed to higher concentrations only. These leaves did not unfold completely and had a needle shape. These severe toxicity symptoms manifested at the high level of exposure required plant collection after 28 days. The phytotoxic level of nickel found in the tissue of these plants varied from 90 to 130 mg/kg DW and in soil from 1630 to 2310 mg/Kg Ni.

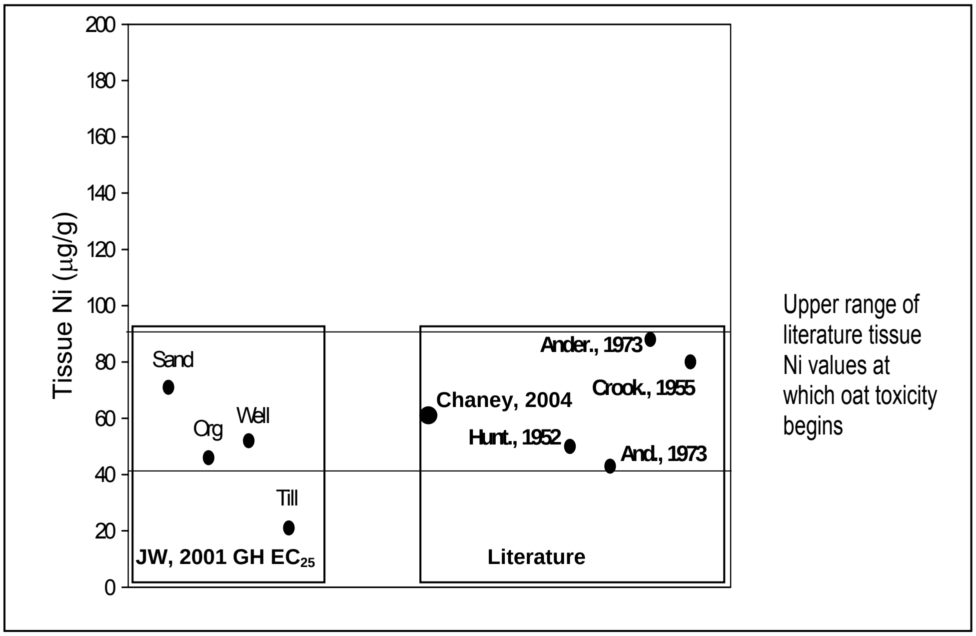
In Welland Clay soils: In the Welland Clay soils, chlorosis was observed in oat seedlings, over the entire leaf surface four days after emergence, and was noted to be most severe in plants grown at the highest nickel concentration. At maturity (after 28 days for radish and 70 days for oats), no phytotoxicity was observed in any of the plants and treatments. This corresponded to a level of about 55 mg Ni/kg in the plants and 1900 mg Ni/kg in soil (the highest dose).

In Organic soils: Chlorosis was noticed mainly in the older leaves and white banding was visible along the leaf blades. In addition to interveinal chlorosis, necrotic lesions were also noticed in older leaves. These symptoms, described as the “gray speck” by Mengel and Kirby (1982) have previously been attributed to manganese deficiencies. Plants growing in soils with the highest levels of total nickel were slender with few tillers as compared to oat growing in the lower soil nickel concentrations. This corresponded to a level of about 40 mg Ni/kg in the oat tissue and 2400 mg Ni/kg in soil (the highest dose).

4.1.3 Comparison of GH 2001 Oat Tissue Ni Concentrations to Literature-Reported Oat Tissue Ni Toxicity Thresholds

A comparison of Year 2001 derived-EC_{25} soil Ni phytotoxicity thresholds with oat toxicity thresholds for Ni from the literature (Figure 2) demonstrates quite clearly that the observed phytotoxicity occurs within the Ni tissue concentration range or even below that observed in other studies (Hunter and Vergnano, 1952; Anderson et al., 1973, Chaney et al. 2003) perhaps indicating the contribution of the other three CoCs (Cu, Co and/or As) and/or chemical and physical soil properties to plant toxicity. Further, since these literature values were determined at the point where deleterious effect was first or expected to be observed, the EC_{25} values for Port Colborne soils may be considered conservative.

Figure 2 Crop Studies Year 2001 show that oat tissue Ni EC_{25} thresholds are within the range of literature-reported oat toxicological thresholds for Ni



Left Column - Greenhouse Yr 2001 soil Ni EC 25 concentrations varying from 1350 to >2400 mg/kg Ni

Thresholds reported in the scientific literature for soil Ni varying from 78 to 2900 mg/kg

4.1.4 Integration of Year 2001 Greenhouse Crop and Naturally-Occurring Plant Findings.

Data on oat tissue Ni concentrations obtained from the Greenhouse (GH) Trials were compared to data on tissue Ni concentrations of a naturally occurring plant, Goldenrod (*Solidago* spp.), in the Port Colborne area. Goldenrod (*Solidago* spp.) tissue data and oat tissue data were pooled and regressed against log-transformed soil total nickel concentration. The quadratic relationship was determined to be quite strong (r^2=0.68; p<0.0001), a result replicated in a similar regression for greenhouse oat tissue data (r^2=0.69; p<0.0001). The strength of both of these relationships, considering the range in soil parameters in both the field and in the greenhouse, provides solid support for the legitimacy of the EC_{25} thresholds generated from plants grown in the soil blends.

4.2 Field Findings

4.2.1 Phytotoxicity Symptoms

Evidence of phytotoxicity was noted for oats and radishes. For oats, a difference was noted between the Clay 2 and Clay 3 Test Sites. At the Clay 2 Test Site (5,000 mg

Ni/kg at pH 6.4), many stems exhibited slight purple discolorations after about three weeks following germination, but these symptoms disappeared at later stages of growth in all of the treatments. By about five weeks, all of the plants were healthy and green. At harvest the level of Ni in the tissue was 58.1 mg/kg Ni in oats, 37.4 mg/kg Ni in soybean, and 2.6 mg/kg Ni for corn (Volume I, Binder 2, AppendixF-1). These results indicate that the sensitivities of the tested plants is oats>soybean>corn, with the oats being more sensitive.

In contrast, symptoms of phytotoxicity were clearly evident on the plots of the Clay 3 Test Site (3,000 mg Ni/kg at pH 5.6). About four weeks after germination, plants showed visible symptoms of phytotoxicity such as chlorosis and longitudinal white banding, mainly on the older leaves. Eight weeks after germination, approximately 50% of the leaves were necrotic and plants were stunted and slender with less foliage. The agronomical tissue samples collected from the Clay 3 Test Site showed a higher level of nickel in the tissue compared to the Clay 2 Test Site. Reasons for the difference in tissue Ni concentration between the 2 clay test sites are discussed in subsection 4.2.2. The difference in oat and soybean tissue concentrations of nickel for the Clay 3 Test Site was not statistically significant and thus the sensitivity of oats and soybean are similar, with corn still being the least sensitive.

4.2.2 Tissue nickel

When comparing the accumulation of nickel in the tissues of oats cultivated at the two field sites (one with a level of approximately 3000 mg/kg Ni and the other one with a level of 5000 mg/kg Ni in the soil), a negative correlation was found (R square= -0.959). This was due to a much lower tissue nickel concentration in tissue of plants at harvest (about 58.1 mg/kg Ni) cultivated at the site with higher nickel level in the soil. When pH was added as a regression variable, the relationship changed significantly. That is, the bioavailability of nickel is much greater at lower soil pH (ie. pH 5.6 at the Clay 3 Test Site compared to pH 6.4 at the Clay 2 Test Site). This new evidence, generated by using the field data from the two field sites in a similar manner as the dose-response experiment conducted under greenhouse conditions, shows clearly that soil metal concentration is *only one* of the factors that is responsible for accumulation of metals in tissue. Other factors such as pH play a significant role in the accumulation or the lack of accumulation of metals in plants.

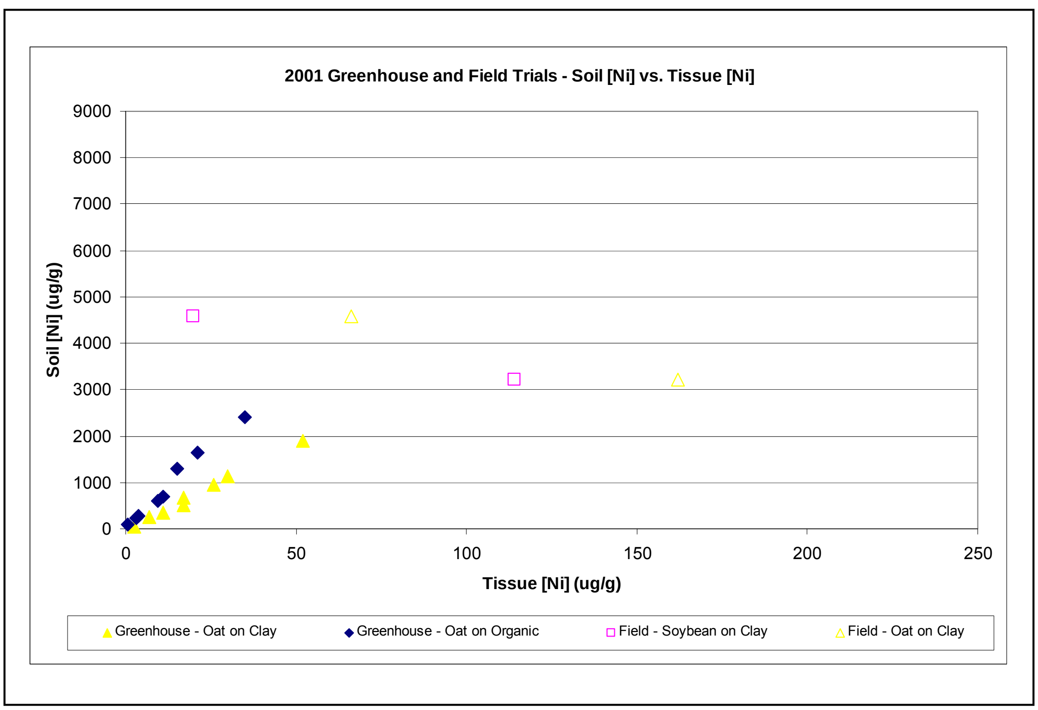
Weng et al. (2003) linked the separate effects of pH on sorption of Ni to soil and plant (i.e. increasing pH enhances shoot Ni accumulation from solution due to reduced competition for root uptake sites between H^+ and Ni^{2+}, balanced against the increased binding of Ni^{2+} to soil particles). Their function (log [Ni-shoot] = 3.66 + 108 log [Ni-soil] – 0.63pH, all concentrations in mmol/kg) describes the bioaccumulation of Ni as a function of total Ni content of the soil, and pH, derived from hydroponic and soil experiments on oat and soluble Ni, as $Ni(NO_3)_2$. It predicts that for 900 mg/kg soil Ni, oat shoots would accumulate over 800 mg/kg Ni in tissue, which compared to the concentration calculated from the function generated by the data in the present CBRA study at this soil Ni concentration (900 mg/kg soil Ni), was approximately 50 mg/kg Ni in tissue. When compared to field data findings from the Port Colborne soils it was found that at only plants growing at levels higher than 5000 mg/kg Ni could accumulate more than 50 mg/kg Ni in the tissue (pH near neutral range).

There are several points to make on this comparison. First, it proves that soluble Ni species cannot be used to set soil criteria for Ni in Port Colborne soils that contain insoluble forms of Ni (see Section 1.1). Second, it seems clear from this comparison that speciation of the Ni in the soil, in addition to the master variable (pH) and binding capacity of the soil must be considered when predicting bioavailability. More importantly the apparent differences can clearly be attributed to differences in exposures in Ni speciation and soil characteristics, proving that Port Colborne soils are unique.

4.2.3 Integration of Year 2001 Greenhouse and Field Crop Findings.

Both field and greenhouse tissue and soil nickel concentration data for the Year 2001 are shown in Figure 3. Compared to the greenhouse 2000 dose response data (Figure 1), the greenhouse 2001 dose response data in Figure 3 produce better fitting lines. The lines representing the greenhouse data in Figure 3 are representative of data points that reflect tissue Ni accumulation in oats that depend only on soil nickel concentration as all the other soil variables are kept constant. The scattered field data points in Figure 3 reflect differences in soil chemistries (e.g. pH, organic matter, cation exchange capacity, etc) which influences plant uptake of CoCs.

Figure 3 Crop Studies Year 2001 - Plant tissue Ni and Soil Ni concentrations in greenhouse studies and field studies.



5.0 BRIDGING THE GAP WITH THE PEER REVIEWER

This section provides a weight of the evidence approach using existing data from the Year 2000 and 2001 Crops Studies to address each of the five concerns identified by the external peer reviewer. As identified earlier in Section 1.0, these concerns included:

1). Usefulness of the Year 2000 data;

2). Use of oat as a single indicator species;

3). Use of plant biomass vs. economic yield;

4). Blending versus unblending and generation of EC_{25} values using unblended soils; and

5). Confidence intervals.

5.1 Usefulness of the Year 2000 data

The external peer reviewer had concerns of deriving soil Ni EC_{25} values using the GH Year 2001 data because these data were based on ‘blended’ soils. An inference was made by the reviewer that perhaps more realistic EC_{25} values could be obtained using the GH Year 2000 data which were based on ‘unblended’ soils.

Notwithstanding the uncertainties in the Year 2000 greenhouse data to establish reliable soil Ni EC_{25} values as mentioned in Section 2, an attempt was made to back calculate or predict soil Ni EC_{25} values in soil using the Year 2000 greenhouse dose response data and several assumptions as stated below.

According to Kukier and Chaney et al. (2004), a decrease of 25% in yield was measured when oat grown on Welland Clay ‘unblended’ soil in Port Colborne accumulates more than 62.7 mg Ni/kg in tissue. This value is in near agreement with Jacques Whitford’s GH Year 2001 findings on tissue nickel accumulation at 52 mg Ni/kg in oat growing on Welland Clay ‘blended’ soil corresponding to a 25% decrease in yield. This literature value is also in near agreement with Jacques Whitford’s GH Year 2001 findings that showed a tissue nickel accumulation of 46 mg Ni/kg in oat growing on Organic ‘blended’ soil corresponding to a 25% decrease in yield. As Jacques Whitford’s EC_{25} values for Welland Clay and Organic soils were both based on the ‘blending’ experiment of Year 2001, the Kukier and Chaney et al. (2004) literature value of 62.7 mg Ni/kg based on ‘unblended’ soils will be used for this calculation to define the point where there is a decrease of 25% in tissue Ni concentration of crops grown on ‘unblended’ soils of the GH Year 2000 experiment.

Regression was done on GH Year 2000 data as a function of soil Ni concentrations versus tissue Ni concentrations representing the dose response data for oat grown on Welland Clay soil (Figure 4) and also on Organic soil (Figure 5). As stated earlier, Welland Clay and Organic soil are representative soil types of the agricultural areas in Port Colborne. For both Figures 4 and 5, the y-axis is the soil Ni concentration and the x-axis is the tissue Ni concentration.

Figure 4 Year 2000 Crop Studies Estimated EC_{25} in Clay soils using threshold tissue nickel concentration.

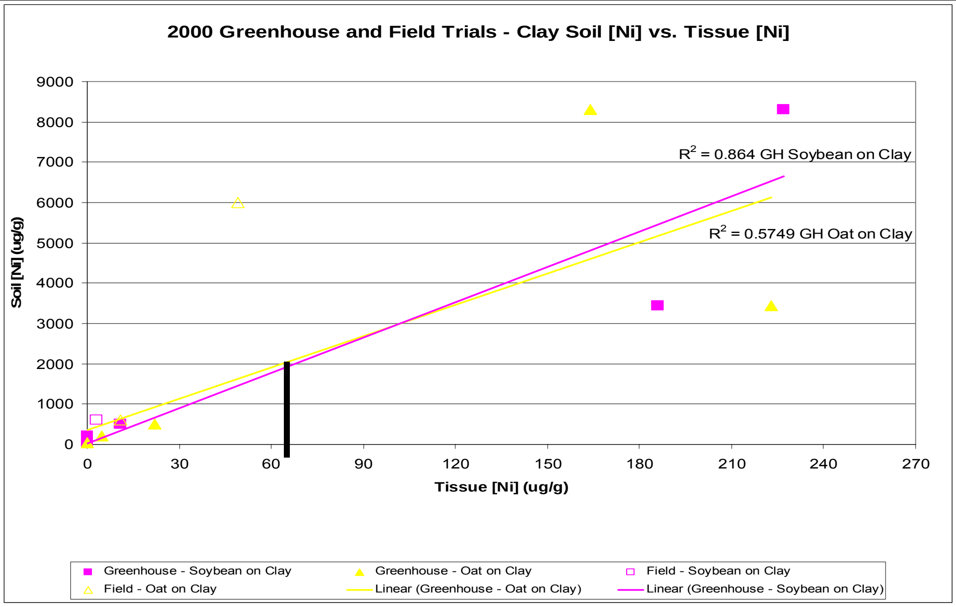
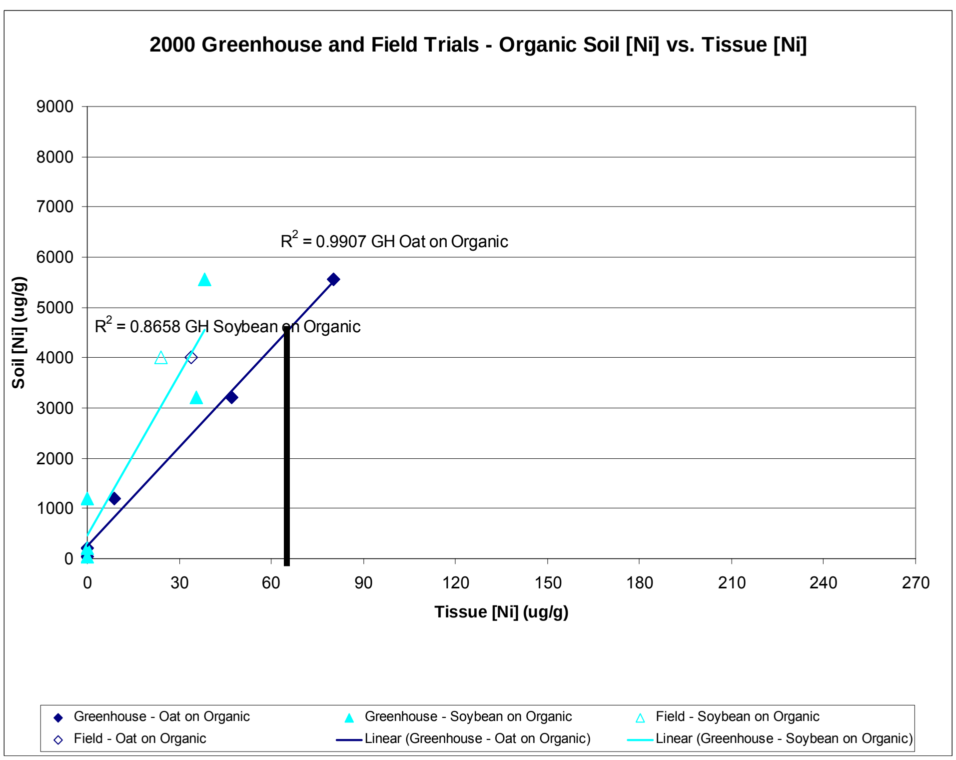


Figure 5 Year 2000 Crop Studies Estimated EC_{25} in Organic Soils using threshold tissue nickel concentration.



The intersection of the slope of each regression line in Figures 4 and 5 with the tissue Ni EC_{25} at 62.7 mg Ni/kg on the x-axis provide a corresponding soil Ni EC_{25} on the y-axis for oat and soybean studied in Year 2000. For soybean, it is assumed for this calculation that soybean would have a similar symptom as oat to Ni toxicity at 62.7 mg Ni/kg causing a 25% decrease in yield biomass. Predicted Ni EC_{25} values for the GH 2000 oat dose response experiment are provided in Table 3.

Table 3 Predicted Ni EC_{25} using Year 2000 Greenhouse Data

Dose-Response Experiment	Ni EC_{25} in Welland Clay ,mg Ni/Kg		Ni EC_{25} in Organic Soil ,mg Ni/Kg	
	Predicted for Yr 2000	Measured in Yr 2001	Predicted for Yr 2000	Measured in Yr 2001
Oat	2000	1880	4500	>2400; 3400*
Soybean	2000	nm	7000	nm

* - derived from meta- analysis using both the 2001 and 2000 oat on Organic Muck soil data.
nm - not measured

The data in Table 3 show no significant differences in soil Ni EC_{25} values between the Year 2000 and Year 2001 dose response data for oat grown on Welland Clay and Organic soil, when they are predicted from tissue Ni concentrations. Table 3 also shows that the predicted values of soil Ni EC_{25} for soybean are similar, if not greater than those of oat.

5.2 Use of oat as a single indicator species

Oat is usually considered the most characteristic plant indicator of nickel phytotoxicity based on research of Vergnano and Hunter (1952) which has been corroborated repeatedly over 50 years of further research (e.g. Anderson et al. 1973). In oat, the visible toxicity symptom specific to nickel phytotoxicity is an alternating pattern of more chlorotic and less chlorotic bands across young leaves and iron deficiency is observed as interveinal chlorosis. It is because of the uniqueness and sensitivity of oat to Ni phytotoxicity, that oat was selected as the crop for the GH 2001 work.

In addition of oats, radishes, soybean, corn and golden rod were evaluated by Jacques Whitford. Integration of phytotoxicity data from all plant species was done. Oats was shown to be more sensitive to the site-specific conditions under both field and greenhouse conditions. This was corroborated by findings in the literature (Chaney et al 2004, Chaney et al., 2003 and references within).

Predicted values of Ni EC_{25} values for soybean on Welland Clay and Organic soils based on the GH 2000 soybean dose response data and the assumptions made in Section 5.1 were shown in Table 3 to be similar, if not greater than the predicted values of Ni EC_{25} for oat.

5.3 Use of Plant Biomass vs. Economic Yield

Chronic effects of metals on plants are usually assessed by long term growth assays and are mostly quantified by measuring plant biomass of the plants after the treatment (exposure) period. Most authors determine the final dry mass of the shoot (more details and references are found in Appendix B). This gives a good indication of a plant's ability to germinate and compete successfully for water, light and nutrients, and play a significant role in ecosystem processes when growing in the presence of elevated metals - these abilities, or endpoints, are identified at the beginning of the risk assessment process.

Plant biomass is a standard measurement used in phytotoxicity studies. The results obtained by using plant biomass data are reliable and comparable with other well documented scientific studies. Interpretation of plant biomass data as it relates to economic yield was validated by discussions with OMAF representatives, local farmers and crop insurance companies.

The farming community in Port Colborne is reporting average yields for the traditional crops cultivated as part of the cash crop rotation. The yields in Port Colborne are comparable with the ones reported by the OMAF for other parts of Southern Ontario, and sometimes even higher. These reports have been confirmed by local farmers (undisclosed farmers) and crop insuring companies (Agricorp Insurance). In Year 2005, the average farm in the Port Colborne area obtained the same average yield for soybean at about 30 bu./acre compared to other farms in Southern Ontario. This reported yield in soybean for Year 2005 represents an increase of 10 bu./acres compared to previous years.

5.4 Blending versus Unblending and Generation of EC_{25} Values using Unblended Soils

Blending of soils to achieve specific Ni concentrations did not result in decreased Ni bioavailability (as measured from water and DTPA soil extractions) and therefore provides confidence that the toxicological thresholds determined in the GH 2001 Trials are relevant for Port Colborne soils (Volume I, Part 3, Section 4.11.4 of the December 2004 Final Crops Report).

The high Ni and background soils used in the 2001 Crop Study were matched for soil properties, including nutrient status. There is no logical basis as supported by the 2001 fertility analyses data (Final Crops Report, Vol. I, Binder 2, App. S 1-1.1, Table 2) to indicate that high-Ni soils are any less fertile than corresponding background soils within each soil type (see Volume V of Appendix A containing the external peer reviewers comments). Furthermore, there are no data that suggest a fertility impact could be generally upheld for high Ni (within the range of soil Ni concentrations tested in 2001) and low-Ni soils in the field. Clear evidence of good fertility was shown when evaluating the EC_{25} values obtained with plants grown in the sand soil with the EC_{25} obtained from the other soils. The sand soil, representing beach sand collected west and east of the Welland Canal and subsequently blended, had limited fertility, but that did not affect the $EC_{25,}$ which was derived at about 1350 mg/kg Ni. This indicates that blending did not change fertility and that the soil blends used in the greenhouse experiments were representative of the natural soils found in Port Colborne area.

Oat grown in the field in Year 2001, in the Clay 2 Test Site, a natural field soil with approximately 5000 mg/kg soil Ni, accumulated tissue Ni approximating 58.2 mg/kg. This concentration of tissue Ni was also observed in the greenhouse blended soils *but* at soil Ni concentrations at 1900 mg Ni/Kg less than approximately one-half of this total soil Ni value (5000 mg Ni/kg), thus indicating that 1) phytotoxicity is more pronounced in the greenhouse compared to the field and 2) the plants grown in blended soils in the greenhouse were not able to avoid the soil Ni because of blending with background soil, in fact, absolutely the opposite occurred.

Also as discussed in Section 5.1 and documented in Table 3, when data from the Year 2000 GH experiment that used 'unblended' soils were used to predict a soil Ni $EC_{25,}$ the predicted soil Ni EC_{25} values were similar to those of the measured Ni EC_{25} values of the Year 2001 GH experiment that used 'blended' soils. Clearly, the process of soil blending did not bias the calculated soil Ni EC_{25} values and thus the reported soil Ni EC_{25} values in the Jacques Whitford December 2004 Final Crops Report remain valid.

5.5 Confidence intervals

As previously explained (see Appendix B for more details) there are two calculations for confidence intervals (CI) around a regression relationship – the population CI's and the data CI's. The former are used to estimate the precision with which the regression relationship will predict Y from the observed values of X, when the intent of the regression relationship is to predict the response of oat plant biomass (Y) to soil Ni (X) over the entire population of Welland Clay soils, which are assumed might vary considerably from those used to determine the relationship. Hence, these CI's are wider than those calculated as the data CI's, which are used to estimate the precision with which the regression relationship predicts Y from the observed Y and observed X (the intent of the CI's depicted with the Weibull functions in the crop report). While it might seem intuitive that the former is more appropriate for risk assessment than the latter, neither actually is correct for the purpose of predicting a value of X for a particular value of Y, as we wish to do by predicting the soil Ni value that is associated with a 75% reduction in plant growth. This is called *inverse prediction* and involves an additional error term to those (errors associated with the estimates of the regression parameters, as well as the unexplained error) used in *prediction*, namely the errors associated with the random, normally distributed variable Y. The confidence intervals for inverse prediction are not symmetrical around the predicted value of X, but like CI's for prediction, get wider with distance from the mean value of Y.

After evaluating the risk to oat growth from soil Ni in the greenhouse trials, a second tier in the crop risk assessment was implemented with higher environmental realism by using field trials. Site-specific weather and biotic factors (microbes, insects, plants etc) contribute to a heterogeneous distribution of the metals in the area (as characterized by soil CoC maps) and affects the phytoavailability of CoCs. It is the findings obtained here that show that greenhouse findings are conservative. These are due to the nature of exposure such as (Chaney et al., 2003): 1) the continuous exposure to metals in the pot-greenhouse environment which is contrast with the natural environment conditions where roots continue to explore the soil environment for nutrients below the 30 cm layer were most of the contamination can be found; 2) the length of the roots is reduced in greenhouse studies compare to field studies due to the reduced phytoavailability of phosphorus, 3) variation in the soil daily temperature and humidity is different in greenhouse studies vs. field studies affecting the flow nutrients from shoots to roots.

Therefore EC_{25} levels measured by using greenhouse pot experiments have already built in a level of protection. Furthermore PNEC values which have been derived from these EC_{25} values are even more protective. In order to address any uncertainties that might not have been accounted for, PNEC values will be used in risk management.

5.6 Summary

The preceding discussion has, in our opinion, satisfactory addressed all five of the external peer reviewer’s concerns of September 26, 2005. We believe that the perceived gap between Jacques Whitford’s findings and interpretation as found in the December 2004 Final Crops Report and those by the peer review in his letter of September 26, 2005 has been considerably narrowed, if not completely eliminated.

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APPENDIX A

Responses from External Peer Reviewer, February 14, 2005

Final Comments on JWEL Response to Reviewer Concerns (February 14, 2005)

M.B. McBride
Cornell University

I have reviewed the responses of JWEL to my concerns about the Port Colborne crops study. I remain unconvinced that JWEL has demonstrated with an acceptable degree of certainty that phytotoxicity is not a concern in soils with total Ni concentrations below the JWEL-derived EC25 values. My argument is based on a weight-of-evidence approach, in looking at all the available and relevant evidence, both directly from JWEL's own studies and other studies of the soils at the impacted site of Pt. Colborne, as well as indirectly from studies of Ni toxicity in the field in other locations. I summarize the 4 main points of my argument below:

A. JWEL's Year 2000 Greenhouse Experiments Provide Evidence for Ni Phytotoxicity at Soil Concentrations Below the EC25 Values Estimated from the Blended-Soil Experiment

There is a critical difference of opinion that comes down to how much weight should be given to the year 2001 Blended soil- greenhouse single crop (oat) study relative to the year 2000 greenhouse study with 3 crops – oats, soybeans and corn. I have reached the conclusion that the year 2000 study cannot be ignored because:

1. it grows crops in soils taken directly from the impacted area, and not modified by blending with large fractions of fertile soil.
2. it measures phytotoxic response in 3 crops, not just one, and does it at 2 lime addition rates as well as for the unamended soil.
3. the yield decreases resulting from increasing total soil Ni levels are consistent with the measured soluble (immediately bioavailable) levels of Ni in the soils,
   and with published studies that indicate the concentrations of soluble Ni expected to be toxic.

Therefore, there are simply more statistically significant data in support of Ni phytotoxic thresholds from the year 2000 studies than from the single-crop blended-soil study of 2001.

The argument by JWEL that the year 2000 results are too confounded by soil effects unrelated to Ni to be useful does not stand up to close scrutiny – the only results from year 2000 that appear anomalous are for one high-Ni Organic soil (3180 mg/kg Ni). This soil showed much better crop growth than would have been expected based on comparison to Organic soils lower and higher in Ni – strongly implicating fertility factors other than Ni toxicity. However, JWEL noted that this soil was collected from a farm field, while the Organic soil even higher in Ni (5550 mg/kg) was taken from a woodlot. Obviously, the farm soil would have historically received substantial fertilizers and probably micronutrients as well (JWEL's soil tests confirm the high nutrient status of the "anomalous" soil).

The anomalous behavior of this one soil does not justify discounting the results for all the soils, particularly since the crop phytotoxicity response for the sand and clay soils are very consistent as soil Ni concentration increases. These yield responses are also consistent with the measured water-soluble Ni in the soils. I have tabulated the data (unlimed soils only) below to show the generally clear trends in crop response to soluble and total soil Ni. The results for the sand soil are particularly clear – all three crops show significant growth inhibition at the 307 mg/kg soil Ni level. This result indicates that the EC25 determined for Ni in sand soil from the Year 2001 Blended Soil Experiment may be much too high.

The summarized results from JWEL's report on the year 2000 corn, soybean and oat crops grown on organic, clay and sand soils are given in Tables 1 , 2 and 3 below (I did not include the lime treatments in these Tables).

Table 1: Summarized Greenhouse Year 2000 Results – Organic Soil

Total Ni (mg/kg)	Soluble Ni (mg/kg)	Soil pH	Corn Dry Wt.	Corn Ni (mg/kg)	Soybean Dry Wt.	Soybean Ni (mg/kg)	Oat Dry Wt.	Oat Ni (mg/kg)
33	< 0.6	5.0	na	na	7.8	<0.1	2.7	<0.1
216	<0.6	6.7	6.62	<0.1	11.6	<0.1	2.6	<0.1
1200	1.2	6.3	3.44	<0.1	13	<0.1	0.6	8.9
3180	**9.1**	**5.4**	**8.51**	**2.8**	**8.5**	**35.6**	**5.1**	**47**
5550	11.2	5.7	1.25	11.6	3.9	38.3	0.5	80.4

Table 2: Summarized Greenhouse Year 2000 Results – Clay Soil

Total Ni (mg/kg)	Soluble Ni (mg/kg)	Soil pH	Corn Dry Wt.	Corn Ni (mg/kg)	Soybean Dry Wt.	Soybean Ni (mg/kg)	Oat Dry Wt.	Oat Ni (mg/kg)
34	< 0.6	7.3	na	<0.1	na	<0.1	na	<0.1
194	0.5	6.7	8.8	<0.1	13.3	<0.1	na	4.8
517	1.7	5.5	9.3	<0.1	8.5	11	na	21.9
3430	8.6	5.4	2.0	73	1.1	227	na	164
8280	13.6	5.8	2.7	112	1.0	186	na	223

Table 3: Summarized Greenhouse Year 2000 Results – Sand Soil

Total Ni (mg/kg)	Soluble Ni (mg/kg)	Soil pH	Corn Dry Wt.	Corn Ni (mg/kg)	Soybean Dry Wt.	Soybean Ni (mg/kg)	Oat Dry Wt.	Oat Ni (mg/kg)
5	< 0.6	7.3	14.6	<0.1	16.4	<0.1	2.4	<0.1
307	<0.6	7.2	10.8	<0.1	11.7	5.6	1.3	37.5
494	0.7	7.6	6.2	<0.1	8.6	8.6	0.9	48.4
1350	3	7.2	6.4	6	6.1	55	0.43	116

In terms of improving the year 2000 greenhouse trials, I agree with JWEL that it would be better to equalize soil fertility parameters to the extent possible – this could have been done by repeating the Year 2000 Greenhouse experiment with soils amended with fertilizer and lime if necessary (lime is not needed for the sand soil as it has a pH above 7).

The discrepancy between the severity of phytotoxicity seen with the Year 2000 soils compared to the Year 2001 Blended soils is somewhat puzzling and unexpected to me. As JWEL points out, the blended soils do seem to represent the soluble Ni levels fairly accurately when compared to unblended soils from the field with similar total Ni concentrations. Nevertheless, there is an assumption built into the Blended-soil concept that has not been tested. Consider, for example, how the blended clay soil with 339 mg/kg Ni was prepared – a grossly Ni-contaminated soil (containing 8655 mg/kg Ni) was mixed with an uncontaminated fertile soil. To get 339 mg/kg final Ni, the soil would be composed of 96 % fertile soil. It seems likely to me that such a mixture would retain many of the desirable properties of the fertile soil. This mixed soil might not represent the degree to which the field-impacted soils were affected by Ni displacement of important micronutrient metals and macronutrients over the decades of pollutant deposition in the field. Other changes in the impacted soils not directly related to Ni content could also have occurred, and these would not be mimicked by the Blended soils.

Possibly related to the effects of blending soils, it is curious that JWEL's measures of $Sr(NO3)_2$-extractable Ni in the blended soils, even at high total Ni, are much lower than that reported by Kukier and Chaney (2001) for the Welland silt loam containing 2930 mg/kg total Ni. Those authors report 54 mg/kg extractable Ni for the Welland soil, much higher than the maximum of 3.1 mg/kg reported for the Blended Welland clay soil (1806 mg/kg Ni) in the JWEL report. In fact, few of the blended soils in the JWEL report show any detectable Sr-exchangeable Ni, which I suspect is not generally the case for impacted soils taken directly from the field in the Pt. Colborne area. It is also disconcerting that the "aqueous" Ni that was extracted from most of the Ni-contaminated soils was higher by a large margin than $Sr(NO_3)_2$-extractable Ni. This does not make chemical sense, and I am concerned that there was an analytical error with Ni determination in the $Sr(NO_3)_2$ extracts.

Because the inadvertent loss of a number of the control crop and one oat crop yield data for the Year 2000 experiments weakened the conclusions, I feel it is important to repeat these greenhouse experiments to strengthen the evidence for phytotoxic thresholds on all three soils. JWEL has argued that a field experiment (the better experiment in my view) is not feasible, but the greenhouse experiment as a substitute would involve considerably less cost.

B. **Oats is Inadequate as a Single Indicator Crop for Ni Phytotoxicity**

- oats are not an economically significant crop in the Pt. Colborne area

- there is no strong evidence in the literature that oats are a particularly sensitive crop. In fact, on pg. 4-35 of the 2004 revised report, JWEL states that "in general, the monocots (oats and corn) were more resistant to phytotoxicity than the dicots (radish and soybean)". JWEL's choice of 50 mg/kg Ni in oat leaves as a threshold of phytotoxicity in itself suggests some tolerance of oats to Ni, as lower thresholds have been reported for several other crops. Dijkshoorn et al.(1979) showed for example, that grass was more tolerant than two broadleaf species, clover and plantain, based on the shoot Ni concentration that caused 50% yield depression.

- oats is a cool-weather crop not well-adapted to greenhouse culture where air and soil temperatures can be quite warm. The low dry-matter greenhouse yields that JWEL obtained for oats compared to corn and soybean attest to this fact. Plants under stress from physical conditions may not show clearly measurable responses to additional stresses from phytotoxic metals.

C. **Field studies on the Ni-impacted organic soils at Port Colborne have already verified severe phytotoxicity at Ni concentrations well below the EC25 values (> 2400 mg/kg Ni) derived by JWEL for these same soils.**

Any objective analysis of the Frank et al. (1982) field crop yields shows frequent severe decreases in yield exceeding 50% at soil Ni concentrations at or below JWEL's EC25 values. For example:

- more than 50% beetroot yield with soil Ni increasing from 1570 to 2180 mg/kg, and zero marketable beet yield at 2075 mg/kg Ni.

- "on soils with 1200 mg/kg Ni, celery production was severely reduced; 59% in 1980 and 66% in 1981"

- in Table 9, reductions in expected yields of head lettuce were measured at all levels of soil Ni from 1300 to 5110 mg/kg, although the reduction in yield at 1300 mg/kg was seen in only one of 2 cropping seasons. Thus, for lettuce, there may be about a 20% average yield reduction at 1000-1500 mg/kg (see Fig. 7 in Frank paper), although there is substantial uncertainty in this estimate. At 1500-2000 mg/kg Ni, however, there is clearly about a 25% yield reduction.

- Note that Frank et al. found considerable differences in year-to-year manifestation of Ni toxicity – this should be expected as generally there is a strong interaction between climatic conditions and plant stresses, whether they be from toxic metals or some other growth-limiting factor.

- Also note that one weakness of the Frank et al. studies is the lack of control plots with low soil Ni, preventing a more certain establishment of the lower threshold of Ni toxicity in these organic soils. Nevertheless, it is indefensible to derive EC25 thresholds for these soils that are above the soil Ni concentration that actually caused severe yield losses in the field.

D. Soluble Ni levels in the Pt. Colborne soils indicate high potential for phytotoxicity when compared to other sites where Ni phytotoxicity was observed.

One published study of a naturally high-Ni soil (Anderson et al., 1973) , for example, showed that as little as 0.3 mg/L of Ni in extracted soil solution was moderately toxic to oats, and severe toxicity was observed when soil solution reached as high as 3.25 mg/L Ni. Oat plants were normal where the soil solution contained 0.13 mg/L Ni. It is useful to compare these solubilities with those reported by JWEL for water-extractable Ni (see Tables 1-3 in this report), which reached the following values:

- 1.2 mg/kg (soil wt. basis) in the organic soil at 1200 mg/kg total Ni

- 1.7 mg/kg (soil wt. basis) in the clay soil at 517 mg/kg total Ni

- 0.7 mg/kg (soil wt. basis) in the sand soil at 494 mg/kg total Ni

These water-extractable Ni numbers are not directly comparable to the true soil solution Ni concentrations of Anderson et al.(1973), but are in fact underestimates of the actual soil solution Ni in the Pt. Colborne soils (because the soil weighs more than the soil solution within the soil). Therefore, we can reasonably predict that the organic, clay and sand soils containing total Ni below the EC25 values derived by JWEL would have several mg/L of Ni in soil solution, which according to Anderson et al. (1973) should be quite toxic to oats. In fact, the lower detection limit of water-extractable Ni of 0.6 mg/kg reported by JWEL (see Tables) could already be within the toxic range, as this level corresponds to soil solution Ni exceeding 1.0 mg/L. Obviously, there could be protective factors (e.g., high Ca) within particular soils that could mitigate the toxicity of soluble Ni, yet this analysis shows that the Pt. Colborne soils have more than enough soluble Ni to cause phytotoxicity in some situations.

References:

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Frank, R., K.I. Stonefield and P. Suda. 1982. Impact of nickel contamination on the production of vegetables on an organic soil, Ontario, Canada, 1980-1981. The Science of the Total Environment, 26, 41-65.

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APPENDIX B

Jacques Whitford's July 26, 2005
Response to External Peer Reviewer's February 14, 2005 Comments

Jacques Whitford

Jacques Whitford Review and Responses to Expert Peer Review Comments of June 13, 2005

prepared on July 26, 2005

Based on the comments and suggestions received at the consultants meeting held in Toronto on June 13, 2005, Jacques Whitford has agreed to review and address the following 6 points:

1). Usefulness of the Year 2000 data
2). Use of oat as a single indicator species
3). Use of plant biomass vs. economic yield
4). Soluble Ni review
5). Blending – unblending; EC_{25} using unblended soils
6). Confidence intervals

Jacques Whitford Responses:

1). Usefulness of the Year 2000 Data

The issue is that the soybean data from the Year 2000 Greenhouse Study suggest that this crop would have a reduction in shoot growth of approximately 35% at soil Ni concentrations of approximately 500 mg/kg, far below the EC_{25} value set for soil Ni using oat in the Year 2001 Greenhouse Study. These data suggest that soybean is more sensitive than oat, and that agronomic production in Port Colborne (typically a corn/soy rotation) will not be protected by the EC_{25} established using oat. This concern must be addressed, as the reviewer is correct when he asserts that Port Colborne farmers are more likely to grow soybean (as part of a corn/soy rotation) than they are to grow oat.

Kukier and Chaney (2004) provide some evidence that soybean is more sensitive than oat. They determined EC_{25}'s for a range of crop species, including corn and oat, using one Welland Clay soil with 2900 mg/kg of Ni, and two levels of amendment with lime to raise the soil pH thus lowering the effective Ni concentration of the soil, thus creating "control" soils. This is not likely to produce identical relationships between plant response and soil Ni as those seen in the field, or in the Jacques Whitford Greenhouse studies, as while the amounts of soluble Ni might be the same, the amount of exchangeable Ca and Mg in the created "control" soils is likely to be considerably higher than in similar field soils that have not been limed. However, this study has some usefulness to this discussion, as the species responses within the study are comparable. In this study, the EC_{25} for soybean is identified as occurring at a $Sr(NO_3)_2$ soil Ni concentration (4.6 mg/kg) which is slightly lower than that for oat (5.7 mg/kg), supporting the reviewer's contention that soybean is more sensitive than oat. However, these values are very close to each other, and are likely statistically not different, and may represent soils with different concentrations of Ca and Mg, thus differing potential for competition with Ni for uptake. The shapes of the dose-response curves in Figure 5 (of the paper) for oat and soybean are essentially identical, and in fact, the authors categorize them both (along with bean and radish) as "sensitive", based on the determination of their fractional yield in limed soil *vs.* calcareous soil. Using this method, corn, ryegrass, wheat and barley were "resistant", and redbeet, Swiss chard and tomato were "very sensitive". This order is in good agreement with the crop sensitivity classification based on the slope of the relationship between plant Ni to soil exchangeable Ni, as follows: (from the least to the most) barley>wheat>ryegrass>corn>bean>soybean> radish>oat>tomato>redbeet>Swiss chard. Their observation suggests that dose response relationships between $Sr(NO_3)_2$ soil Ni concentration and toxicity might also be more acute for oat than for soybean and corn, if the assumption that tissue accumulation of Ni and toxicity are linearly related, is true.

There are several pieces of evidence that suggest that the Year 2000 Greenhouse Study data for soybean are not predictive of what will happen in the field. One piece of evidence that the 2000 Greenhouse (GH) Study may not reflect correctly what would be expected in the field, is the tissue concentration of Ni in soybean plants grown at 500 mg/kg which is associated with approximately 35% loss of growth in comparison to control plants. This value was only 11 mg/kg dry weight, somewhat low to be the sole cause of a 35% yield reduction for soybean. Kabata-Pendias lists 1-10 mg/kg tissue Ni as the tolerable range for agronomic crops, and Kukier and Chaney (2004) determined that tissue Ni for soybean at 25% reduction of shoot growth was 32.6 mg/kg, which is three fold greater than that associated with a 35% reduction in shoot growth observed in Year 2000 GH study for soybean. Thus, we conclude that it is likely that factors other than soil Ni caused a considerable portion of the growth loss in soybean in Year 2000. Those factors are unidentified at this time, beyond noting that soil water relations influence availability of Mn and Fe, more than of Ni, and there is a significant interaction among these in their effect on plant growth. There are no remaining tissue samples with which to explore the possible cause, nor are there archived soils. There is additional evidence that something in the cultural practices of the Year 2000 GH soybean plants resulted in far greater tissue concentrations of Ni relative to soil Ni concentrations, than would be expected in the field. As noted earlier in this section, soybean grown in Welland Clay with 500 mg/kg soil Ni had tissue Ni concentrations of 11 mg/kg. Soybean plants grown in the field in the Year 2000 at C1 (one of the Clay sites) with an average soil Ni concentration of about 600 mg/kg Ni had tissue concentrations of Ni that were less than 3 mg/kg. The same difference was noted for oat, which in Year 2000 GH accumulated approximately 22 mg/kg tissue Ni on 500 mg/kg soil Ni in Welland clay, whereas the same species grown in the field at C1, with 600 mg/kg soil Ni, accumulated half that concentration of Ni in the tissue. This difference between field and Year 2000 GH is likely due to the rooting zone of field-grown plants which extends below the upper layer of soil where Ni concentrations are the highest. *Thus, we would anticipate that the toxicity observed in the pot-grown soybean, and oat, in both Year 2000 and Year 2001 GH studies, would be greater than that expected in the field-grown crops, relative to soil Ni concentration.*

2). Use of Oat as a single indicator

The usefulness of oat vs. soybean has been addressed above. Additionally, Jacques Whitford designed and conducted the Year 2001 Greenhouse with more than one crop. Radish was chosen as an alternate crop species to provide context for the EC_{25} values, as Frank et al. (1982) demonstrated that it was the most sensitive of a group of horticultural crops, to elevated soil Ni in organic soil. Jacques Whitford conducted a dose response experiment with radishes in the Welland Clay soil. The results of this experiment were presented in Final Report of the Crop Studies in volume I, Part 3, Appendix GH-4-2. Specifically a 100% seed germination was achieved at all soil CoC levels. Visual toxicity symptoms were observed only on plants at the highest level of exposure where plants exhibited mild interveinal chlorosis. Plant yield (measured by two indicator parameters: above ground biomass and globes) was unaffected by the increasing CoC exposure. Although it could have been stated that for radish the EC_{25}> 1900 mg/kg soil Ni, Jacques Whitford has used the conservative approach and stated that "the EC_{25} values applied to radish will be adopted based on those determined for the oat grown on Welland Clay soil".

3). Use of plant biomass *vs.* economic yield

For some crops, of course, the plant biomass is the economic yield. For other crops, such as oat, the biomass influences the economic yield, but is not the economic yield. Chronic effects of metals on plants are usually assessed by long term growth assays and are mostly quantified by measuring plant biomass of the plants after the treatment (exposure) period. Most authors determine the final dry mass of the shoot (Baker et al., 1983; Dueck et al., 1987; Bernal et al., 1994; Pollard and Becker, 1996). This gives a good indication of a plant's ability to germinate and compete successfully for water, light and nutrients, and play a significant role in ecosystem processes when growing in the presence of elevated metals - these abilities, or endpoints, are identified at the beginning of the risk assessment process. When the endpoint to be protected is grain yield, the best predictor of that is to actually measure that endpoint in the toxicity testing. However, there is a vast amount of research that has been undertaken to predict the relationships between marketable yield and above-ground biomass, the ratio of which is known as the "harvest index". The harvest index is genetically controlled, but environmental factors can influence its value; for example, the harvest index of semi-dwarf varieties of oat is higher than conventional sized varieties, and too much nitrogen will reduce the harvest index of a crop by promoting vegetative growth. We could find no published literature describing the effect of elevated soil metals on harvest index of grain crops, thus the Crops Study assumes that the harvest index remains the same across the range of reduction in above-ground growth. This is likely a conservative assumption (unless the number of flowers or spikes per plant is reduced by elevated soil Ni) as carbon is usually re-allocated within the shoot to favour reproductive structures, when shoot growth is reduced by environmental stress.

The dry weight responses of the above-ground biomass can be misleading as plants can react to stresses during vegetative stages that compensate for early effects symptoms such that grain yields would not be as significant as what vegetative biomasses might suggest (Ian Collins, Applied Research Coordinator - Field Crops, Ontario Ministry of Agriculture, personal communications). The study by Lynch and Frey (1993) compared agronomic characteristics of oat varieties that were released pre 1963 with those released since 1963. The older cultivars had lower mean harvest index than the newer cultivars (35% *vs.* 47%), demonstrating the genetic improvement in harvest index through selective breeding. In a year where soil moisture was limited, reducing shoot growth, the harvest index increased to 46% and 57%, respectively, for older and newer varieties. These data demonstrate the principal that reduction of shoot growth does not necessarily lead to a similar reduction in grain yield in oat, thus protecting oat at Port Colborne against a 25% loss in shoot biomass will likely protect against a smaller loss in marketable yield.

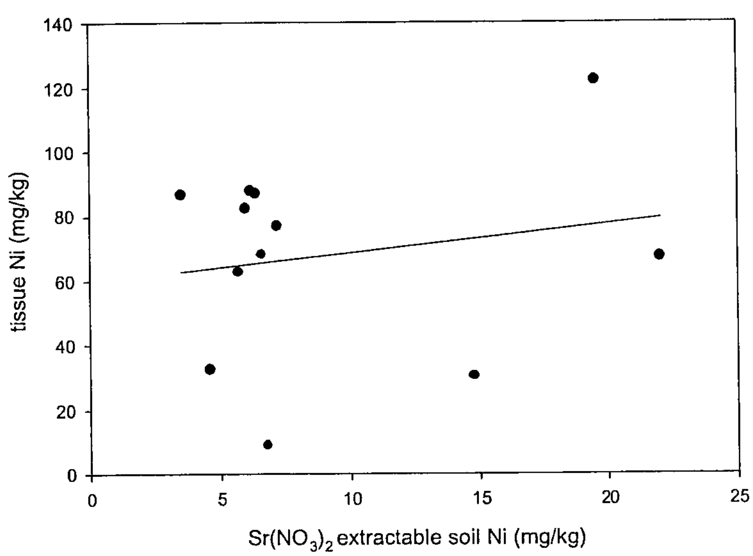
4). Soluble Ni

The reviewer raised the possibility that $Sr(NO_3)_2$ – extractable soil Ni was a useful approach to compare studies of Ni phytotoxicity, as it quantifies the bioavailable Ni in soil, thus normalizing the data among studies. On the basis of this contention, the reviewer has identified a study by Anderson *et al.* (1973) in which soluble soil Ni concentrations causing phytotoxicity were lower than those expected for the EC_{25} values derived from Jacques Whitford's blended soil experiment. The reviewer does allow that differences in calcium, for example, among studies, could interfere with the predictive power of soluble soil Ni.

Before the actual details of the Anderson paper are examined, the general merits of soluble soil Ni as a predictor of phytotoxicity or bioaccumulation or bioavailability, should be discussed. There is no question that some estimate of soil solution metal will be a better estimate of what is bioavailable than is total soil metal, as extracted by a very strong acid, such as nitric or hydrofluoric acid. The latter agents remove metal from insoluble mineral forms, a process which plants can do, but one which is very, very slow – thus to extract all the metal in this form in soils instantaneously is to over-estimate on a biological time scale, the amount of metal that the plant can access. Accordingly, soil scientists then developed a number of soil metal extraction protocols with the intent that they quantify "the water soluble and easily exchangeable" metals in soils. There is no doubt that these extraction methods (isotopic dilution, or extraction with $CaCl_2$ or $Sr(NO_3)_2$ are three examples) produce values for soil metals that are less than those extracted using strong acid. Hough et al. (2005) labelled these estimates as 'metal capacity' of the soil, meaning the "metal reservoir in the soil which re-supplies metal to the soil following depletion". This is as distinguished from the 'metal intensity' of the soil, meaning "the activity of a particular metal species in the soil solution, for example, free metal ion activity." These authors point out that the ratio of intensity to capacity will vary among soils (and presumably among elements), and so both are important considerations for bioavailability. The paper by Hough et al. (2005) related the accumulation of Zn and Cd by successive cuttings of grass grown in a very wide variety of urban or metal-spiked soils containing Zn or Cd, to total metal in soil, exchangeable metal in the soil (termed "labile" in this study), metals in soil pore water collected under negative pressure, and predicted free metal ion activity in soil solution. As expected, exchangeable soil Zn or Cd was significantly related to accumulation in tissue, whereas total soil Zn or Cd was not. However, exchangeable soil metal explained, at best, 43% of the variation in this relationship, and at worst, less than one-quarter. Much stronger were the relationships between tissue accumulation of Zn and Cd, and either soil pore water metal, or free metal ion activity; either of these estimates of soil metal accounted for between 60 and 87% of the variation in the plant accumulation of metal. That "water soluble and easily exchangeable" soil metals or "capacity" do not predict uptake or toxicity by plants is based on much experimental evidence that there is competition between ions in soil solution, and the metal ion of interest, for uptake by the plant, something that is not captured by "capacity". Specifically, the presence of H^+, and perhaps Ca^{2+} and Mg^{2+} (evidence in hydroponic culture, but not so evident in soils), in soil solution potentially interfere with the uptake of metal free ion (Me^{2+}) from soil solution. Hough et al. (2005) stated that in addition to Me^{2+}, H^+ was a significant modifier of plant tissue accumulation of Zn and Cd, although they noted that Ca^{2+} was not. Although not explained in the paper, the corresponding author suggests that this was likely an artefact of the lack of precision in WHAM predictions of Ca^{2+} in soil solution, as well as a very narrow range of Ca^{2+} in the experimental soils (Young, pers. comm.), rather than a true absence of Ca^{2+} effect on plant accumulation of Cd and Zn. Based on this work, and a large body of other studies, there is general agreement that a soil Me^{2+} activity (intensity) accompanied by low Ca^{2+}, Mg^{2+} in solution, and a high pH, is likely to result in greater tissue accumulation (leading to potentially greater toxicity) than the same soil Me^{2+} activity with high Ca^{2+}, Mg^{2+} in solution, and low pH. Neither the "intensity" of soil metal, nor this potential for modification is captured by "water soluble and easily exchangeable" soil metal. The study by Hough et al. (2005) summarizes the situation: *that "water soluble and easily exchangeable" soil metal normalizes data from studies on different soils better than total soil metal, but not very well, and not as well as soil pore water or free ion activity does; and, even if free ion activity is measured or modelled, solution pH, as well as the activities of Ca^{2+} and Mg^{2+} in the solution need to be known.*

And even if this information is available, there are biological mechanisms that further complicate a simple relationship between "water soluble and easily exchangeable" soil metal and plant uptake of the metal, and response to it, namely that the plants themselves influence the chemistry of metals in the soil immediately surrounding the roots (the rhizosphere). This altered chemistry is not captured by "water soluble and easily exchangeable" metal extraction of bulk soil, but profoundly influences plant uptake of metals. The specifics of the biological mechanisms are that plant roots exude organic acids, and protons, for the specific purpose of solubilizing microelements from soil solids. These mechanisms are induced particularly in the case of low soluble iron in soils, but it is important to note that plants exude organic acids in most soils, as these support microbial populations, which in turn are important for plant productivity as microorganisms also solubilize essential elements from soil solids and organic matter. As well, plant roots exude protons if the nitrogen supply is ammonium, rather than nitrate, which can result in a rhizosphere pH several units lower than that of the bulk soil. Finally, the induction of these mechanisms varies considerably among plant species, a point which is illustrated well using data from Kukier and Chaney (2004) who reported the tissue Ni concentration at EC25, and the $Sr(NO_3)_2$ soil Ni concentration at that EC25 (See Figure 1). It is important to note that it is the same soil for all data points, but the pH of the soils was varied with limestone to achieve the range of $Sr(NO_3)_2$ soil Ni concentrations.

Figure 1. Relationship between $Sr(NO_3)_2$ soil Ni concentration and tissue Ni concentration at EC_{25} (from Kukier and Chaney, 2004)



This figure (Figure 1) demonstrates that within a very narrow range of $Sr(NO_3)_2$ soil Ni concentrations, the concentrations of Ni in tissue vary 15-fold. These data indicate that $Sr(NO_3)_2$ soil Ni concentration is not a useful predictor of tissue Ni concentration among plant species, within soil type, indicating that the effect of biological species on the amount of Ni taken up from soil is larger than the effect of the amount of $Sr(NO_3)_2$ Ni in soils. Further, Kukier and Chaney (2004) compared the slopes of the regression relationships linking $Sr(NO_3)_2$ soil Ni concentration with tissue Ni concentrations, among species, and found a 6-fold difference. Thus, even within species, $Sr(NO_3)_2$ soil Ni concentration is less or more related to tissue Ni concentration, and subsequent toxicity. It was tempting to consider doing $Sr(NO_3)_2$ extraction of the blended soils for the 2001 study, to compare with those values achieved by Kukier and Chaney (2004). However, that idea has been rejected because at the time of proposing the idea, it was not realized that these authors achieved their range of soil Ni concentrations by liming, rather than by field collection. Should the $Sr(NO_3)_2$ extraction of the blended soils for the 2001 take place, it would be very difficult to attribute differences in plant response to soil Ni vs. differences in soil Ca and Mg between the blended soils, and Kukier and Chaney (2004).

The reviewer quoted Weng et al. (2003, 2004) as evidence that $CaCl_2$ extraction of soil Ni is a good predictor of toxicity. The authors of this paper state that expressing toxicity relative to $CaCl_2$ – soil Ni, over a range of pH from 4 to 7, and among different soils, results in a two-fold range in critical threshold (EC_{50}). This is much smaller than the range that was determined on the basis of total soil Ni, over those pH ranges, within, let alone among, soils; this is not surprising, as, demonstrated by Hough et al. (2005), some measurement of soluble soil Ni is more predictive than total. However, most of this improvement is because $CaCl_2$ extraction normalized for the effect of pH on competition for metal binding to soil solids. If the EC_{50}'s at pH ~ 6 in this study are compared among soils, the range of those expressed as total soil metal is similar to those expressed as $CaCl_2$ extracted metal, leaving some unexplained variation in the relationship between soil Ni and toxicity, that is specific to soil. (And, if the larger standard errors of the EC_{50}'s determined from $CaCl_2$ extraction (compared to those from total soil Ni) are considered, the "range" in EC_{50} for pH ~ 6 is actually larger for $CaCl_2$ extraction than for total soil Ni.) The authors point out that there is an additional effect of pH on competition between H^+ and Ni^{2+} for uptake by the plant, that would likely be part of the variation among the EC_{50}'s determined from $CaCl_2$ extraction. It is important to note that three of the soils were sandy (one of which had higher organic matter and Fe content), and one of the soils was clay – thus one would expect a good deal of similarity among them. Interestingly, when Weng et al. (2004) plot the relative yield data for all soils and pH's vs. $CaCl_2$ – soil Ni, *more than half of the data for clay soil have to be omitted, as they actually demonstrate growth stimulation in response to soil Ni. Had they included these data, the relationship between $CaCl_2$ – soil Ni and toxicity would have been much weaker, if at all.* In summary, Hough et al. (2005), and Weng *et al.* (2003, 2004) support our contention that measuring the "soluble and easily exchangeable metal" in soil is neither a simple nor obvious method to compare data among studies – would that it were so.

The reviewer also invokes the "soluble soil Ni" concentrations expressed by Anderson *et al.* (1973), who demonstrated toxicity thresholds at low soluble Ni concentration ($\leq$16 ppm extracted by N ammonium acetate; total soil Ni 266 ppm). Part of this concern revolves around the reviewer's belief that the Jacques Whitford 2001 estimates of soluble soil Ni at the EC_{25} (3 mg/kg extracted by $Sr(NO_3)_2$; total soil Ni 1900 mg/kg) are much too low, a point that is addressed later in this section, and in Table 1. In any event, it appears that the reviewer's concern is that, based on Anderson's study, the blended soil study by Jacques Whitford should have demonstrated much higher soluble Ni concentrations, and, toxicity at much lower soil Ni concentrations, presumably corresponding to a soluble Ni concentration similar to that measured by Anderson *et al.* (1973). Anderson *et al.*'s study was of serpentine soils, where, as they acknowledge and demonstrate in Table 5, exchangeable Ca and Mg are much lower than in soils in general; this is a critical point of uncertainty, as Ca and Mg have been demonstrated in hydroponic culture to compete with cationic metals for uptake and are a credible reason for apparently greater uptake relative to total or soluble soil Ni (and thus greater toxicity) of oat in this study, relative to that observed for the Jacques Whitford blended soil study. We acknowledge that Hough *et al.* (2005) did not find that Ca improved the fit of the relationship between soluble or free ion metals and plant accumulation, but that may be because of the narrow range in Ca^{2+} in their soils. Anderson *et al.* (1973) point out that calcium deficiency could not be the reason for the Ni toxicity at the higher concentrations, as similar exchangeable soil Ca was observed at lower soil Ni concentrations. The point they omit is that the exchangeable Ca concentrations in all their soils were low (less than 30% of total CEC), relative to what is common for non-serpentine soils (60-80% of total CEC). The soils of Port Colborne were not analyzed for Ca as percent of total CEC, so a direct comparison cannot be made; however, the Port Colborne soils are not serpentine, so we would not expect the results of Anderson *et al.* (1973) to simulate what would be seen in Port Colborne soils.

In summary, if the unexplained variations noted in the Hough *et al.* (2005) and Weng *et al.* (2003, 2004) studies for the relationships between soluble soil metal and plant accumulation of these metals are generally true, then this uncertainty must be considered when comparing pairs of point estimates of toxicity relative to soil soluble metal (such as a comparison between Anderson et al. and the Jacques Whitford data). It is our contention that if the uncertainty is fairly calculated, the Jacques Whitford determinations would fall within the bounds of previously observed toxicities.

The reviewer questioned the level of $Sr(NO_3)_2$ extractable Ni measured by Jacques Whitford (3.1 mg/kg extractable Ni) and compared it with the levels measured (54 mg/kg extractable Ni) by Kukier and Chaney (2001). Jacques Whitford is in partial agreement with the reviewer that the $Sr(NO_3)_2$ extraction data are reduced in comparison to the levels reported by Kukier and Chaney (2001). However it is important to note that this would be expected, as in the comparison to which the reviewer is referring, *the soil pH differs*. The reader is referred to Table 1 below that gives the strontium nitrate data and corresponding pH data from both Kukier and Chaney (2001,2004) and Jacques Whitford, 2004. In Kukier and Chaney (2001), the Ni-extractable values range from a high of 54.2 mg/kg at a corresponding low soil pH (pH 5.11), to a low of 2.42 mg/kg at a corresponding high soil pH (pH 7.36). Additionally, when the same soil type (Welland Clay) collected in Port Colborne by Kukier and Chaney in 2004 with a concentration of 2930 mg/kg Ni was subjected to a strontium nitrate extraction, the amount of strontium nitrate extractable Ni was 5.7 mg/kg at pH 7.7. In comparison, the Ni-extractable value in Jacques Whitford (2004) was 3.1 mg/kg at a soil pH of 6.3, which is in line with the trend developed in Kukier and Chaney (2001). Therefore it is reasonable to explain the reviewer's observation of a lower-than-expected extractable soil Ni value by Jacques Whitford (2004) in comparison to those reported in other studies as being influenced by a difference in soil pH.

Table 1. Comparison of extractable nickel (using strontium nitrate extractions) on Port Colborne soils in various studies

Kukier and Chaney (2001) - Unblended -			Jacques Whitford (2004) - Blended -		
Total Ni in Soil (mg/kg)	Ni-extractable in $Sr(NO_3)_2$ (mg/kg)	pH	Total Ni in Soil (mg/kg)	Ni-extractable in $Sr(NO_3)_2$ (mg/kg)	pH
2930	54.2	5.11			
2930	2.42	7.36	1900	3.1	6.3
Kukier and Chaney (2004) - Unblended -					
2930	5.7	7.7			

It is important to note that in the Welland Clay soil blend used by Jacques Whitford (2004), the High Nickel soil at 8,000 mg/kg collected in Port Colborne at pH 6.2 was not subjected to any pH adjustment, therefore the solubility of nickel in the blends obtained by using this soil in the Year 2001 experiment would be similar to the levels measured in the field.

5). Blended soils

Determination of EC_{25} was done using measurements from two sets of data points for the two types of soils (Till Clay and Welland Clay) where relevant data were found. The reviewer's concern is that plants are more tolerant of Ni in blended soils than in unblended soils as occur in the field, as the Ni in the blended soils could occur in aggregates that the plant roots can avoid. In our opinion, this avoidance of Ni by the plant roots is actually MORE applicable to the field situation than it is to the greenhouse pot studies, as in the field, plant roots colonize the soil below the plough layer, where the concentrations of Ni and other CoC's are diminished.

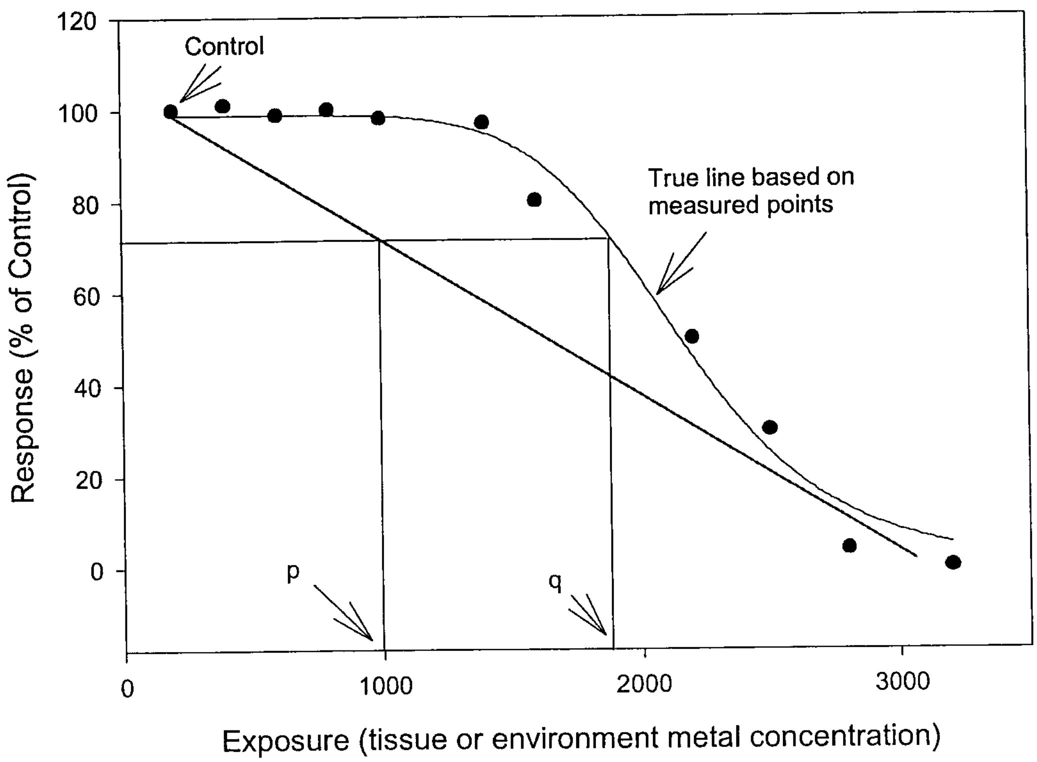
A second response to this concern by the reviewer is to question how the reviewer visualizes that the roots can avoid the high-Ni particles (thus expressing lower toxicity), yet quantification of the soluble (which will include soil Ni from the theoretically avoided and non-avoided soil in the pot) and easily exchangeable soil Ni would be a good basis upon which to validate the blended soil studies.

A third response to this concern by the reviewer is that oat grown in the field in Year 2001, in the clay C2 site, an unblended soil with approximately 5000 mg/kg soil Ni, accumulated tissue Ni approximating 30 mg/kg. This concentration of tissue Ni was observed in the greenhouse blended soils at soil Ni concentrations less than approximately half of this value, suggesting that the plants grown in blended soils in the greenhouse were not able to avoid the soil Ni because of blending with fertile soil, in fact, absolutely the opposite occurred.

As an additional verification of the accuracy of the documented EC_{25}'s in the Jacques Whitford (2004) report, in supplement to the sensitivity analysis given in this report, we have done 2 calculations that project crude EC_{25}'s values for the Port Colborne soils based on a linear extrapolation between only 2 data points, i.e. an unblended soil Ni concentration for the Port Colborne control used by Jacques Whitford and (a) an unblended high soil Ni concentration of Port Colborne soil (Till Clay) used by Jacques Whitford as calculation 1) and (b) an unblended high soil Ni concentration of Port Colborne soil (Welland Clay) used by others as found in the literature as calculation 2).

This crude estimation of EC_{25} is based on the method used by Davis *et al*. 1978. The estimates of plant response at EC_x obtained by fitting a linear function to just two values of X, are lower than the "true" values (Davis *et al*. 1978), as biological response to increasing concentration of an essential element, such as Ni, is typically logistic or log-logistic. In the example graph in Figure 2, the value 'p' measured by a regression analysis based on only two metal concentrations (control and highest) can be seen to be lower than the actual EC_x at value 'q' , which would have been obtained if all of the eleven (11) 'real' data points on Figure 2 had been considered and a more appropriate (Weibull) regression had been used.

Figure 2: True vs. False Line (modified from Davis et al., 1978)

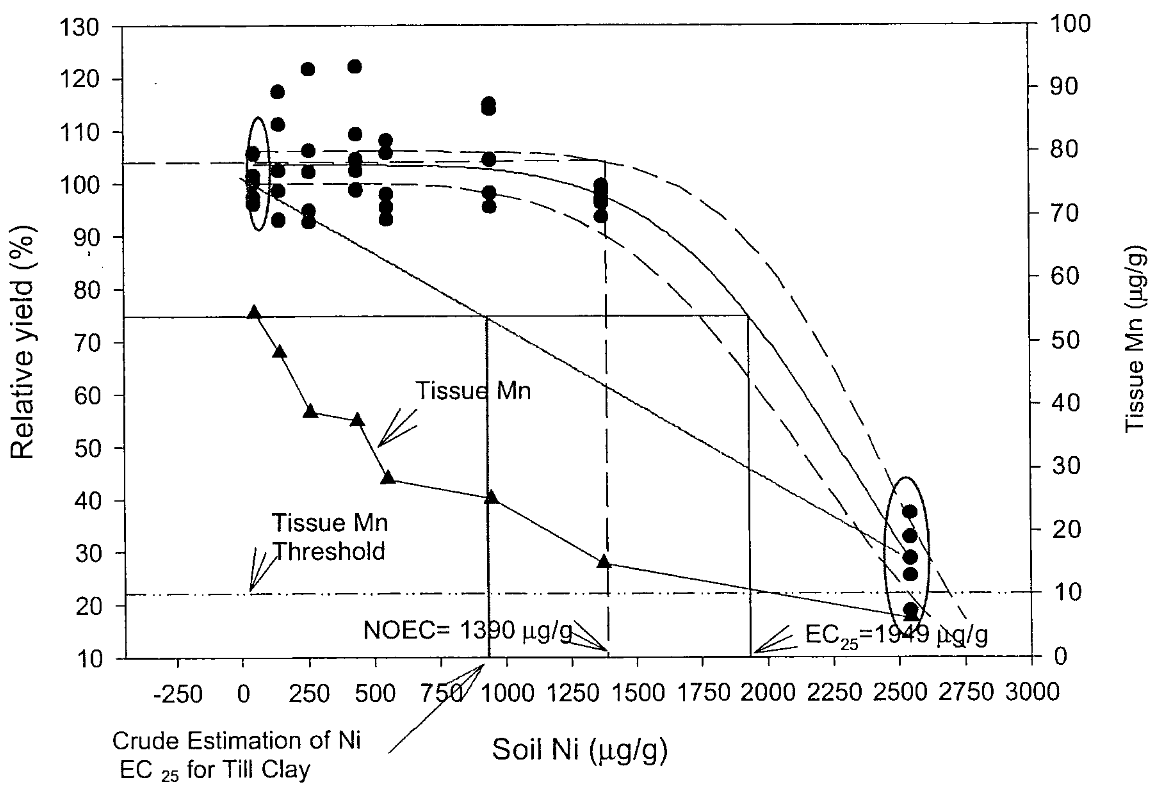


Calculation 1) Crude Estimation of Ni- EC_{25} for Till Clay

In the case of the Till Clay, the highest level tested in the Year 2001 in the Greenhouse was in unblended soil collected from the field. By using only two set of data points (unblended Control and unblended High), a linear regression was obtained, which as previously mentioned is not true for biological systems. However, this method does permit a crude approximation of an EC_{25} without using any of the data points generated from plants growing in blended soils. The results obtained must be interpreted with caution as confidence intervals could not be generated due to the lack of data points and relying only on the CTL (control) and High Ni Till Clay soil (2500 mg/kg Ni). A linear regression line has an equation of the form Y = a + bX, where X is the explanatory variable (soil nickel) and Y is the dependent variable (plant biomass). The slope of the line is b, and a is the intercept (the value of y when x = 0); where:

Y= 101-0.0286(soil Ni)
75=101-0.0286(soil Ni)
soil Ni= (101-75)/0.0286
crude soil Ni- EC_{25} = 907.09 ug/g (no confidence intervals)

Figure 3. Crude Estimation of Ni EC_{25} for Till Clay

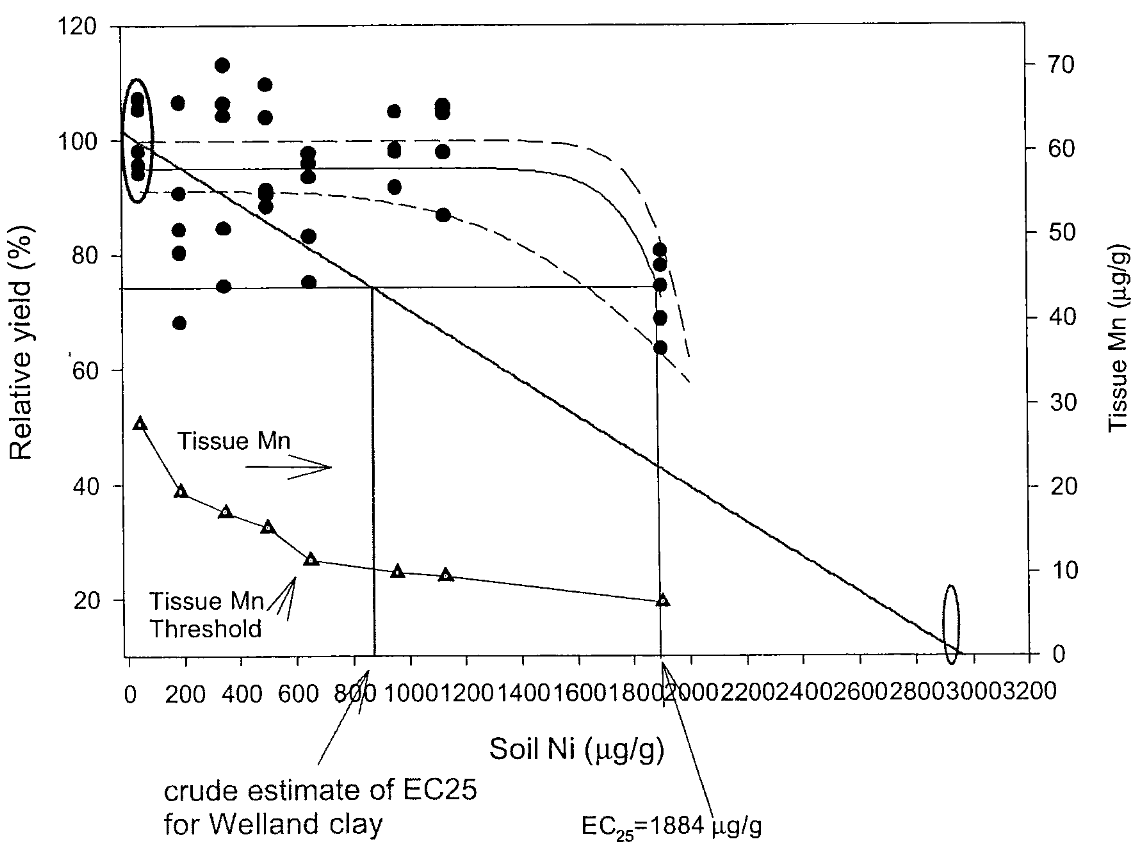


Calculation 2) Crude Estimation of Ni- EC25 for Welland Clay

The EC_{25} for Welland Clay soils was calculated using the unblended control soil data points from the 2001 Greenhouse experiment, and using Kukier and Chaney, (2004) oat relative growth data (approximated from their graph) for unblended Welland Clay soil with approximately 2900 mg Ni/kg. There was no unblended high Ni Welland Clay soil to use from the 2001 experiment as the highest level of Ni exposure was a blend of about 1900 mg/kg Ni. The calculated soil Ni for the Welland Clay is as follows:

Y= 101-0.0307(soil Ni)
75=101-0.0387(soil Ni)
soil Ni= (101-75)/0.0387
crude soil Ni- EC_{25} = 847.90 ug/g (no confidence intervals)

Figure 4. Crude Estimation of Ni EC_{25} for Welland Clay



The EC_{25} values derived by the crude estimation method give **very conservative** values of 907 mg/kg for Till Clay and 847 mg/kg for Welland Clay.

Are these crude estimations of EC_{25} applicable as soil cleanup numbers for Port Colborne? Absolutely not. They do not factor in all of the dose-response information collected from the Jacques Whitford 2001 greenhouse experiments, and they do not conform to the logistic function which is typical for toxicity studies. What these crudely-estimated EC_{25} values do is remove the effect of blended soils, and their associated artefacts, from the calculation of EC_{25}.

6). Confidence intervals.

The reviewer has questioned the use of confidence intervals for the Weibull dose response functions. There are two calculations for confidence intervals (CI) around a regression relationship – the population CI's and the data CI's. The former are used to estimate the precision with which the regression relationship will predict Y from the observed values of X, when the intent of the regression relationship is to predict the response of oat plant biomass (Y) to soil Ni (X) over the entire population of Welland Clay soils, which are assumed might vary considerably from those used to determine the relationship. Hence, these CI's are wider than those calculated as the data CI's, which are used to estimate the precision with which the regression relationship predicts Y from the observed Y and observed X (the intent of the CI's depicted with the Weibull functions in the crop report). While it might seem intuitive that the former is more appropriate for risk assessment than the latter, neither actually is correct for the purpose of predicting a value of X for a particular value of Y, as we wish to do by predicting the soil Ni value that is associated with a 75% reduction in plant growth. This is called *inverse prediction* and involves an additional error term to those (errors associated with the estimates of the regression parameters, as well as the unexplained error) used in *prediction*, namely the errors associated with the random, normally distributed variable Y. The confidence intervals for inverse prediction are not symmetrical around the predicted value of X, but like CI's for prediction, get wider with distance from the mean value of Y.

There are three considerations to calculating and/or using CI's for predictions of soil Ni concentration that correspond to 75% reduction in plant growth, from the Weibull functions. First, the appropriate CI's for inverse prediction would need to be calculated, and at this point, we have a method for doing so only on linear functions. Second, good estimates of CI's result from X data that are evenly distributed – and in the Weibull functions for both Till and Welland clay, there is a gap in soil Ni concentrations between the highest and second highest. This is unfortunately where much of the plant response of greatest interest to the CBRA, but where the least precision is, thus to use CI's generated for the EC_{25} would result in safety factors that are inflated as a result of experimental design, rather than as a true reflection of uncertainty.

7). What should the Soil Number be?

We believe that there is no credible evidence that the use of blended soils, or greenhouse-grown potted plants, or oat as the indicator species, has resulted in the identification of artificially high soil Ni concentrations for the protection of agronomic production in Port Colborne. The evidence from the published literature is that oat is similarly sensitive to soybean, and the anecdotal evidence from the community is that soybean is not as sensitive as oat. We speculate that due to root exploitation of uncontaminated soil below the plough layer, less Ni was accumulated in tissue from the field plants than was accumulated, or would be expected to accumulate, from blended greenhouse soils. From this it can be inferred that less toxicity would be expected in the field than in the blended soils, at similar soil Ni concentrations. Thus, we believe that using the EC_{25}'s as calculated in the Jacques Whitford (2004) report from the regression relationships for oat grown in the blended soils with elevated Ni concentrations will more than protect agronomic production (corn/soy rotation, and oat or mixed grains) in the field from a 25% reduction in growth. As the MOE normal practice is to set soil cleanup guidelines in the Province based on EC_{25} values, our position is to recommend at a maximum that the EC_{25} values as found in the Jacques Whitford (2004) report be used as the safe intervention levels for Ni in agricultural soils in Port Colborne. At a minimum, we would recommend the PNEC values for remediation to lend another layer of conservatism.

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APPENDIX C

Responses from External Peer Reviewer, September 26, 2005

September 26, 2005

Response to July 26, 2005 Review by JW

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JW has provided some useful comments based on my critique of their conclusions from the Port Colborne crop study. I agree with some points presented in these comments, but not with others. I do fear, however, that we are getting bogged down into technical details, and perhaps missing the main points about the weaknesses of the original JW crops report. Nevertheless, I will respond to each of the 6 specific points that JW has addressed.

1.Usefulness of the year 2000 data –
JW attempts to show from the data of Kukier and Chaney (2004) that soybean may be not much more sensitive than oat. Here, they calculate the tissue Ni concentrations at which 25% yield reduction occurred in those experiments. I must point out, however, that the Kukier and Chaney yield data are based, not on controlled studies, but on one soil (Welland Clay) with very high Ni (2900 mg/kg) that was limed to several levels to reduce available Ni and potentially improve growth. There is no control soil with low Ni in this study that can be used for yield comparison. Therefore, in my view, the JW analysis is not valid, as the yield reductions they are calculating are relative to yield on a limed soil with very high Ni content. Unless the limed high-Ni soil is just as productive as a similar soil uncontaminated by Ni, which I seriously doubt, this analysis is flawed. Thus, when JW state that tissue Ni for soybean at 25% reduction of shoot growth was 32.6 mg/kg, three-fold higher than that associated with 35% reduction in the year 2000 greenhouse trials, they are making an invalid comparison. In the first case, the yield reduction is relative to a limed high Ni soil, in the second (2000 greenhouse) case, the reduction is relative to a control soil with low Ni. I would be very surprised if 32.6 mg/kg Ni in above-ground tissue only produced a 25 % yield reduction, as Marschner (Mineral Nutrition of Higher Plants, 1995) states that sensitive crops have critical Ni toxicity thresholds around 10 mg/kg in the tissue.

I do agree with JW that, in the field, Ni in above-ground tissue is likely to be lower than in the greenhouse because of greater rooting depth. However, this could well mean that the surface soil is still phytotoxic, and shallow-rooting crops may not fare so well.

All of this discussion is missing the point that above-ground tissue Ni levels may be a poor predictor of growth response and yield, because physiological damage is focused at the root, Ni accumulates at the root, and in many crops is largely excluded from the top. A controlled field experiment could have dealt with these uncertainties without resorting to after-the-fact suppositions.

2. Use of Oat as a single indicator crop
JW argues that radish was also used as an indicator crop in 2001, in addition to oat. However, I believe that this was still a blended-soil experiment, and has the same drawbacks of the 2001 Blended Soil experiment. Besides, radish is not an economically important crop of the area.

3. Use of plant biomass vs. economic yield –
This point deals with the question of whether a relatively short-term greenhouse trial with oat, or any other crop, can estimate likely reductions in economic yield (seed, grain, etc.). In most greenhouse trials, only vegetative growth is being measured. But the important measurement to growers is seed production, i.e. reproductive growth, in the case of many important crops. I agree with JW that there is very little published literature on the effect of toxic soil metals on the harvest index, the ratio of economic yield to plant biomass. However, based on very limited evidence in the literature (e.g., Piccini and Malavolta, J Plant Nutrition, vol. 15, 2343-2350). I think it is quite possible that economic yield will be more severely affected by metal toxicity than plant vegetative growth. Seed can only be produced in significant yield if there is excess photosynthate for the plant leaves to export to the reproductive tissues. This does not happen with a plant stunted by a poor root system. The plant cannot reallocate photosynthate to seeds if it is chlorotic, as photosynthesis is severely limited.

The JW example of shifts in harvest indices in newer cultivars is of no relevance to the question of what happens to harvest index when soils contain toxic metals.

Again, we are engaging in educated guesses on complex plant responses here, when the logical approach would be to test plant response to Ni in the field under real conditions.

4. Soluble Ni-
I agree with much of the discussion here on soil metal chemistry and plant uptake of Ni. I also agree that measuring water-soluble or exchangeable Ni in the soil is only a crude predictor of Ni uptake in crops. It is not a substitute for a plant assay, preferably in the field. Note however, that Datta and Young (Water Air and Soil Pollution, 2005, vol.163, 119-136) were able to predict Cd, Zn and Cu uptake into plants without using either a H ion or Ca ion factor, only an estimate of the free metal ion activity in the soil solution.

JW has provided a reasonable explanation for the much higher $Sr(NO_3)_2$ extractable Ni reported by Kukier and Chaney as due to soil pH. This shows the extreme importance of pH in determining the readily-available quantity of Ni in soil. This raises an important question of how soil pH will be maintained at 7 or higher in the Port Colborne area, as soils tend to generate acidity over time.

Anyway, the debate over how best to extract or measure soluble Ni, and how useful it is, is fairly academic as the real test of plant uptake of Ni should be a field trial. Also, as I pointed out before, the amount of Ni in the top of the plant may not be a good indicator of toxicity. (This principle is well known for Cu toxicity). Some plants are clearly Ni excluders, such as oat, corn and soybean, based on Figure 5 in Kukier and Chaney (2004). Leaf Ni in these plants may be an insensitive indicator of toxicity. The wide range of plant tissue Ni at any given $Sr(NO_3)_2$-extractable soil Ni level for different crops (Figure 1 in the JW comments) seems to confirm this point, so that available Ni in the soil is not necessarily transported to the top of the plant. It may, however, be accumulating in the root and doing damage there.

5. Blended Soils-
JW argues here that Blended Soils, where Ni may be concentrated in aggregates, not homogeneously distributed in the soil, is relevant to the field situation in Port Colborne because in the field, the crop roots could to some degree avoid the high-Ni topsoil,

growing into the low-Ni subsoil. Thus, the impact of Ni on crops in a greenhouse trial would be more severe than that in the field. While this may well be true, it implies that it is acceptable to have a phytotoxic topsoil, which the roots would avoid by growing into and proliferating in the low-Ni subsoil. Such a situation is undesirable because it could affect yield for even a deep-rooting crop, as roots could not function in the more nutrient-rich topsoil, and certainly would reduce yield in shallow-rooting crops. I think that a topsoil phytotoxic to roots is not an acceptable agricultural situation – otherwise, any level of toxic metal in topsoils could be defended because plant roots could avoid the toxic layer.

As a comment on the estimates of EC25 shown in Figures 3 and 4, the analysis seems reasonable, but has some weaknesses. One is that apparently random variability in relative yield at soil Ni levels not thought to be causing any yield reductions is quite large in some soils. For example in JW's Figure 4, the yield varies from about 70% to 110% of the average. Obviously, it would be very hard to detect even a 25% yield reduction given this background variability. I am concerned then that an economically important yield reduction could easily be obscured. A second problem is that there are few if any measurements of yield between the "no-effect" Ni level and the severe crop yield reduction (see JW Figure 3). This means that the data set can not provide very certain EC25 soil Ni levels for moderately small, say, 10% yield reduction.

6. Confidence Intervals-
JW point out here that there is "a gap in soil Ni concentrations ..... this is unfortunately where much of the plant response of greatest interest to the CBRA, but where the least precision". I commented on this weakness in the Blended Soil data in point 5 (see above). JW states that they do not want to "inflate" safety factors in soil Ni limits as a result of this uncertainty. But it seems to me that this is exactly what you must do, increase the safety margin, when you lack data to support a more precise measurement of the EC25. In risk assessment, lack of data invariably leads to increased safety margins, at least until better data can be obtained.

7. In summary, I disagree with JW's conclusion that there is no credible evidence that the approach used in the year 2001 Blended Soil greenhouse study with oats resulted in soil EC25 limits that are too high. Their own data from the 2000 greenhouse studies with several crops provide credible, if not wholly satisfactory, evidence that the calculated EC25 values are too high. This leaves an unacceptable degree of doubt, in my opinion, about the validity of using the Blended Soil trials in isolation to estimate EC25 limits for soil Ni.

APPENDIX D

Jacques Whitford's October 12, 2005
Response to External Peer Reviewer's September 26, 2005 Comments

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October 12, 2005

M.B. McBride
Dept. of Crop and Soil Science
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Re: Response to September 26, 2005 Reviewer Comments

Dear Dr. McBride:

Thank you for reviewing the Community Based Risk Assessment, Port Colborne, Crop Studies 2004 Report, and for providing Jacques Whitford with your comments.

All the comments received were carefully evaluated and addressed. The ones which Jacques Whitford considered to be relevant to the Port Colborne Community Based Risk Assessment were incorporated in the final interpretation of the Crop Studies Report.

We believe that the weight of evidence both from our study, and the broader literature, supports the applicability of the EC_{25}'s as calculated to the site-specific conditions of the Port Colborne environment as scientifically-defendable alternates to the generic soil quality guideline for nickel.

Yours Very Truly,

JACQUES WHITFORD LIMITED

Original to be signed & retained on file

Tereza Dan, Ph.D.
Phytotoxicologist

Original to be signed & retained on file

Eric Veska, Ph.D., P.Geo., C.Chem.
Project Manager

cc: Beverly Hale, Ph.D., University of Guelph
Bruce Conard, Ph.D., Inco
Rob Watters, Ph.D., Watters Environmental Group

TAB 2

Jacques Whitford's Response Letter to Murray B. McBride's May 4, 2006 Comments on the Protectiveness of the Proposed Ni - PNEC values for Soils of Port Colborne. Response Letter is dated September 1, 2006

Jacques Whitford's Response to Murray B. McBride's May 4, 2006 Comments on the Protectiveness of the Proposed Ni - PNEC values for Soils of Port Colborne

(September 1, 2006)

The preamble to Jacques Whitford's answers to Dr. McBride's comments of May 4, 2006 (below in italics) is to remind readers that in previous exchanges of information (Jacques Whitford, 2004a, 2006), we have provided evidence both from the field in Port Colborne, and from fundamental knowledge of plant physiology and culture, that estimates of plant growth response to soil metals, obtained from pot studies in the greenhouse and expressed as PNECs, are likely to **overestimate** sensitivity. The primary reason for this is that metals in the field soil of Port Colborne are most concentrated in the upper 15 cm, a layer that many plant roots quickly grow through in their search for water. The roots of greenhouse-grown plants are confined to soils with elevated metal concentration, thus their accumulated dose throughout their lifetime is greater than that in field-grown plants. So, we would argue that Dr. McBride's insistence that the Y2000 greenhouse study predicts what would happen in the field is wrong, in light of the supporting results of the field studies undertaken by Jacques Whitford on soybean, the Y2001 study of oat, and the fundamental mechanisms of plant growth in the field. Having said this, each of the reviewer's points is addressed, below:

***McBride**: Are INCO's proposed PNEC values for soil Ni (750 mg/kg for sand, 1400 and 1650 mg/kg for Till Clay and Welland Clay, respectively, and 2350 for organic soil) protective of the most sensitive crops grown in Port Colborne? That is, can soils at or below the proposed PNEC Ni levels be used in an unrestricted manner ?*

***McBride**: The answer in my opinion is no, based on evidence both within the JW crops report itself as well as from other observations and published information on Ni toxicity in soils. Specific evidence that much lower PNECs are needed to protect important sensitive crops includes :*

1. ***McBride**: The year 2000 greenhouse study of JW shows statistically significant yield reductions in corn, soybeans and oats at soil Ni concentrations well below the PNEC's in the sand and clay soils. Thus, for sand, 300 mg/kg Ni was shown to produce significant yield reductions for all three crops. For clay, the yield data were lost for oats, but 500 mg/kg Ni appeared to be toxic for soybeans,whereas toxicity for corn was not evident at 500 mg/kg. For peat, yield results were more erratic, and it is difficult to establish a threshold for toxicity. For this organic soil, the study of Frank et al.(1982) is informative, as vegetable crops such as celery showed severe reduction in yield at 1200 mg/kg total Ni. This is obviously well below the proposed PNEC of 2350 for this soil, and suggests that an acceptable PNEC for vegetable crops would need to be below 1200 mg/kg.*

Jacques Whitford: The reductions in growth measured in the 2000 greenhouse study are not supported by the field experiments that were carried out during the CBRA, nor by the general successes of farming practice in the Port Colborne area. If soil Ni concentrations between 300 and 500 mg/kg genuinely caused poor crop growth, it would be evident from the many farming operations that are carried out on soils with similar concentrations of Ni; clearly this is not the case.

Dr. McBride's referenced studies of the Frank et al. (1982) paper pertain to crops grown on organic soil some 25 years ago (1980-1981) at a time when the Port Colborne Ni refinery was in full operation (the refinery halted operations in 1984) and when aerial deposition of Ni and the effects of SO_2 emissions to foliage likely would have increased the exposure of plants to Ni relative to the currently naturally weathered or aged soil Ni concentration. Thus the data from the Frank et al. (1982) paper should not be applied to the Port Colborne CBRA, as the exposure to plants then and now are not comparable. Considering the years that have transpired since the closing of the refinery and the cessation of atmospheric Ni input and emissions of $SO_{2,}$ the current pool of bioavailable fraction of soil Ni available to crops has been greatly reduced. It is the exposure of plants to the current soil conditions with current soil Ni concentrations that is relevant in this CBRA.

2. ***McBride**: Observations in the field in the Port Colborne area show evidence of phytotoxicity at Ni concentrations well below the proposed PNEC values. These observations are illustrated by photographs in the JW Crops report. For example, the pictures of young soybean plants on a field labeled "Rae Farm" showed clear evidence of the type of severe chlorosis in emerging leaves that is consistent with Ni toxicity (Figure F4-11, Crops Report, Vol. 1, page F4-A7). Toxicity symptoms remained evident even with liming (Figure F4-12). I understand that this soil contained approximately 500-600 mg/kg total Ni , much lower than the proposed PNEC for clay soils.*

Jacques Whitford: It is well known that chlorosis of leaves is consistent with both deficiencies and toxicities of many plant nutrients, and that to diagnose as Dr. McBride has on the cause of observed chlorosis in soybeans based on a single photograph at a single point in time without supporting data for tissue Ni concentrations, is speculative at best. The chlorosis that the reviewer is describing could be caused by a deficiency or excess of a nutrient that is independent of elevated Ni in the soil. It is possible that chlorosis in soybean grown in Ni-elevated soils could be caused by deficiency of an essential element as a secondary effect of Ni toxicity, but that is speculative.

The soils of the Rae Farm are a Till Clay with a soil Ni concentration of 636 mg/kg. Table 4-6 on page 4-24 of Volume 1 of the Crops report indicates that the tissue Ni concentration in soybean, i.e. the same soybeans shown in the photo referred to by Dr. McBride, is only 3 mg/kg in dry weight, or less than 0.46% uptake of Ni from soil.

This level of uptake is very low but not unusual, as soybean plants require nickel for normal growth (Shimada and Ando, 1980; Krogmeier et al., 1991). It should be noted that the measured uptake of 3 mg/kg is 10 (x) times lower than and well below the reported Kukier and Chaney (2004) value of 32.6 mg/kg of tissue Ni that would result in a 25% reduction in shoot growth in soybean. It can be concluded from this that the observations made by Dr. McBride on the chlorosis of soybean leaves from this one picture was not related to soil Ni toxicity.

3. ***McBride**: Existing data in the literature show that soil Ni concentrations much lower than the PNEC values can be phytotoxic.*

   3.i) The study of Frank et al., (1982) <u>was conducted on the contaminated organic soils of Port Colborne</u>, and therefore should have been considered highly relevant in deriving PNEC values for that soil type.

<u>**Jacques Whitford**</u>: The limited relevance of the Frank et al. paper to the derivation of PNEC values has been addressed earlier in this document under item 1), and so those points won't be repeated here. Generation of PNEC values at the time when Inco's nickel refinery prior to 1985 was in full operation was not the objective of the CBRA work.

*3.ii) **McBride**: In my initial 2003 review of the Crops Report I cited other studies with oats, such as that of Sauerbeck and Hein (1991). That study showed that oat straw concentrations of 20 to 30 mg/kg Ni produced yield losses in greenhouse pot experiments, those yield losses occurring when soil total Ni was in the 100-200 mg/kg Ni range (for non-acid soils), much less than the PNEC Ni values for any of the soils of Port Colborne. It could be argued that the Ni was more soluble and available in the Sauerbeck and Hein study (they used Ni-contaminated sewage sludge as well as Ni salts to add Ni to their soils). However, even at the highest level of soil Ni tested in their greenhouse trial (200 mg/kg Ni), the 0.005 M DTPA-extractable Ni never exceeded 50 mg/kg. If we compare this result to data for soils of the Pt. Colborne area, total soil Ni has to be below about 200-300 mg/kg in order for DTPA-extractable Ni to be less than 50 mg/kg (see JW Crops report,Table 11, page 230-231). In other words, the Ni of the Port Colborne soils is only somewhat less extractable by DTPA than Ni of the Sauerbeck and Hein study. Since DTPA is considered by many soil scientists to estimate the size of the plant-available pool (and is conducted by the soil testing lab at the University of Guelph for that purpose), this comparison of studies suggests that some soils of Pt. Colborne should be phytotoxic at 200-300 mg/kg total Ni – a suggestion to some degree supported by the year 2000 greenhouse study (see comments above).*

<u>**Jacques Whitford:**</u> We agree that DTPA is one of many soil extractions that gives an estimate of the soluble and readily labile metal that a plant could access; however, an inspection of the Sauerbeck and Hein (1991) paper amply demonstrates why soil

scientists shouldn't (and plant physiologists don't) consider DTPA extractable soil metals as a predictor of actual plant accumulation of metals, and their subsequent toxicity. Table II of this paper reports an r-value (not an R^2) of 0.5. While it is anticipated that this is a significant correlation (no value for *n* is reported), as the authors state "…DTPA and DTPA/TEA did not indicate the Ni availability sufficiently well, as they dissolved a large portion of the Ni irrespective of the origin or soil type". What this means is that DTPA-extractable soil Ni is a poor predictor of plant accumulation of Ni, which is the principal cause of toxicity. Thus, concluding that a soil in Port Colborne with a particular DTPA-extractable soil Ni concentration should be phytotoxic because another study demonstrated phytotoxicity at the same DTPA-extractable soil Ni concentration but in a different soil with a different source of Ni, has no credibility.

Further, the yield data to which the reviewer refers are not presented in the Sauerbeck and Hein (1991) paper, so who knows what degree of yield loss they attribute to soil Ni, and what relation that might have to the losses shown in the year 2001 greenhouse study of the CBRA?

Most of the scientific basis of the arguments made in the comments by Dr. McBride in his review of the Crops report are in respect to the findings and conclusions of the scientific study carried out by Sauerbeck and Hein (1991). Sauerbeck and Hein (1991) examined Ni uptake in plants grown on sewage sludge and compared that data to Europe's maximum permissible level of nickel in soils from sewage sludge application. A similar comparison to the Port Colborne situation as inferred by Dr. McBride cannot be made in our opinion, but as the reviewer believes that the paper by Sauerbeck and Hein (1991) is relevant, a copy of their paper is provided in Appendix A and a brief summary of their work is provided below for the edification of the reader. The Sauerbeck and Hein (1991) paper documents experiments conducted in the greenhouse on two types of soils, one a pH 7.25 Luvisol soil and the other a pH 5.45 Cambisol soil. The pH 7.25 Luvisol soil was adjusted to pH 6.5 by the addition of aluminum sulphate. The pH 5.45 Cambisol soil was increased to pH 6.3 by the addition of calcium carbonate. A sewage sludge originally low in metals was enriched with soluble nickel chloride and incubated for 30 days at 30 degrees Celsius. Mixing (or 'blending' using a CBRA term) the two types of soils with this 30 day aged artificially spiked nickel chloride sewage sludge produced nickel contents of 0, 17, 34, 50, 75, 100, 150 and 200 mg/kg in the resulting soil/sludge mixture. Experiments were also conducted on: 1) two nickel-enriched sewage samples from industrial waste, 2) a nickel-enriched industrial filter dust sample and 3) a geogenic naturally-high nickel containing soil basalt mixed with the Cambisol soil to the same range of soil nickel concentrations obtained through mixing clean soil with soluble nickel containing sewage sludge.

The reader will come to the realization that there are some parts of the methodology described in the Sauerbeck and Hein (1991) paper that mirror some of the methods carried out in the CBRA greenhouse experiments. Those parts include soil pH adjustment and soil mixing (or 'blending') for purposes of dose-response experiments.

Like the Jacques Whitford greenhouse experiment of Y2001, Sauerbeck and Hein (1991) used the **same** method of pH adjustment with aluminum sulphate and calcium carbonate. Also like the Jacques Whitford greenhouse experiment of Y2001, Sauerbeck and Hein (1991) used the **same** method of mixing a highly-contaminated nickel material with control soil with background levels of nickel to various proportions such that a range of soil nickel concentrations could be achieved for the dose-response experiments.

Where the reader will notice a large difference between the methodologies is that Jacques Whitford used naturally weathered or aged (greater than 15 years since date of last atmospheric CoC deposition) and representative soil types from Port Colborne prior to mixing with each type of control soils from uncontaminated areas of Port Colborne; whereas, Sauerbeck and Hein (1991) used an **unaged** (1 month only) soluble nickel chloride spiked sewage sludge (not soil) prior to mixing with their two types of control soils. The authors of the Sauerbeck and Hein (1991) paper even themselves express some doubt on page 863 of their paper (see Appendix A) as to whether the soils were aged sufficiently after amendment of soluble nickel chloride to simulate field bioavailability of Ni. The ageing of soils after amendment by a soluble metal salt to simulate bioavailability is a topic of considerable current research and discussion. At the International Workshop on Metals-in-Soils held in Ottawa in February 2005, a presentation by Dr. Erik Smolders entitled: "Differences in Metal Toxicity between Spiked- and Field- Contaminated Soils" revealed that there are many cases in the literature of greenhouse trials using unaged contaminated soils created by spiking with soluble metal salts where the metal bioavailability measured is much greater than those derived from experiments using field contaminated soils that are naturally weathered or aged. Dr. Smolders gave an example where the time dependence of fixation of zinc spiked as a salt in soils in one experiment took more than 200 days before equilibrium conditions could exist. The conclusion from the Smolders presentation is that proper ageing of a spiked soluble metal soil is important for any meaningful dose-response experiment. It has also been reported that studies carried out on Ni for the European Risk Assessment have demonstrated that soil pore water Ni only stabilized after 3 months of ageing of a nickel chloride spiked soil; a considerably longer period than the 1 month period used in Sauerbeck and Hein. The inference here is that **had** Sauebeck and Hein aged their nickel chloride spiked sewage sludge soils for a total of 3 months instead of 1 month as they did in 1991, the true measured bioavailability of Ni in their soils would have been much less than the apparent bioavailability that they had observed.

Findings and conclusions of the Sauerbeck and Hein (1991) did reveal that in comparison to the spiked sewage sludge samples, the filter dust samples and basaltic soil samples showed very low Ni uptake in plants (i.e. low Ni bioavailability). The authors concluded that in the case of the basaltic soil sample, Ni is bound in the soil minerals from where it can be only released very slowly by gradual weathering. It is Jacques Whitford's opinion that bioavailability of the relatively insoluble Ni identified in Port Colborne soils would be somewhere between their experimented filter dust samples and their basaltic soil samples. A further discussion on this topic of Ni solubility in Port Colborne soils is presented in response to Dr. McBride's comment 3.iii).

Lastly, we would like to point out to the reader yet another reason as to why the Sauerbeck and Hein (1991) paper that studied Ni uptake in plants grown on sewage sludge and comparison of that data to Europe's maximum permissible level of nickel in soils from sewage sludge application at 50 mg/kg is not a relevant comparator to our Crops study. As the reader knows, one of the main objectives of Crops study was to derive soil nickel PNEC values that would be applicable to the Port Colborne agricultural soils; soils which to our knowledge do not contain any biosolids. Maximum concentration limits in biosolids set by the MOE are not directly comparable to, or related to, MOE soil standards for the simple reason that they are two very different things. Biosolids are organic material that are derived from a stabilized waste product of sewage treatment and digestion of sewage sludge. These materials can be land applied in a number of forms ranging from liquid slurry to dried pellets. Most land applied forms have a very high water content and all have a very high organic content. The organic material serves as a plant nutrient which is the reason for land application. Over time, the biosolids become integrated with the soil matrix, the organic matter decays and is used by plants which are harvested, necessitating the continuous replacement of soil nutrients. The extra moisture content is also lost. This removal of moisture and decay/uptake of organic matter and nutrients substantially reduces the mass of the initially applied biosolids and thereby leads to the potential build up of heavy metals in soils. Even dried biosolids pellets applied to a lawn have been observed to rapidly integrate into the soil matrix (Jacques Whitford, 2004). Numerous publications are available on the potentially toxic build up of heavy metals in soils as the result of biosolids land application (e.g. Jacques Whitford 2004; WERF 2002). Biosolids regulations are designed to address this potential for build up of metals through the repeated application of these materials to the same land, year after year. Limits are based on a low initial metal content in the soil and a limit on the metal concentrations in the sludge so that long term concentrations in soil do not become elevated. The same concerns and hence limits are not directly applicable to soil concentrations where biosolids are not being applied, which is the reason that the maximum permissible Ni concentration in soils for biosolids application (32 mg/kg – MOE, 1996) is much less than the generic standard for nickel in soil (200 mg/kg – MOE, 2004).

> *3.iii **McBride**: Other tests of extractable metals in Port Colborne also suggest that a large fraction of the total soil Ni is potentially susceptible to dissolution, and therefore <u>not</u> in an inert Ni oxide form. For example, the Crops Report shows from 30-40% of the total soil Ni to be soluble in acid oxalate. In addition, water-soluble Ni in these soils is not negligible, ranging from 3 to 13 mg/kg in the organic soil, 3-5 mg/kg in the high-Ni clay 2 test site, and about 0.8-1.3 mg/kg in the low-Ni (500-700 mg/kg total Ni) Clay1 test site (Appendix F-2, Crops Report). This immediately available form of Ni is expected to*

damage roots, based on published reports of Ni effects on roots (e.g., Gabbrielli et al., 1999).

Jacques Whitford: The comment that a "large fraction of the total soil Ni is potentially susceptible to dissolution" is disingenuous, as that is a conclusion from the oxalic acid extraction. Unless it starts to rain oxalic acid in Port Colborne, it is difficult to understand under what field condition this large degree of dissolution would occur. And, of course the water soluble Ni in the soils is the fraction that is immediately available, and that which is expected to cause phytotoxicity, but this will largely depend on soil pH, organic matter, and dissolved Ca and Mg, among other cations that have the potential to compete with Ni for sites of root toxicity. For these latter reasons, it is misleading by Dr. McBride to link soil Ni concentrations in one study to effects at that concentration in another study – unless all else is held constant, the studies are not comparable.

It is not known to Jacques Whitford as to whether Dr. McBride is cognizant of other Jacques Whitford studies carried out in this CBRA such as the Human Health Risk Assessment that has identified the relative insoluble forms of Ni in Port Colborne soils. If not, Jacques Whitford would refer Dr. McBride to review the May 2005 Human Health Risk Assessment report (Jacques Whitford, 2005), in particular the following volumes:

Volume III, Appendix 8 – Oral Bioavailability and Bioaccessability of CoCs in Port Colborne Soils
Volume IV , Appendix 12 – Nickel Speciation in Soil and Air Filter Samples

Within Volume IV , Appendix 12, Scanning Electron Microscopy (SEM) examination of Port Colborne soils showed the following distribution of Ni forms as summarized in Table 1 below. Most of the nickel in soil is found as insoluble oxidic nickel.

Table 1 Nickel speciation of Port Colborne Soils by SEM

	Organic Muck	Sand	Till Clay	Welland Clay
Total Ni (mg/kg)	10,045	3,920	2,545	8,655
Percentage as Oxidic Ni[1]	99.6%	91.7%	99.1%	89.9%
Percentage as Iron oxide/oxy-hydroxide with trace Ni	0.4%	8.3%	0.9%	10.1%

[1]Oxidic Ni includes all forms of Ni oxide/hydroxide, Ni-Fe oxide/hydroxide and Ni-Fe-Cu oxide/hydroxide

3.iv) ***McBride****: In contrast to the above evidence supporting lower PNECs, JW's Blended Soil Greenhouse Trail (2001) provides data (based on oats only) that support the proposed higher PNEC's. These results are not consistent with other data in the JW Crops Report (see point 1 above), where unblended soils from the Port Colborne area were tested for toxic effects on crops. There are a number of reasons why the blended soils could have provided misleading evidence favoring higher PNECs. Weaknesses in the blended soil approach to establishing PNEC values include :*

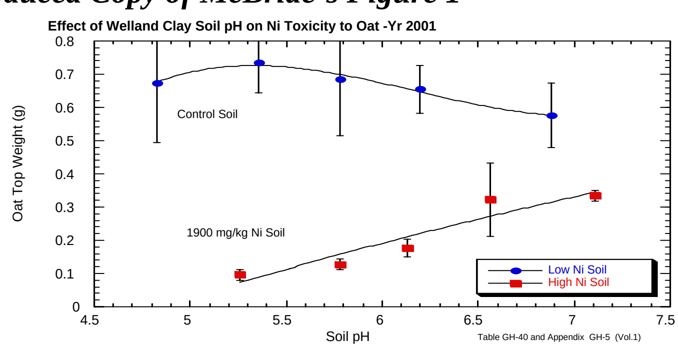
Jacques Whitford: The arguments and deduction by Dr. McBride on lower PNECs is simply not correct and not supported by evidence provided within the Crops report (Jacques Whitford, 2004a) for the reasons stated in our Overview of Evidence Paper (Jacques Whitford, 2006) and our rebuttal within this text. Our responses to Dr. McBride's points a, b and c under this item *3.iv)* are provided below.

a. ***McBride****:The blending process added lime to bring the pH measured in 0.01 M $CaCl_2$ to about 6.0- 6.2, which in my experience means that the soil pH in water is probably close to 7.0.*

Jacques Whitford: Dr. McBride's personal experience that a soil pH in water measurement is actually one whole pH unit greater than that measured in 0.01 M $CaCl_2$ was not experienced in work done by Jacques Whitford or others conducting similar recent studies (Canadian Land Reclamation Conference, 2006). Values of soil pH were presented in the Crops report as both water and calcium chloride measurements. The actual difference in soil pH between these two independent methods was only 0.3, i.e., definitely not an entire 1.0 pH unit.

McBride*: Therefore, the derived PNECs must be considered to be pH-conditional, and could only apply to soils with near-neutral pH. PNEC's for Ni are expected to be highly sensitive to soil pH because of the well-known strong effect of soil pH on Ni solubility and plant toxicity. pH in the contaminated area varies considerably, as shown by data in the JW report. As pH decreases in the soil, PNEC values are likely to be drastically lowered. Figure 1, taken from data in the JW crops report, shows the dramatic effect of soil pH on Ni toxicity to oats. This toxicity is diminished, but not eliminated, by raising soil pH.*

Reproduced Copy of McBride's Figure 1



Jacques Whitford: We agree with Dr. McBride's observation on Figure 1 that there is a definite pH effect on soil Ni bioavailability and thus, logically, toxicity of soil Ni – no doubt. At no time has Jacques Whitford taken the position that the PNEC values are not pH dependent. PNECs were developed at specific narrow pH ranges representative of the averages of soil pH measured in the four soil types of Port Colborne. Table 2 shows the specific soil pH range per soil type for the crop dose-response greenhouse experiments of year 2001 and corresponding soil Ni EC_{25} and PNEC values.

Table 2 PNEC and EC_{25} values of Soil Ni Derived at Specific Soil pH Ranges

Soil Type	Greenhouse 2001 Soil pH Range	Reference for given Soil pH Range	Soil Ni EC_{25}, mg/kg	Soil Ni PNEC, mg/kg
Welland Clay	5.86 - 6.38	Table GH 27	1880	1650
Till Clay	5.49 – 6.48	Table GH 36	1950	1400
Organic Muck	5.81 – 5.91	Table GH 22	3490	2350
Sand (Dune)	7.14 – 7.39	Table GH 17	1350	750

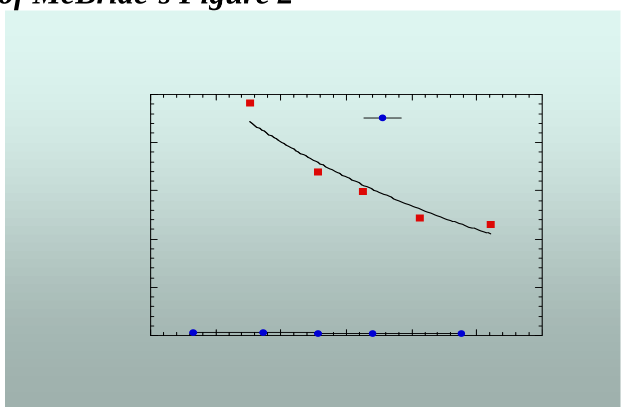
For areas of Port Colborne with soils above the upper limit of the experimental soil pH ranges of pH 5.9 -6.4 for Welland Clay, pH 5.5 to 6.5 for Till Clay and pH 5.8 to 5.9 for Organic Muck (Table 2) ie. above pH 6.5, higher PNECs than those presented above in Table 2 would result. This is based on the observed lower bioavailability of nickel at pH values above 6.5 in the upper end of the curve in the reproduced Figure 1 (ie. the pH experiment results).

There are no agricultural areas within the Ni-impacted area of Port Colborne for a particular soil type that may be found below the lower limit of the experimental soil pH ranges of pH 5.9 -6.4 for Welland Clay, pH 5.5 to 6.5 for Till Clay and pH 5.8 to 5.9 for Organic Muck (Table 1), ie. there are no measured or recorded soil pH values below pH 5.5. Examination of the soil pH map for the area of Port Colborne on Drawing No. 2.2 in Part 2 of Volume 1 of the Crops Report (Jacques Whitford, 2004) show no pH values below pH 5.5 in Port Colborne. The lowest pH value shown on Drawing No. 2.2 is pH 5.64 in Till Clay at test pit location J.

If hypothetically there are agricultural areas in Port Colborne for a particular soil type that may be found below pH 5.5 (the lower limit of the experimental soil pH ranges), then theoretically, based on the lower end of the curve in the reproduced Figure 1, more conservative or lower PNECs may result. (This is a theoretical assumption only as there were no greenhouse studies conducted below pH 5.5 and thus we have no measured PNECs at lower pH values for comparison). But in practice, farming at soil pH values below pH 5.5, or below the lower limit of the above-mentioned experimental soil pH ranges, would not be desirable and thus not applicable to this CBRA. The Agronomy Guide for Field Crops (Ontario Ministry of Food and Rural Affairs, 2002, publication 811) recommends to farmers interested in optimizing their growing conditions and obtaining maximum crop yield that their soil pH of agricultural lands be maintained above pH 6.5 (for coarse and medium-textured mineral soils) and pH 6.0 (for fine-textured mineral soils). Port Colborne agricultural soils within the top 15 cm of the tilling zone are coarse and medium-textured mineral soils (Section 4.2 of Vol IV of the Crops Report). Thus derivation of a PNEC for a Port Colborne soil pH below the lower limits of the experimental pH ranges of pH 5.9 -6.4 for Welland Clay, pH 5.5 to 6.5 for Till Clay and pH 5.8 to 5.9 for Organic Muck (see Table 2) and thus below the OMAFA-recommended soil pH of 6.5 would be only of pure academic interest and not of particular assistance to the prudent Port Colborne farmer interested in obtaining maximum crop yield.

> ***McBride:*** *Figure 2, from the same JW experiment, confirms the very high Ni uptake by oats from this clay soil containing 1900 mg/kg total Ni, so the bioavailability of Ni in the Port Colborne clay soil is clearly quite high.*

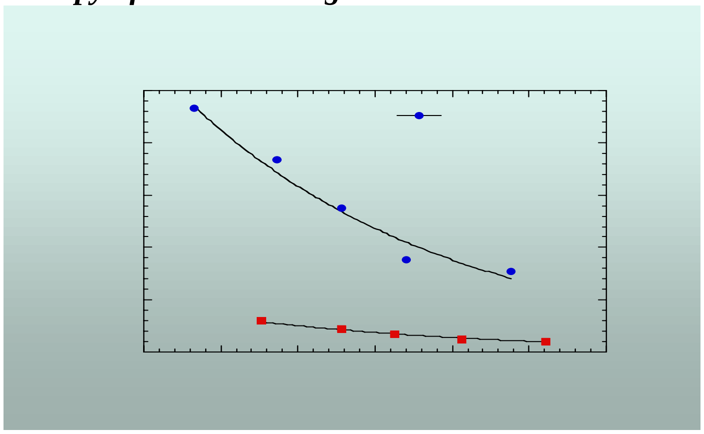
Reproduced Copy of McBride's Figure 2



<u>Jacques Whitford</u>: Dr. McBride's comment on Figure 2 is <u>his</u> description of oat grown on Welland Clay at a soil Ni concentration of 1900 mg/g, i.e. at a soil Ni concentration <u>greater than</u> the reported PNEC of 1650 µg/g (see above Table 2) for this soil type. Jacques Whitford has issues with the reviewer's use of relative terms describing Figure 2, such as "very high Ni uptake" and bioavailability that is "clearly quite high" which in our view are not particularly appropriate, unless of course a frame of reference is given. If the data in the Crops report were to be compared to those gathered from a hypothetical study where 1900 mg/kg of Ni as $NiCl_2$ was added to sand, the data in the Crops report would demonstrate "very low Ni uptake" and bioavailaibility that was "clearly quite low". In short, of course there is toxicity resulting from Ni uptake (i.e. some of the soil Ni was bioavailable), at soil Ni concentrations greater than the PNEC.

b. ***McBride**: The blending process replenishes certain essential nutrients , such as Mn, that may have been severely depleted by long-term exposure to aerial deposition of various toxic metals and sulfur from the refinery stack. Figure 3, based on data from a greenhouse experiment in the JW crops report, shows the critical effect of Ni contamination in the clay soil of Port Colborne in depressing Mn uptake by oats and inducing severe Mn deficiency in the crop. Clearly, Ni toxic effects cannot be understood without at the same time considering the secondary effects that Ni has on uptake of essential micronutrients by the crop.*

Reproduced Copy of McBride's Figure 3



<u>Jacques Whitford</u>: The blending process that Dr. McBride mentions in this comment had followed the same mixing process described by Sauerbeck and Hein (1991), an investigation which by the very nature of Dr. McBride's previous comments above appears to be his gold standard.

Dr. McBride's logic on the blending process replenishing deficient nutrients is flawed. If the experimental blending, or mixing, replenishes certain essential elements that have been "depleted [from soil] by aerial deposition of various toxic metals" (note that all metals are toxic, depending on the dose), then it follows that the depletion of the field soils must be proportional to the dose via aerial deposition, resulting in a range of depletion in the field. So why would experimental blending of high- and low- Ni soils not reasonably simulate the range of depletions (if in fact such depletions are happening at all) that would occur in the field, and which would be correlated with total soil Ni?

c. ***McBride:*** *In creating the blended soils, a single high-Ni soil taken from one specific location was blended with fertile soil and lime to generate soils with a range of Ni concentrations. This results in reliance of the entire PNEC determination for the region on a single soil sample from one location to represent all Ni-contaminated Port Colborne soils, a questionable approach given variability in soils of the region.*

Jacques Whitford: Greenhouse experiments in the year 2001 derived PNEC values for the 4 major soil types to be applied to the impacted agricultural areas in Port Colborne. Regarding the methodology used in generating these PNEC values, this protocol of blending a highly contaminated Port Colborne soil with a control Port Colborne soil has been recently endorsed by Environment Canada (2005) after considerable consultation and experimentation. And as mentioned earlier in our responses, the blending process is essentially the same protocol as used by Sauerbeck and Hein (1991), the methodology and results in which the reviewer has considerable confidence.

*4. **McBride**: The proposed PNEC values have an additional degree of uncertainty because the only crop used by JW to establish the PNECs , oats, are not a particularly Ni- sensitive crop, as Kukier and Chaney (2004) have shown for the Port Colborne soils. They state unequivocally that "grass species were more resistant to Ni toxicity than dicots". As oats is a grass species and soybeans are dicots, this observation argues against using oats to establish PNEC's for sensitive crops, even if oats were a significant crop in the region. At a minimum, soybeans should have been added to the greenhouse tests as a potentially sensitive and economically important crop of the region. Ideally, a number of crops with different rooting depths and lengths of growing season should have been tested.*

Jacques Whitford: The key data from the cited paper of Kukier and Chaney (2004) are in Table 3, which lists the concentration of 0.01 $Sr(NO_3)_2$-extractable soil Ni corresponding to the EC_{25} for shoot mass for each species examined in this study. For soybean, the value is 4.6 mg/kg and for oat, the value is 5.7 mg/kg; so, based on these absolute values, soybean is slightly more sensitive than oat. However, the authors of the paper present no variability for their measurements of $Sr(NO_3)_2$-extractable soil Ni, and it is very likely that these two values are not statistically different from each other. Further, Table 3 of the Kukier and Chaney (2004) study does support the use of oat as an indicator for Ni toxicity in soils, as, despite being a monocot, its $Sr(NO_3)_2$-extractable soil Ni concentration for EC_{25} is quite similar to that of corn, both of which are far lower than those for barley, wheat or ryegrass. So, indeed, monocots are generally more tolerant to Ni than dicots, but oat is an exception to this generalization. It should also be noted that the original data for these values are presented in Figure 5 of the Kukier and Chaney (2004) study, and while these regressions represent a tremendous amount of work, many of them would be considered statistically weak.

Specifically, a number of the relationships are two clusters of data joined by a straight line, or extrapolations from very steep parts of a curve.

The reviewer although suggesting the use of soybean, has not considered how the results obtained would have been evaluated in comparison to other studies in the scientific literature and accepted by the scientific and regulatory community as soybean plants have not been used as a plant system for characterizing metal toxicity. As described previously, for oat the nickel induced toxicity is unique and allows scientists to be able to clearly establish a cause-effect relationship. This is not the case with soybean where specific phototoxic symptoms that can be attributed to nickel have not been identified. There is a very extensive literature scientific data base for Ni phytotoxicity research using oats.

Integration of phytotoxicity data from all plant species including oat, radish, soybean, corn and goldenrod was done within Jacques Whitford's Crops report. Oat was shown to be most sensitive to the site-specific conditions under both field and greenhouse conditions. This was corroborated by findings in the literature (Kukier and Chaney, 2004, Chaney et al., 2003 and references within). Oat is usually considered the most characteristic plant indicator of nickel phytotoxicity based on research of Vergnano and Hunter (1952) which has been corroborated repeatedly over 50 years of further research (e.g. Anderson et al. 1973). In oat, the visible toxicity symptom specific to nickel phytotoxicity is an alternating pattern of more chlorotic and less chlorotic bands across young leaves and iron deficiency is observed as interveinal chlorosis. It is because of the uniqueness and sensitivity of oat to Ni phytotoxicity, that oat was selected as the crop for the GH 2001 work.

Summary Response to Dr. McBride's May 4, 2006 Comments

Jacques Whitford is of the opinion that the solution chemistry of the nickel in Port Colborne soils is unique and cannot be compared to any literature studies using more soluble forms of Ni amended to soils as the reviewer has made in his foregoing comments.

At the January 31st 2006 CBRA Technical Subcommittee (TSC) Meeting in Port Colborne where Jacques Whitford presented their evidence supporting the Crops-derived PNEC values, a TSC member did a quick "logic check" on the validity of these PNEC values. The logic check involved adjusting the Ontario Generic Soil Cleanup Criterion of 200 mg/kg for soluble nickel divided by the measured bioaccessibililty fraction for insoluble forms of nickel in Port Colborne soils to derive adjusted (predicted) MOE Soil Ni cleanup values for comparison to CBRA greenhouse-derived soil Ni PNEC values. The reader should keep in mind that the MOE Soil Ni cleanup 200 mg/kg value was based on greenhouse experiments by Davis et al. (1978) of barley grown on a quartz sand culture and exposed to varying concentrations of soluble nickel chloride. Thus the MOE Soil Ni cleanup 200 mg/kg value is representative of phytotoxicty of crops to only soluble nickel in soil, but not representative of crop exposure to the identified insoluble forms of Ni (Jacques Whitford, 2005) in Port Colborne soil.

Input values on nickel bioaccessibility in soils for this logic check calculation was taken from data in the MOE (2002) Rodney Street Report and the Jacques Whitford (2005) Human Health Risk Assessment Report. Using the above-described equation of dividing the Ontario Generic Soil Cleanup Criterion of 200 mg/kg for soluble nickel by the measured bioaccessibility for soil types including Welland Clay, Fill Material from the Rodney Street area and Organic Muck, led to the calculation of PNEC values as shown in Table 3. Good agreement between the derivated and measured PNEC values was found for Welland Clay.

Although a PNEC was not measured for the Rodney Street Fill soil samples, it can be assumed that the fill material was comprised of a mixture of Welland Clay and Organic Muck, ie. the two major soil types in the immediate area of the East Side Community. Thus, it can be assumed that had a soil Ni PNEC been measured on the fill, it would have had been between 1650 mg/kg (Welland Clay) and 2350 mg/kg (Organic Muck). Under this assumption, good agreement between derivated and measured PNEC values was found for the Rodney Street Fill material.

The measured soil Ni PNEC value of 2350 mg/kg for Organic Muck soil is twice as high than that derivated (952 and 769 mg/kg). Explanation for the difference is that PNEC derivations using the above method is only valid for mineral soils, which was the host medium used by the Davies et al.(1978) experiment, not Organic Muck soils containing 40% of organic carbon that would have scavenged more of the soluble Ni than a quartz sand would. The sand culture used by Davies et al. (1978) cannot model the buffering influence of organic matter, so it over-predicts the potential for toxicity in Organic Muck soils, hence the poorer fit with the Crops-derived PNECs for this soil type.

Overall the findings from this logic check calculation illustrates that the Crops-derived PNEC values for the mineral soil types are comparable to the MOE generic standard when the measured bioaccessibility of the soil is accounted for.

Lastly, Jacques Whitford would like to point out that the PNECs derived for the CBRA Crops Study are based on observation and measurement of Ni phytotoxicity in oat using CoCs occurring in naturally aged Port Colborne soil types and thus are conservative of what would be observed in the field, and will be equally protective of other economic crops grown in Port Colborne soils. In our opinion, the characteristics of the natural Port Colborne soil types used to develop the PNECs are representative of the majority of agriculture producing soils in Port Colborne.

Table 3 - Derivation of PNECs by Adjustment of the Ontario Generic Soil Clean-up Criterion for Nickel Employing Bioaccessibility Values Determined Under Acidic Conditions

Source	Soil Type	Nickel Bioaccessibility Test Type	Nickel Bioaccessibility (%) mean (range) (mg/kg)	Derivated PNEC [Adjusted Generic Soil Clean-up Criterion 200[f]/bioaccessi-bility fraction] (mg/kg)	Measured PNEC[e], JW 2004 (mg/kg)
MOE 2002	Fill	Simulated stomach leach	16.5 (11.8-23.3)	1212 (1694-858)	nm[g] {1650-2350}
MOE 2002	Fill	SBRC Acid Extract	14 (8-21)[a] 19 (11-28)[b]	1429 (2500-952) 1052 (1818-714)	nm[g] {1650-2350}
JW, 2002	Fill	SBRC Acid Extract	5.4[c] 6.8[d]	3703 2941	nm[g] {1650-2350}
JW, 2002	Welland Clay	SBRC Acid Extract	14[c] 14[d]	1429 1429	1650
JW, 2002	Organic Muck	SBRC Acid Extract	21[c] 26[d]	952 769	2350[h]

a. Ground Port Colborne soil

b. Sieved fine Port Colborne soil

c. Without glycine: Welland clay (14%); organic soil (21%); fill soil (5.4%)

d. With glycine: Welland clay (14%); organic soil (26%); fill soil (6.8%)

e. Predicted No Effect Concentration (PNEC) based on experimental data, in mg/kg: 1650 (heavy clay); 1400 (fill clay); 2350 (organic); 750 (sand)

f. 200 mg/kg is representative of phytotoxicty of crops to soluble Ni in soil, but not representative of crop exposure to insoluble forms of Ni in Port Colborne soil. The 200 mg/kg value for soluble Ni was based on greenhouse experiments by Davis et al. (1978) of barley grown on a quartz sand culture and exposed to varying concentrations of soluble nickel chloride. The number 200 mg/kg for soluble Ni is divided by the measured bioaccessibililty fraction for insoluble forms of Ni in Port Colborne soils to provide adjusted (predicted) MOE soil Ni cleanup values for comparison to CBRA measured greenhouse-derived soil Ni PNEC values. Good agreement between derivated and measured PNEC values is found for Welland Clay.

g. nm - A PNEC was not measured for the non-agricultural fill type soils collected from the East Side Community for bioaccessibility testing. Although a PNEC was not measured for the fill soil samples, it can be assumed that the fill material was comprised of a mixture of Welland Clay and Organic Muck, ie. the two major soil types in the immediate area of the East Side Community. Thus, it can be assumed that had a soil Ni PNEC been measured on the fill, it would have had been between 1650 mg/kg (Welland Clay) and 2350 mg/kg (Organic Muck). Under this assumption, good agreement between derivated and measured PNEC values is found for the fill material.

h. The measured soil Ni PNEC value of 2350 mg/kg for Organic Muck soil is twice as high than that derivated (952 and 769 mg/kg). Explanation is that derivations using the above mentioned calculation is only valid for mineral soils, which was the host medium used by the Davies et al. experiment, not Organic Muck soils containing 40% of organic carbon that would scavenge more of the soluble Ni than a quartz sand would.

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APPENDIX A

Sauerbeck and Hein (1991) Paper

THE NICKEL UPTAKE FROM DIFFERENT SOILS AND ITS PREDICTION BY CHEMICAL EXTRACTIONS

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ABSTRACT. The uptake of Ni by 13 plant species was investigated from two soil types containing Ni in different concentrations and forms. Absorption was highest from $NiCl_2$, less from Ni containing sewage sludge or industrial filter dust, and least from a soil containing geogenic Ni. The 13 species grown can be classified into four groups differing in Ni uptake and toxicity. The Ni contents in grain and in storage organs were larger than in the vegetative plant parts. The highest Ni contents were found in the roots. Plants grown in pots absorbed more Ni than from the same soils in the field. During consecutive years the Ni availability did not decrease. Only soil extractions with unbuffered salt solutions reflected the availability of pollution-derived Ni sufficiently well.

1. INTRODUCTION

Besides Cd and Zn, Ni is considered to be one of the more mobile heavy metals in soils. In contrast to Cd its zootoxicity is relatively low, but similar to Zn it can readily reach phytotoxic concentrations. However, compared with other trace metals the knowledge about its behavior and uptake depending on soils, chemical forms and crop plants is still relatively scarce. This causes some uncertainty in assessing the acceptable maximum Ni concentrations for soils.

It was, therefore, decided to run a series of experiments in which various Ni forms were offered to different plants in two types of soil, so as to study uptake, and to compare several chemical extractants for predicting the Ni availability. Some representative results are presented he-re, whereas the full set of data has been reported elsewhere (Hein, 1988; Hein and Sauerbeck, 1988).

2. MATERIALS AND METHODS

2.1 Soils and xperimental treatments

Two different soils were compared, a Loess-derived neutral Luvisol and a more acid sandy Cambisol (table I).

a) A sewage sludge originally low in heavy metals was enriched with $NiCl_2$ and incubated for 30 d at 30^{o}C. Mixing the soils with this sludge resulted in additional Ni contents of 0, 17, 34, 50, 75, 100, 150 and 200 mg kg^{-1}. All pots received identical amounts of 100 g sludge dw / 10 kg soil.

Water, Air, and Soil Pollution **57–58**: 861–871, 1991.

b) In order to compare this sludge treatment with a readily soluble salt, other pots received $NiCl_2$ at the same concentrations.

c) Two sewage sludges contaminated with Ni from industrial waste were also compared.

d) An industrial filter dust was also imncluded in quantities yielding identical Ni concentrations.

e) The pH value of the Luvisol (7.25) was lowered in some treatments to 6.5 by $Al_2(SO_4)_3$, while the acidity of the Cambisol (pH 5.45) was in some cases reduced with $CaCO_3$ to pH 6.3.

f) A geogeneously Ni enriched soil from basalt was mixed with the above mentioned Cambisol in order to obtain similar Ni concentrations.

TABLE I. Analytical data of the 2 soils

	Luvisol	Cambisol
pH (0.01 M $CaCl_2$)	7.25 (6.5)	5.45 (6.3)
% $CaCO_3$	1.3	-
mg P_2O_5 (CAL)	18	10
mg K_2O (CAL)	25	7
% C	1.81	1.25
CEC	16.4	9.3
% sand	3	35
% silt	82	57
% clay	15	8

2.2 Experimental plants

During 3 consecutive seasons, a series of 7 vegetables, 3 cereals and ryegrass were grown ander greenhouse conditions in polyethylene pots, each holding 9.5 kg soil.

2.3 Analytical techniques

The plant material was dried at 105 ^{o}C and wet-digested using $HNO_3/HClO_4$ (4:1). Ni measurements were carried out by a Perkin Elmer atomic absorption spectrophotometer.

Soil samples were extracted with (1) 0.005 M DTPA; (2) 0.005 M DTPA/TEA (Mitchell et al., 1978); (3) 0.005 M DTPA/0.05 M $CaCl_2$/0.05 M TEA (Lindsay and Norvell, 1978); (4) 0.005 and 0.01 M $(NH_4)_2$EDTA (Rietz and Soechtig, 1981; (5) 1 M NH_4OAc (Rietz and Soechtig, 1981); (6) 0.025 and 0.125 M $CuCl_2$ (Horst and Bruene, 1987); (7) 1 M $NaNO_3$ (Haeni and Gupta, 1983); (8) 2 M KCl (Roth et al., 1971); (9) 0.05 M $CaCl_2$ (Sauerbeck and Styperek,1985; Styperek, 1986); and (10) 1 M $MgCl_2$ Pietz et al., 1983).

3. RESULTS AND DISCUSSION

3.1 Ni availability and distribution (Fig. 1 to 6)

The uptake of Ni depends on a) its form and origin, b) soil pH, c) plant species, d) plant organ, and e) can be influenced by time. There was f) a difference depending on whether the plants were grown in pots or in the field.

a) Ni form and origin (Figure 1)

From the different Ni forms, the mineral $NiCl_2$ was always the most available one. Mixing and incubating this with sewage sludge reduced its availability only slightly, which may have to do with the fact that its reaction time with the sludge was only 30 d. Similar results have also been published by Cunningham et al. (1975), Dijkshoorn et al. (1983a, b) and Cottenie et al. (1983).

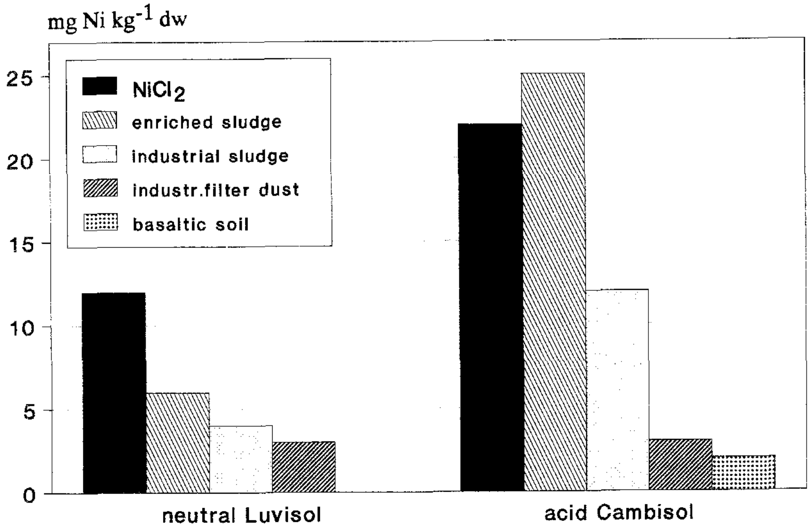

Fig. 1. Ni content of lettuce plants (1985) on soils which were polluted with 50 mg Ni kg^{-1} from different sources

The Ni uptake from the industrial sludges and especially from the filter dust was much less. This can be explained by the fact that the sewage-derived Ni was incorporated and stabilized during the anaerobic sludge treatment (Bloomfield and McGrath, 1982). The Ni uptake from the filter dust was rather low, and the geogenic Ni in the basalt soil was hardly available at all, because it is bound in the soil minerals from where it can only be released very slowly by gradual weathering (Schlichting, 1979; Horst and Bruene, 1987).

b) pH value of soils (Figure 2)

The lower the pH value of the soils, the higher was the Ni uptake by the plants. This has already been shown by other investigators (Cottenie and Kiekens, 1981); Dijkshoorn et al., 1981; Fassbender and Seekamp, 1976; Kuntze et al., 1984. Lowering the pH of the neutral Luvisol increased Ni uptake, whereas liming the acid Cambisol decreased the availability.

c) Plant species (Figure 2):

Yield depresssions were registered both for the vegetables and for oats, but toxicity symptoms were only shown on the acid Cambisol. The Ni concentrations in the plants depended very much on the plant species.

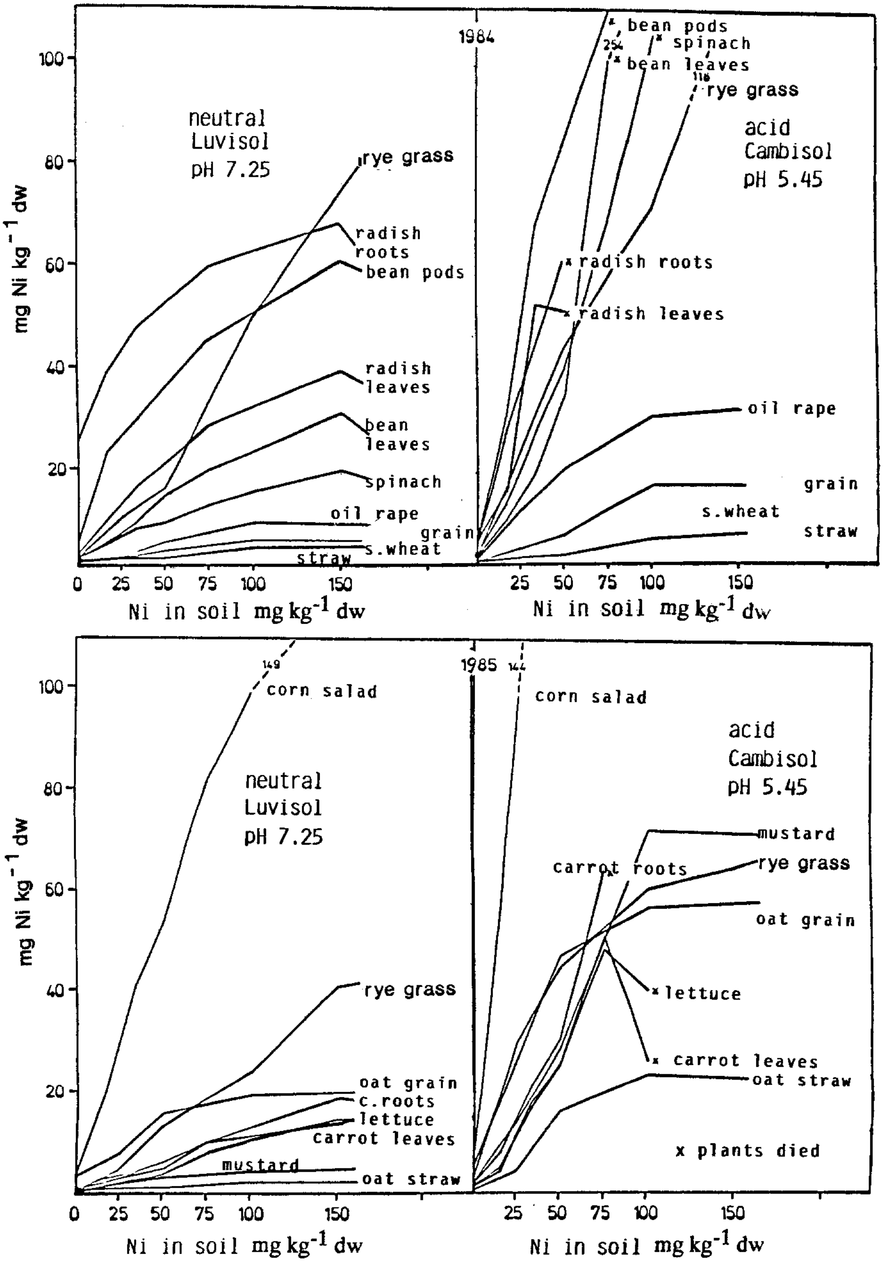

Figure 2. Ni in different plant tissues depending on soil pH and plant species

The plants tested can be classified for their Ni sensitivity as follows:

- Plants which absorb only small amounts of Ni and do not show any toxicity symptoms:
  * Spring wheat * spring barley * oil rape * mustard *
- Plants which absorb medium amounts of Ni and may sometimes show toxicity symptoms:
  * Spinach * lettuce * carrots * kohlrabi * oats *
- Plants which absorb high amounts of Ni and are severely damaged:
  * Phaseolus beans * radish * corn salad *
- Plants which absorb high amounts of Ni but do not show any toxicity symptoms:
  * Ryegrass *

d) Plant organs (Figure 2 to 4)

The Ni contents of the reproductive and the storage organs were considerably higher than in the vegetative plant parts (e. g. bean pods > bean leaves; cereal grain > straw). This suggests a physiological relationship between the transport of photosynthates and of Ni.

There are contradictory statements in the literature about this Ni distribution: According to Diez and Rosopulo (1976) this preferential accumulation in the cereal grains occurs only at elevated Ni concentrations in soil.. Phaseolus beans, on the other hand, were reported to accumulate Ni in their pods irrespective of the Ni supply (Foroughi et al., 1981).

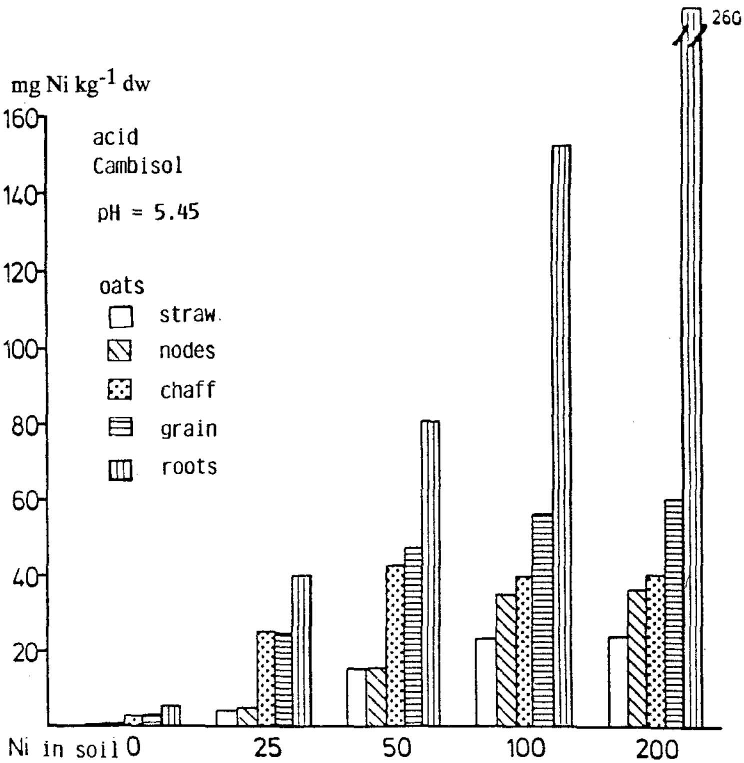

Figure 3. Ni contents of different plant parts of oats when grown in Ni contaminated soils

Figure 3 is an example for this Ni distribution in oat plants including their roots. The most striking Ni accumulation and retention occurred in the plant roots. Their Ni contents increased almost proportionately with the Ni in the soil, whereas the contents in the above-ground plant parts increased much less.

Since this phenomenon was associated with decreasing plant yields, it may be safely be assumed that, in cases with more than 50 mg Ni kg^{-1} in the acid Cambisol, oat roots were damaged. Thus, uptake and translocation were probably impeded which, in turn, reduced plant yields and the Ni transfer to shoots.

e) Influence of time (Figure 4)

Time can influence the uptake of heavy metals in various ways. One is the growth period, which may be the reason for the different contents in plant varieties. The other ome is time for the particular heavy metal to be incorporated into natural binding forms in the soil (Sauerbeck, 1985). While the first question can not be clearly answered from the results of this work, the 3 years of grass cropping have provided information concerning the latter.

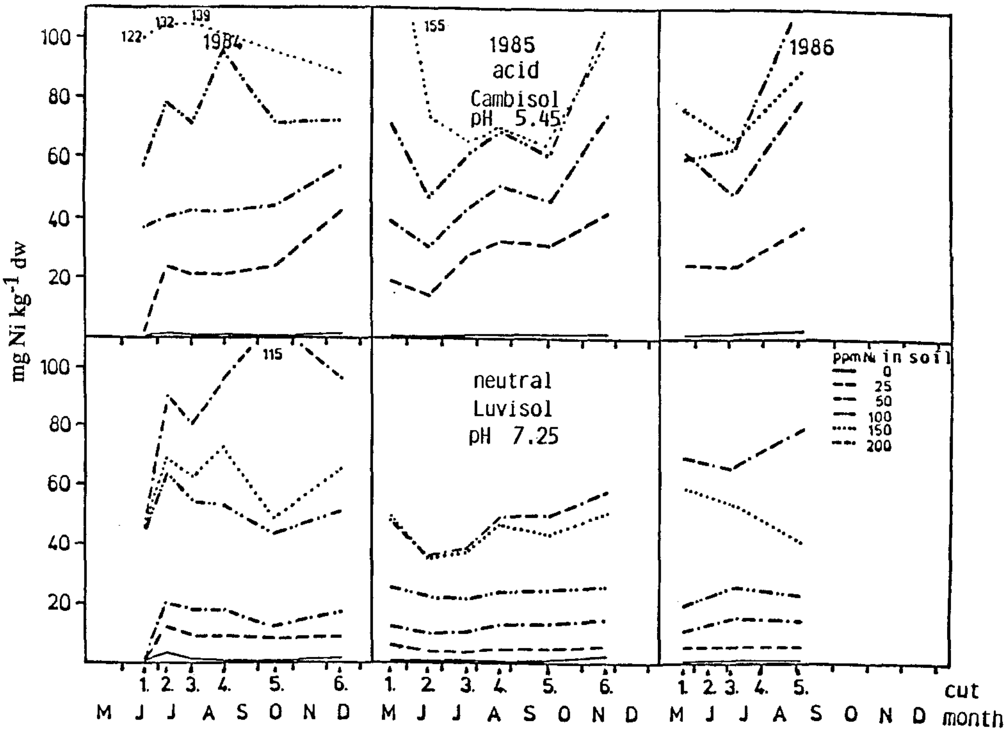

Figure 4. Ni contents of the 5 -6 ryegrass cuts during three consecutive years

In all years there was a tendency of increasing Ni contents in later grass cuts, especially on the more acid Cambisol, but this differed with the degree of Ni contamination. However, there was little decrease in the Ni uptake during consecutive years, which indicated that in both soils little fixation occured.

3.2 Extraction procedures

As with all other heavy metals, the total Ni content of soils reflects their contamination but does not indicate plant availability, which depends on soil properties and on the Ni sources and forms. However, to get an idea about the suitability of different chemical extractants to predict availability, a number of solutions were tested, using the soils with the $NiCl_2$ enriched sludge.

TABLE II. Correlation coefficients between extractable Ni and the Ni uptake by carrots on 2 different soils treated with $NiCl_2$ polluted sewage sludge

extractant		DTPA	DTPA +TEA	DTPA+ TEA+ $CaCl_2$	.01N $NH_4)_2EDTA$	0.05N $NH_4)_2EDTA$	1M H_4OAc
corr.	roots r =	0.50	0.50	0.80	0.50	0.37	0.60
coeff.	leaves r =	0.50	0.50	0.80	0.50	0.43	0.70

extractant		0.025M $CuCl_2$	0.125M $CuCl_2$	1M $NaNO_3$	2M KCl	0.05M $CaCl_2$	1M $MgCl_2$
corr.	roots r =	0.60	0.60	0.98	0.99	0.98	0.99
coeff.	leaves r =	0.60	0.70	0.80	0.90	0.90	0.90

According to Table II, the DTPA and DTPA/TEA did not indicate the Ni availability sufficiently well (r = 0.50), because they dissolved a large proportion of the Ni irrespective of its origin and the soil properties. The mixture of DTPA/$CaCl_2$/TEA was more suitable, which can be concluded from the correlation coefficient of 0.80.

A poor correlation also was found for $(NH4)_2EDTA$, because its dissolution capacity did not depend on the individual soil properties (r = 0.37 to 0.50). The amounts dissolved by NH_4OAc were lower than those released by DTPA and EDTA, but the correlation (r = 0.60 to 0.70) was still not very good. Also the extraction with $CuCl_2$ (r = 0.60 to 0.70) did not appear particularly promising.

The other four unbuffered salt solutions were, in fact, the most suitable ones (r = 0.80 to 0.99). Similarly good correlations have already been shown by others for Cd and Zn. However, the $CaCl_2$ is preferred because it dissolves considerably larger amounts without impairing the correlation, wich offers the advantage of an easier measurement (Sanders et al., 1986a,b; Sauerbeck and Styperek 1985).

The data in Table II are not yet sufficient to draw definite conclusions. However, they indicate at least the principal superiority of the unbuffered salt solutions. Accordingly, $CaCl_2$, $CuCl_2$ and DTPA/$CaCl_2$/TEA were additionally tested using a larger collection of samples, which at this time included the full set of data which had been obtained from the entire treatments in the plant growth experiment.

The results in Figure 5 a-c are for carrot roots and leaves. According to these graphs, the overall correlation for $CaCl_2$ (r = 0.88-0.90) was considerably better than for $CuCl_2$ (0.80) or for DTPA/$CaCl_2$/TEA (r = 0.80). However, this apparent superiority of the $CaCl_2$ was mainly due to the large number of data at lower concentrations, whereas in the upper concentration range there was considerably more scatter.

The main reason for this large scatter must be that at high Ni concentrations the plants were injured, with the result that the Ni contents did not increase in proportion to the Ni contents of the soil (see, e. g. Figure 1 and Table II). However, notwithstanding these limitations, the data show that an extraction with unbufferd salts such as $CaCl_2$ will be the most promising way to predict the availability of Ni in polluted soils.

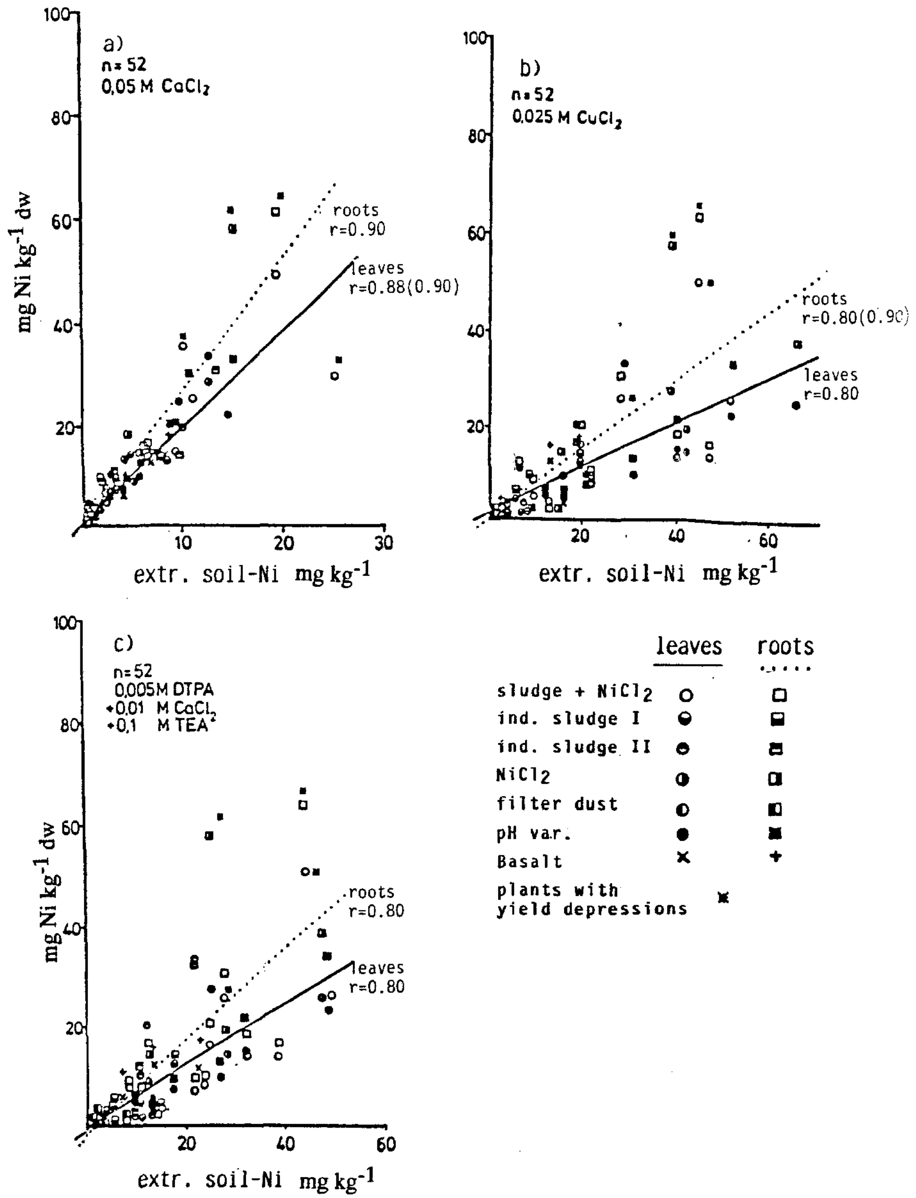

Figure 5. Correlation between Ni contents of carrot and the Ni amounts extracted by a) $CaCl_2$, b) $CuCl_2$ and c) $DTPA/CaCl_2/TEA$

3.3 Consequences for the allowable Ni concentrations

The German sewage sludge ordinance (BMI, 1982) and the corresponding EC directive (CEC, 1986) both state about 50 (30 to 75) mg Ni kg^{-1} as the maximum permissible level for soils on which sewage sludge may be applied. From the results obtained it is clear that this 50 mg kg^{-1} value is unnecessarily strict for the neutral Luvisol. Here 100 mg kg^{-1} would still be sufficiently safe, even if this soil were to drop to pH values between 6.0 and 6.5 .

However, as the results for the more sandy Cambisol have schown, not only some of the vegetables, but even oats, can be damaged if 50 mg Ni kg^{-1} soil are surpassed. This is at least so for the more soluble Ni of antropogenic origin, whereas the geogenic Ni can be safely assumed to have a most limited availability (Figure 1).

Hence, if the significance of certain Ni contents in soils is to be judged, one either has to take into consideration the individual soil properties or to use an additional extraction with unbufferd salt solution. The 50 mg Ni kg^{-1} limit, which has been discussed, can not be considered an unrealistic worst case value, although under normal circumstances it is reasonably safe (Sauerbeck, 1989).

4. CONCLUSIONS

- Among the different forms of Ni, inorganic $NiCl_2$ proved to be the most available one, followed by an artificially Ni enriched sewage sludge, a Ni containing industrial sludge, an industrial filter dust, and the geogenic Ni from a basaltic soil.
- The lower the pH of the soil, the higher was the Ni uptake by plants. Acidification of neutral soil increases, and liming of acid soil decreased, this Ni availability.
- The Ni content in different plant species varied considerably and was higher in the reproductive than in the vegetative plant parts.
- The highest Ni accumulation occurred in plant roots, which seemed to be the prime reason for yield depression, even though Ni contents in plant shoots were not extremely high.
- Extraction with unbufferd salt solutions provided the best prediction of available Ni. This indicated that extractability with these solutions is governed by the same soil properties as is the uptake by plants.
- Of the salts tested as extractants, $CaCl_2$ was the most suitable one, at least for assessing the significance of anthropogenic soil Ni pollutions.
- The aaceptable Ni contamination of soils depends on their properties. As far as the Ni of anthropogenic origin is concerned, its content should not exceed about 50 mg kg^{-1} in light sandy soils.

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TAB 3

Jacques Whitford's Response Letter to Watters Environmental July 13, 2006 Comments/Questions on the Jacques Whitford Crops Report. Response Letter is dated September 12, 2006

Jacques Whitford's Response to Watters Environmental July 13, 2006 Comments/Questions on the Jacques Whitford Crops Report (September 12, 2006)

This document constitutes the response from Jacques Whitford Limited (Jacques Whitford) to the July 13, 2006 Watters Environmental Group Inc. (WEGI) comments on their review of the 2004 Port Colborne CBRA Crops Report (Jacques Whitford, 2004). Each of the comments/questions from WEGI have been thoroughly reviewed and answered in this text. WEGI's comments have been reproduced in the text below in an italized Times New Roman font with Jacques Whitford's response following each major point in an Arial font.

• *Please provide the reference to information in the Crops Report that details evidence for your position that the plants were over-watered in the 2000 study.*

In Year 2000, greenhouse testing was done in a closed pot environment where the inside of each pot was artificially lined with a plastic bag. It was the opinion of Jacques Whitford at the time of the design of the Year 2000 experimental setup, that lining inside the pots would prevent the escape of any soluble salts and CoCs from being washed out of the pots. However, as the experiments progressed, it became evident, to Jacques Whitford that the use of an artificial liner produced a growth limiting factor to the crops sown, as created by the closed environment that lowered the redox potential of the soils and produced reducing conditions at the bottom of the pots. This evidence was based on observations made on the growth habit on both treated and control plants. Thus the absence of drainage caused reducing conditions at the base of the pots even though careful watering had been carried out. The lack of oxygen in the root zone created phytotoxic conditions and these reducing conditions are not normally found within active agricultural soils of Port Colborne. At the advice of the University of Guelph scientists, this design flaw was rectified by Jacques Whitford in the greenhouse experiments of 2001. As the design of the Year 2000 experiment did not simulate actual oxidizing conditions of Port Colborne soils in an open environment, the Year 2000 findings must be interpreted with <u>caution</u>.

• *Comment on whether there is any information in the 2000 study that can be used to interpret the overall findings of the Crops Study. Specifically comment on the findings of the following tests:*

a) Sand data for all 3 crops used

b) Soybean data on clay soils

Jacques Whitford has responded to the above comments in previous responses as outlined our Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a) that addressed aspects of the experiments such as phytototoxicity for plants grown on sand and even integrated findings from the experiment conducted with soybean grown on clay soils [see graphs presented on page 11 of the Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a)].

- *The 2000 data for unlimed sand appears to show a significant yield reduction (vrs controls) for oats, soybean and corn at 300 ppm soil Ni. Please comment on this information compared with your proposed PNEC values.*

As stated earlier in our first response, the Year 2000 findings must be interpreted with <u>caution</u>. WEGI's interpretation "….appears to show a significant yield reduction at 300 ppm soil Ni…" is incorrect and misleading. The reader is referred to Section 5.1 of the Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a) that discusses the usefulness of the Year 2000 data and comparisons that were drawn to the PNEC values.

• *The 2000 data for soybeans on clay appears to show a significant reduction in "yield" from 200 to 500 ppm Ni that appears not to be corrected by lime. Please comment.*

As stated earlier in our first response, the Year 2000 findings must be interpreted with <u>caution</u>. WEGI's interpretation " …appear to show a significant reduction in yield…" is incorrect and misleading. The reader is referred to Section 5.1 of the Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a) that discusses the usefulness of the Year 2000 data.

• *Did Jacques Whitford assume that plant yield is the same as top weight? What do local Port Colborne farmers use to measure crop "success"?*

Jacques Whitford used plant biomass or "top weight" to use WEGI's definition for measurement of plant yield. Plant biomass is a standard measurement technique used in phytotoxicity studies. The results obtained by using plant biomass data are reliable and comparable with other well documented scientific studies. Chronic effects of metals on plants are usually assessed by long term growth assays and are mostly quantified by measuring plant biomass of the plants after the treatment (exposure) period. Most plant scientists determine the final dry mass of the shoot. This gives a good indication of a plant's ability to germinate and compete successfully for water, light and nutrients, and play a significant role in ecosystem processes when growing in the presence of elevated metals - these abilities, or endpoints, are identified at the beginning of the risk assessment process. Interpretation of plant biomass data as it relates to economic yield was validated by discussions with OMAF representatives, local farmers and crop insurance companies.

The local Port Colborne farmers as well as other farmers in Southern Ontario measure their crop "success" against reported average yields of the traditional crops cultivated as part of the cash crop rotation. The yields in Port Colborne are comparable with the ones reported by the Ontario Ministry of Food and Rural Affairs OMAF (2002) for other parts of Southern Ontario, and sometimes even higher. These reports have been confirmed by local farmers (undisclosed farmers) and crop insuring companies (Agricorp Insurance). In Year 2005, the average farm in the Port Colborne area obtained the same average yield for soybean at about 30 bu./acre compared to other farms in Southern Ontario. This indicates that the yields produced by Port Colborne farmers have not been negatively impacted by the historically deposited soil Ni contamination.

• *Using Jacques Whitford's 2001 soil pH study, it appears that, although liming can increase pH to reduce Ni effects, the plant weights ("yields") do not approach those of control plants grown on soils with a similar pH. Does this mean that, despite efforts to "correct" the Ni issues on plants, agricultural systems can never be returned to "precontamination" conditions (at least by liming)? Please comment.*

Based on the observations and measurements from the pH experiment of oat grown on Welland Clay at a soil Ni concentration well above soil Ni PNEC level and at various soil pH levels (page 9 of Jacques Whitford, 2006b), the beneficial effect of liming on the tissue Ni accumulation is evident. The full effect of lime application to soil contaminated with the CBRA CoCs can only be quantified after some time from its application. This was obvious from the results presented in the Crop Report (Jacques Whitford, 2004) and in the Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a) for the clay C2 and C3 sites where agricultural limestone was applied at different points in time, ie. 1999 and 2001, respectively. At the clay C2 site which was limed three years before the clay C3 site, the beneficial effects of liming was more evident (ie. in the clay C2 site), starting with the second year of cropping.

• *Comment on whether Jacques Whitford utilized the results of its 2001 pH study in its overall interpretation. If so, please provide reference(s) where used.*

PNECs were developed at specific narrow pH ranges representative of the averages of soil pH measured in the four soil types of Port Colborne, not for soil pH values outside (ie. above and below) these narrow pH ranges. Table 1 shows the specific soil pH range per soil type for the crop dose-response greenhouse experiments of year 2001 and corresponding soil Ni EC_{25} and PNEC values.

Table 1 PNEC and EC_{25} values of Soil Ni Derived at Specific Soil pH Ranges

Soil Type	Greenhouse 2001 Soil pH Range	Reference for given Soil pH Range	Soil Ni EC_{25}, mg/kg	Soil Ni PNEC, mg/kg
Welland Clay	5.86 - 6.38	Table GH 27	1880	1650
Till Clay	5.49 – 6.48	Table GH 36	1950	1400
Organic Muck	5.81 – 5.91	Table GH 22	3490	2350
Sand (Dune)	7.14 – 7.39	Table GH 17	1350	750

• *Comment on whether Jacques Whitford's EC_{25} values are pH-dependent, and whether the tested soil pH(s) represent the most sensitive soil pH condition found in Port Colborne. If not, how can test results be interpreted to satisfy the study objective(s)?*

The results of the Jacques Whitford 2001 pH study indicated that the soil Ni bioavailability is affected by soil pH. For areas of Port Colborne with soils above the upper limit of the experimental soil pH ranges of pH 5.9 -6.4 for Welland Clay, pH 5.5 to 6.5 for Till Clay and pH 5.8 to 5.9 for Organic Muck (Table 1), ie. approximately above pH 6.5, higher PNECs than those presented above in Table 1 would result based on the Jacques Whitford 2001 pH study that showed lower bioavailability of nickel as soil pH increased above the soil pH experimental range.

There are no agricultural areas within the Ni-impacted area of Port Colborne for a particular soil type that may be found below the lower limit of the experimental soil pH ranges of pH 5.9 -6.4 for Welland Clay, pH 5.5 to 6.5 for Till Clay and pH 5.8 to 5.9 for Organic Muck (Table 1), ie. there are no measured or recorded soil pH values below pH 5.5. Examination of the soil pH map for the area of Port Colborne on Drawing No. 2.2 in Part 2 of Volume 1 of the Crops Report (Jacques Whitford, 2004) show no pH values below pH 5.5 in Port Colborne. The lowest pH value shown on Drawing No. 2.2 is pH 5.64 in Till Clay at test pit location J.

However, if there were hypothetically such areas of lower soil pH than the experimental pH ranges (Table 1), then theoretically, based on the findings of the Jacques Whitford 2001 pH study, more conservative or lower PNECs would result at the lower pHs, ie. below pH 5.5. <u>But in practice,</u> farming at soil pH values below the above mentioned experimental soil pH ranges would not be desirable and thus not applicable to this CBRA. The Agronomy Guide for Field Crops (Ontario Ministry of Food and Rural Affairs, 2002, publication 811) recommends to farmers interested in optimizing their growing conditions and obtaining maximum crop yield that their soil pH of agricultural lands be maintained above pH 6.5 (for coarse and medium-textured mineral soils) and pH 6.0 (for fine-textured mineral soils). Port Colborne agricultural soils within the top 15 cm of the tilling zone are coarse and medium-textured mineral soils (Section 4.2 of Vol IV of the Crops Report). Thus derivation of a PNEC for a Port Colborne soil pH below the experimental pH ranges of pH 5.9 -6.4 for Welland Clay, pH 5.5 to 6.5 for Till Clay and pH 5.8 to 5.9 for Organic Muck (see Table 2) and also below the OMAFA-recommended soil pH of 6.5 would be only of pure academic interest and not of particular assistance to the prudent Port Colborne farmer interested in obtaining maximum crop yield.

• *Comment on why Jacques Whitford measured soil pH in a solution of calcium chloride. Why wasn't water used? What method would be used for soils provided by local farmers? How would the 2 results compare?*

Jacques Whitford used both the water and the calcium chloride methods in measuring soil pH as this is common practice in soil and plant science when soil metal availability is being assessed. Within the Crops Report (Jacques Whitford, 2004), values of soil pH were presented as both water and calcium chloride measurements in Tables GH1- 7, 22, 27, 36, 40 (Volume I, Binder 2 out of 3). The actual difference in soil pH found between these two independent methods was only 0.3. This is consistent with other studies (Canadian Land Reclamation Conference, 2006).

• *Comment on whether oats is the most sensitive species for a study to establish level(s) of soil Ni that are to be protective of all agricultural crops in Port Colborne.*

Integration of phytotoxicity data from all plant species including oat, radish, soybean, corn and goldenrod was done within the Crops report (Jacques Whitford, 2004). Oat was shown to be most sensitive to the site-specific conditions under both field and greenhouse conditions. This was corroborated by findings in the literature (Kukier and Chaney, 2004, Chaney et al., 2003 and references within). Oat is usually considered the most characteristic plant indicator of nickel phytotoxicity based on research of Vergnano and Hunter (1952) which has been corroborated repeatedly over 50 years of further research (e.g. Anderson et al. 1973). In oat, the visible toxicity symptom specific to nickel phytotoxicity is an alternating pattern of more chlorotic and less chlorotic bands across young leaves and iron deficiency is observed as interveinal chlorosis. It is because of the uniqueness and sensitivity of oat to Ni phytotoxicity, that oat was selected as the crop for the GH 2001 work.

Kukier and Chaney (2004) reported the concentration of 0.01 $Sr(NO_3)_2$-extractable soil Ni corresponding to the EC_{25} for shoot mass for each species examined in this study. For soybean, the value is 4.6 mg/kg and for oat, the value is 5.7 mg/kg; so, based on these absolute values, soybean is slightly more sensitive than oat. However, the authors of the paper present no variability for their measurements of $Sr(NO_3)_2$-extractable soil Ni, and it is very likely that these two values are <u>not statistically different</u> from each other. Further, Table 3 of the Kukier and Chaney (2004) study does support the use of oat as an indicator for Ni toxicity in soils, as, despite being a monocot, its $Sr(NO_3)_2$-extractable soil Ni concentration for EC_{25} is quite similar to that of corn, both of which are far lower than those for barley, wheat or ryegrass. So, indeed, monocots are generally more tolerant to Ni than dicots, but oat is an exception to this generalization. It should also be noted that the original data for these values are presented in Figure 5 of the Kukier and Chaney (2004) study, and while these regressions represent a tremendous amount of work, many of them including the relationship between oat and soybean, are <u>statistically weak</u>. Specifically, a number of the relationships are two clusters of data joined by a straight line, or extrapolations from very steep parts of a curve.

If Jacques Whitford in designing the Y2001 greenhouse trials had used soybean instead of oat, it would be uncertain as to how the results obtained would have been evaluated in comparison to other studies in the scientific literature and accepted by the scientific and regulatory community as soybean plants have not been used as a plant system for characterizing metal toxicity. As described previously, for oat the nickel induced toxicity is unique and allows scientists to be able to clearly establish a cause-effect relationship. This is not the case with soybean where specific phytotoxic symptoms that can be attributed to nickel have not been identified. There is a very extensive literature scientific data base for Ni phytotoxicity research using oats.

• *Comment on whether the Confidence Intervals provided by Jacques Whitford in its report are based on (a) the data itself, or (b) a "best-fit line Explain the difference and how each can be interpreted.*

As previously explained in the Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a), there are two calculations for confidence intervals (CI) around a regression relationship – the population CI's and the data CI's. The former are used to estimate the precision with which the regression relationship will predict Y from the observed values of X, when the intent of the regression relationship is to predict the response of oat plant biomass (Y) to soil Ni (X) over the entire population of Welland

Clay soils, which are assumed might vary considerably from those used to determine the relationship. Hence, these CI's are wider than those calculated as the data CI's, which are used to estimate the precision with which the regression relationship predicts Y from the observed Y and observed X (the intent of the CI's depicted with the Weibull functions in the crop report). While it might seem intuitive that the former is more appropriate for risk assessment than the latter, neither actually is correct for the purpose of predicting a value of X for a particular value of Y, as we wish to do by predicting the soil Ni value that is associated with a 75% reduction in plant growth. This is called *inverse prediction* and involves an additional error term to those (errors associated with the estimates of the regression parameters, as well as the unexplained error) used in *prediction*, namely the errors associated with the random, normally distributed variable Y. The confidence intervals for inverse prediction are not symmetrical around the predicted value of X, but like CI's for prediction, get wider with distance from the mean value of Y.

• *What is the variability (+/-) for each EC_{25} value?*

The variability (+/-) for each EC_{25} value can be found in Table 3-6, page 3-61 of Volume I (binder 1 out of 3) of the Crops Report (Jacques Whitford, 2004).

• *Comment on whether any of the field plots used by Jacques Whitford were previously used by others. If so, please provide details, and comment on whether (and how) Jacques Whitford's results might have been influenced by these previous studies.*

The clay C1 and C2 Test sites as well as the organic muck site were previously prepared and used by others prior to Jacques Whitford's involvement in Y 2000. Specifically at the C1 Test site, liming occurred in 1999, while cultivation occurred in 1999 and,2000. At the C2 Test site, liming occurred in 1999 and cultivation in 1999, 2000 and 2001. The only field site originally prepared by Jacques Whitford and not by others was the clay C3 Test site. At the C3 Test site, lime application was conducted in late spring of Y 2001. Details on site preparation by Jaques Whitford and others are included in Part IV of the Crop Studies Report (Jacques Whitford, 2004). It is not anticipated that the Jacques Whitford results have been influenced by previous studies as Jacques Whitford used the same field plots for unamended and amended as used by the previous investigators. In similar manner, Jacques Whitford conducted work on field plots on the C2 and organic muck sites consecutively in years 2000 and 2001 and it is not anticipated that Jacques Whitford's results in 2001 were influenced by Jacques Whitford's results in 2000.

• *A significant amount of time and effort was expended to collect field data. Please confirm why this data was collected and how it was to be used.*

Field data including biomonitoring data were collected to verify and ground truth the Greenhouse data that was eventually used to calculate the soil Ni - EC_{25} values. Field data and biomonitoring data can be found in Parts 4 and 5 respectively in the Crop Report (Jacques Whitford, 2004). The reader is referred to the sensitivity analysis of the derived soil Ni - EC_{25} values in Section 4.11.3 of Part 3, Volume 1 of the Crops Report (Jacques Whitford, 2004) that compared Greenhouse data with the Biomonitoring data. The reader is also referred to the integration of the Greenhouse and Field findings in Sections 4.2.3 and 5.1 of the Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a).

• *Did Jacques Whitford use any of the collected field data in its overall interpretations? If so, please provide references/details on how the field data were used.*

As per above, reader is referred to Section 4.11.3 of Part 3, Volume 1 of the Crops Report (Jacques Whitford, 2004) and Sections 4.2.3 and 5.1 of the Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a).

• *Comment on the inherent risks/limitations of not having thorough field studies data to confirm the greenhouse study results.*

Jacques Whitford believes there are no inherent risks of "not having thorough field studies data to confirm the greenhouse study results" as inferred above by WEGI. Evidence provided both from the field in Port Colborne, and from fundamental knowledge of plant physiology and crop cultivation, indicate that estimates of plant growth response to soil metals, obtained from pot studies in the greenhouse and expressed as PNECs, are likely to **overestimate** sensitivity. The primary reason for this is that metals in the field soil of Port Colborne are most concentrated in the upper 15 cm, a layer that many plant roots quickly grow through in their search for water. The roots of greenhouse-grown plants are confined to soils with elevated metal concentration, thus their accumulated dose throughout their lifetime is greater than that in field-grown plants.

We agree that if practical and achievable, field dose-response experiments in the derivation of PNECs could have offered a comparison to the greenhouse-derived PNEC values. However the impracticality of conducting dose-response experiments in the field was explained in detail under Option 2 on page 4 of Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a).

In conclusion, the greenhouse-derived PNEC values are conservative and overestimate the crop sensitivity in the field, and would be much greater than field-derived PNEC values.

• *Please provide details on the effort employed by Jacques Whitford to locate suitable field control sites. Were options provided to Jacques Whitford by TSC members?*

Details of the design of the Y2000 and Y2001 field trials and the selected clay C1, C2, C3 sites and the Organic Muck site are outlined in Sections 2.3.2 and 2.4.1, respectively within the Crops Report (Jacques Whitford, 2004). As stated within these Sections, neither of the Y2000 nor the Y2001 field trials were designed as dose-response experiments and thus, the use of field control sites were not considered. Soil nickel concentrations at the clay C1, C2, C3 sites and the Organic Muck site were 636, 4950, 3590 and 3210 mg/kg; that is, at soil nickel concentrations well above the PNEC values with the exception of the clay C1 site. The impracticality of conducting dose-response experiments in the field in Port Colborne was explained in detail under Option 2 on page 4 of Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a).

• *Comment on whether the Jacques Whitford blended soils represent well-aged actual Port Colborne soils*

The Jacques Whitford blended soils of the Y2001 greenhouse trials most definitely represent well-aged actual Port Colborne soils. Jacques Whitford used naturally weathered and aged (ie. greater than 15 years since date of last atmospheric CoC deposition) representative soils from Port Colborne from the nickel impacted area and these were mixed with control soils for the major soil types from uncontaminated areas of Port Colborne. The issue with aging of soils is not with this study, but instead with other literature published studies of greenhouse experiments using soils spiked with a soluble metal salt, aged for a short period of time and then the mixed with control soils.

• *Comment on the inherent risks and limitations of using a single soil sample to manufacture all the blended soil ranges for that soil type.*

Jacques Whitford used a protocol of blending a highly contaminated Port Colborne soil with a control Port Colborne soil; a protocol that was later endorsed by Environment Canada (2005) after considerable consultation and experimentation. Details on the rationale on the selection of this protocol are found under Option 4 on page 5 of the Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a).

• *Comment on the impact of mixing a highly nutrient-rich (control) soil with a nutrient deficient contaminated soil. How does the resulting "blended" soil compare with actual in-situ soils?*

If the experimental blending, or mixing, replenishes certain essential elements that have been "depleted [from soil] by aerial deposition of various toxic metals" (note that all metals are toxic, depending on the dose), then it follows that the depletion of the field soils must be proportional to the dose via aerial deposition, resulting in a range of depletion in the field. So why would experimental blending of high- and low- Ni soils not reasonably simulate the range of depletions (if in fact such depletions are happening at all) that would occur in the field, and which would be correlated with total soil Ni?

In the case of deriving a PNEC for sand taken along the dunes off Lake Erie, the nutrient levels were relatively similar in both the control sand and the contaminated sand. Thus the concern by the reviewer regarding mixing high nutrient rich soil with low nutrient deficient soil is not valid and the PNEC value for sand is valid. Because the nutrient question is not an issue in the derivation of the PNEC for sand, and because of our reasoning on the whole nutrient issue as given above, Jacques Whitford is of the opinion that the blended soil compares well with the actual insitu soils.

• *Were any proposals/options provided to Jacques Whitford that offered an opportunity to test actual Port Colborne soils with a range of soil Ni levels? (i.e. Without the need for blending)*

Our files indicate no documentation of any such offers to test actual Port Colborne soils with a range of soil Ni levels. It can be said however, that prior to the initiation of the Y2001 field work, that there were extensive meetings and discussion at both on the TSC and PLC level regarding opinions on the design of this work. The final design took all such views into consideration and were carefully evaluated. The impracticality of conducting dose-response experiments in the field in Port Colborne is summarized under Option 2 on page 4 of Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a).

• *Describe the efforts employed by Jacques Whitford to assess the representativeness of blended soils to actual field soils in Port Colborne.*

The blended soils used in the Y2001 greenhouse trials were actual soils from Port Colborne. Every effort was made in the preparation of these blended soils to ensure consistency of the representative field averages in values of soil chemistry and physical parameters and that the only variable was a range in soil Ni concentrations. Details can be found in Section 4.0 of Part II of Volume I of the Crops Report (Jacques Whitford, 2004).

• *Comment on whether a 'weight-of-evidence' approach might have been used for overall interpretation of findings (i.e., using the "best" of the 2000 & 2001 datasets)*

A 'weight of evidence' approach using 'all' of the Y2000 and Y2001 datasets, not just the 'best' datasets, was undertaken by Jacques Whitford; the findings and conclusions of which are documented in Sections 4 and 5 of the Overview of Evidence Addendum to the Crops Report (Jacques Whitford, 2006a).

• *Comment on whether the proposed PNEC values are intended to represent unrestricted land use values.*

We are not sure of WEGI's use of the term "unrestricted land use" in relevance to the Crops report. The Official Plan of the City of Port Colborne has designated land use types. If WEGI's question was meant to ask if the proposed PNEC values apply to agricultural areas in Port Colborne in an unrestricted manner, the answer would be yes. PNECs were developed for agricultural crops grown on agricultural zoned soils in Port Colborne.

It was discussed earlier in this letter that PNECs may be pH dependent. For areas of Port Colborne with soils above pH 6.5, higher PNECs than those documented in the Crops report would result. For areas of Port Colborne with soils below pH 5.5, lower PNECs than those documented in the Crops report would result. However, there are no documented evidence that there are such areas in Port Colborne where the soil pH is less than pH 5.5.

As previously mentioned in this letter, if hypothetically there were such areas of soil pH below pH 5.5, then theoretically lower PNECs would result. <u>But in practice,</u> farming at soil pH values below pH 5.5 would not be desirable nor applicable to the spirit of this CBRA. The Agronomy Guide for Field Crops (Ontario Ministry of Food and Rural Affairs, 2002, publication 811) recommends to farmers interested in optimizing their growing conditions and obtaining maximum crop yield that their soil pH of agricultural lands be maintained above pH 6.5 (for coarse and medium-textured mineral soils) and pH 6.0 (for fine-textured mineral soils). Port Colborne agricultural soils within the top 15 cm of the tilling zone are coarse and medium-textured mineral soils (Section 4.2 of Vol

IV of the Crops Report). Thus derivation of a PNEC for a Port Colborne soil pH below pH 5.5 and also below the OMAFA-recommended soil pH of 6.5 would be only of pure academic interest and not of particular assistance to the prudent Port Colborne farmer interested in obtaining maximum crop yield.

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TAB 4

Jacques Whitford's Response to Public Comments of Jacques Whitford Final Crops Report by deadline of July 14, 2006

No Public Comments were received by the July 14, 2006 Deadline.

TAB 5

Compilation of Public Notices, TCS and PLC meetings for documenting the public process for the CBRA

Eric Veska - Confirmation of Technical Subcommittee Meeting...Thursday May 26, 6.00 pm

From: <chuckmiller@portcolborne.com>
To: <mbellantino@inco.com>, <jbonaldo@regional.niagara.on.ca>, <bjornc@regional.niagara.on.ca>, <doug.durant@dsbn.edu.on.ca>, <rmbisson@sympatico.ca>, <malonj@parl.gc.ca>, <dave.mclaughlin@ene.gov.on.ca>, <mmonette@csdccs.edu.on.ca>, <paul.nieweglowski@ene.gov.on.ca>, <paul.perron@ncdsb.com>, <bob.slattery@ene.gov.on.ca>, <eveska@jacqueswhitford.com>, <rwatters@wattersenvironmental.com>, <thomas.saintivany@ncdsb.com>, <bconard@inco.com>, <pdayboll@niagarac.on.ca>, <tvlmom@yahoo.com>, <musky_924@hotmail.com>, <mi3sons@iaw.on.ca>, <2mwbw@qlink.queensu.ca>, <e.sellers@ventana-crc.com>, <l.kasprzak@ventana-crc.com>, <HWells@contactpsc.com>, <johnarm@oeb.com>, <timhudak@niagara.net>, <tmccallum@deloitte.ca>, <ianheerdegen.deloitte@sympatico.ca>, <appraisers@deloitte.ca>, <jgheller@jghcons.com>, <mtaylor@stantec.com>, <b.desousa@ventana-crc.com>, <chuckmiller@portcolborne.com>, <rosemari@interpc.ca>, <tgrabell@sympatico.ca>, <vivmos@cogeco.ca>, <nieboere@mcmaster.ca>, <dfraipont@inco.com>, <pavroz@idirect.com>, <speddle@cwren.com>, <agabor@urbanstrategies.com>, <tsmith@urbanstrategies.com>, <wprice@urbanstrategies.com>, <lpavlov@cogeco.ca>, <tribune@wellandtribune.ca>, <mi3sons1@iaw.on.ca>, <CWillert@jacqueswhitford.com>, <tfoster@oeb.com>, <inport@wellandtribune.ca>
Date: 5/19/2005 7:34:48 PM
Subject: Confirmation of Technical Subcommittee Meeting...Thursday May 26, 6.00 pm
CC: <MarthaToscher@Portcolborne.com>, <cao@portcolborne.com>, <janbeckett@portcolborne.com>, <City_Council% PORT__NOTES@portcolborne.com>, <stevenrivers@portcolborne.com>, <portcolborneleader@interpc.com>, <djohnso1@becon.org>, <mayor@portcolborne.com>, <lauralee@portcolborne.com>, <joanmiller83@hotmail.com>, <susanmabee@portcolborne.com>, <msturman@niagarathisweek.com>

Further to my e-mail of May 2 outlining the overall CBRA schedule for May, this will confirm the Technical Subcommittee will meet next Thursday as follows:

Date: Thursday May 26, 2005
Time: 6.00 pm
Place: Committee Room Three, City Hall

At the meeting the ERA Crops Report will be formally tabled and introduced by Jacques Whitford. The report will be the subject of technical and public review in the coming months.

I believe the report has been distributed to everyone who requested. However, I have additional hard copies available in my office.

Chuck

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"Serving You to Create and Even Better Community"

NOTICE OF MEETING

PUBLIC LIAISON COMMITTEE
for the
COMMUNITY BASED RISK ASSESSMENT
for Soils Contaminated in the Port Colborne Area

Thursday June 16, 2005...7.00 pm
Council Chambers, City Hall

AGENDA

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1 **Approval of Agenda**

2 **Approval of Minutes**

-PLC Meeting of March 24, 2005
-PLC Meeting of April 21, 2005
-PLC Meeting of May 19, 2005

3 **Delegations**

4 **"Tabling" and "Overview" of the ERA - Crops Report**
-Jacques Whitford

5 **Updates of CBRA Activities**
- Status of CBRA Activities
- General CHAP Activities
- Status of Lead Task Force Activities

6 **General Question and Answer Session**

7 **Next Meeting**

8 **Adjournment**

Persons wishing to be "delegates" to the Committee should register, in advance, with Martha Toscher at (905) 835-2900 ext. 319

CROP STUDIES

PORT COLBORNE CBRA

June 16-th, 2005

Presented by
Jacques Whitford Limited,
Consultants to Inco Ltd.

Jacques Whitford

Study Objective

The main objective:

To determine the concentrations of historically-deposited CoCs in Port Colborne soil that present an unacceptable risk (phytotoxicity) to agricultural crops.

Jacques Whitford

Design and Review Process

The Crop Studies were subjected to continuous input and review from experimental design to final interpretation from the Year 2000 to Year 2001.

Reviewers:

•Technical subcommittee (MOE, NPH, PLC, PLC Consultant).

•Internal expert review: Dr. B. Shelp, Dr. L Evans and Dr. B. Hale, University of Guelph; Dr. R. Chaney, US Department of Agriculture.

Jacques Whitford

Background

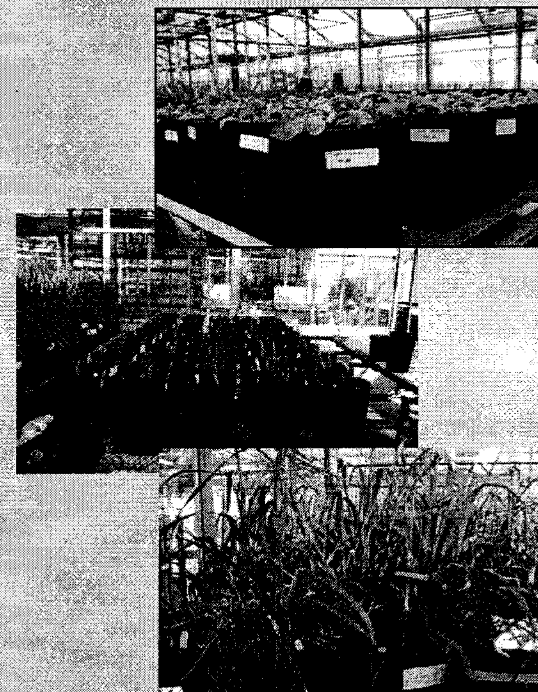
- Preliminary GH and Field Trials – 2000
- GH and Field Trials – 2001
- Data Analysis - 2002

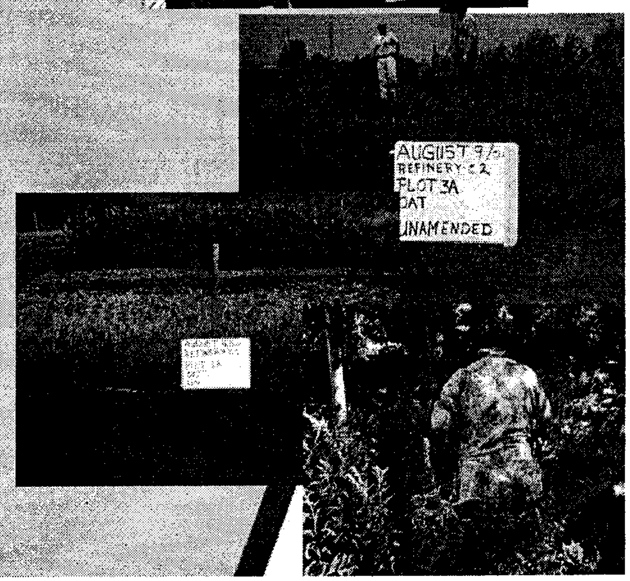
- First Draft Report– April 2003
- Revised Draft Report – July 2003
- Final Crop Report – December 2004

Jacques Whitford

YEAR 2000

YEAR 2001





YEAR 2001 TRIALS

Un-Amended
175 Pots

Amended
175 Pots

One Amendment Levels

- **"Prudent Farmer" (pH 7)**
- **$CaCO_3/MgCO_3$**
- **Compost**

Four Soils: Heavy Clay, Till Clay, Sand and Organic
Blends: Six CoC levels up to 2500 mg Ni/kg

Biomass Harvest

Plants: Oats and Radish*

Welland Clay Soil
~ 2000 mg Ni/kg

Un-Amended

Amended Plots

Welland Clay Soil
~ 6000 mg Ni/kg

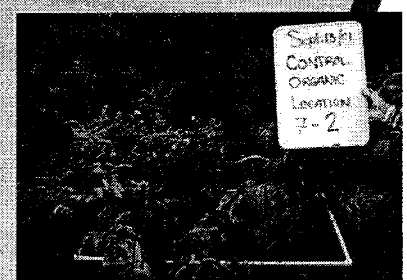
Welland Clay Soil
~ 3000 mg Ni/kg

Three Amendment Levels

- **OMAF**
- **2X OMAF**
- **Calcareous**

Dolomitic Limestone

Agronomic Biomass Harvest
Toxicological Biomass Harvest
Crop Yield Harvest
Plants: Oats, Soybeans, Corn and Radish

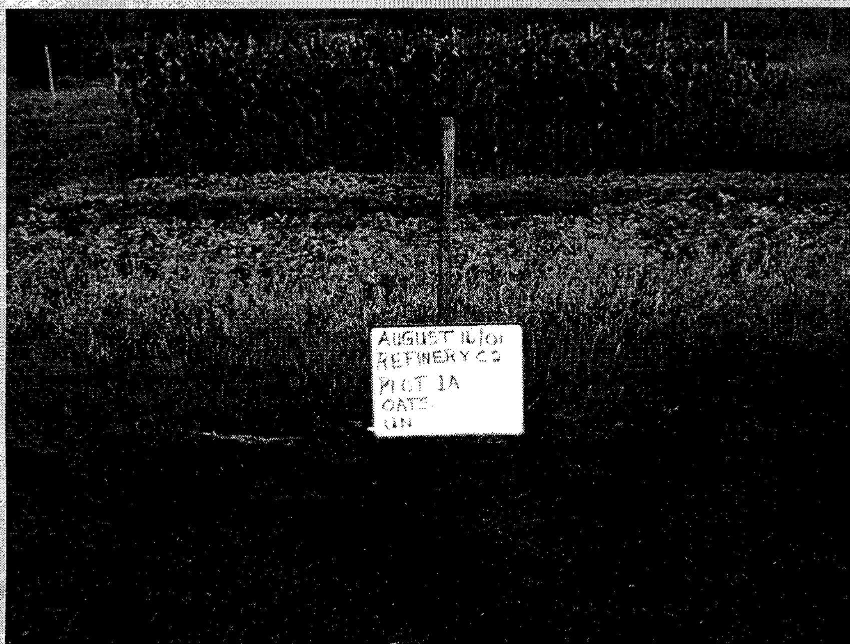


Biomonitoring

Jacques Whitford

Field Results:

Clay Soil | Organic Soil

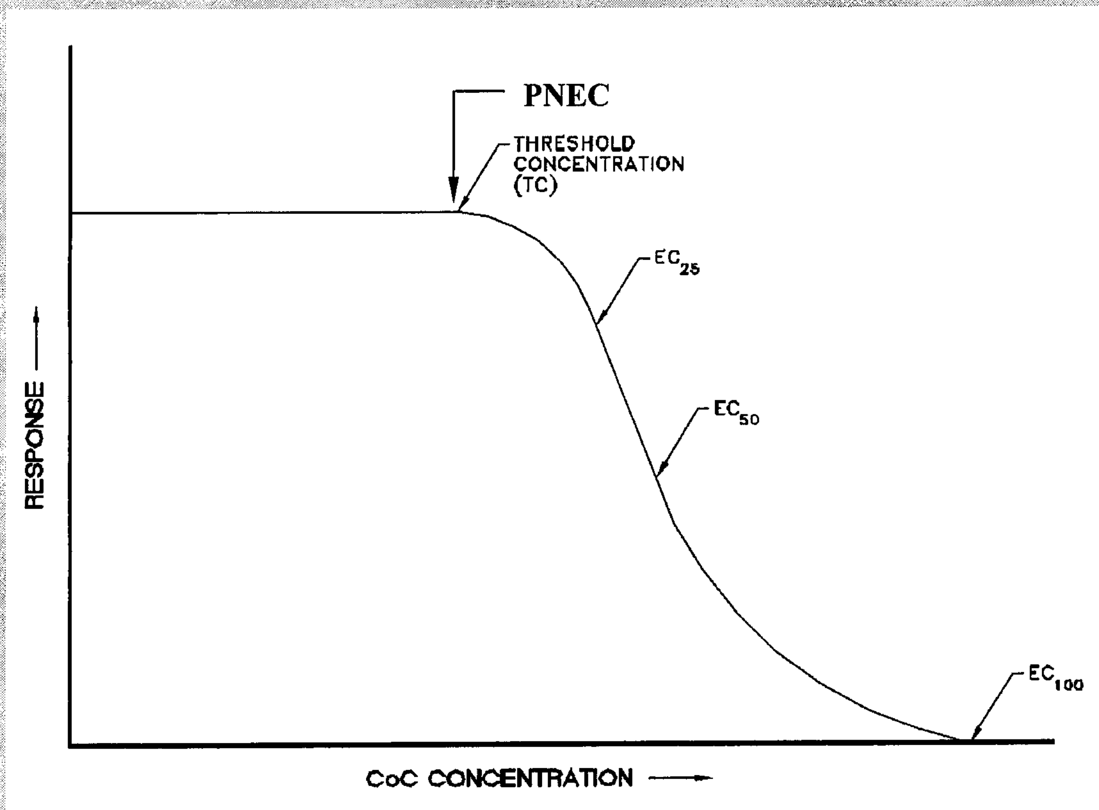


Jacques Whitford


AUG 30/01
REFINERY
C2 SITE

Evaluation of Phytotoxicity

PNEC = Predicted No Effect Concentration
TC = Threshold Concentration
ECx% = Effective concentration for x% impact of toxic effect



Jacques Whitford

Summary of EC_{25} Values and Confidence Intervals (5%, 95%)

Experiment	EC_{25} (mg/kg Ni in Soil)	EC_{25} (mg/kg Ni in oat tissue)
Oat on Sand	1350 (1100,1490)	71 (60,80)
Oat on Organic	>2400 (3490 meta-analyses)	>35 (46 meta-analyses)
Oat on Welland Clay	1880 (1600,1950)	52 (46,58)
Oat on Till Clay	1950 (1650,2000)	21 (19,23)

Jacques Whitford

Summary of EC_{25} and PNEC Calculations Based on Soil Total Ni

Soil Type	EC_{25} (μg/g)	PNEC (μg/g)
	Ni	Ni
Sand	1350	750
Organic	>2400, 3400*	2350
Till	1950	1400
Welland	1880	1650

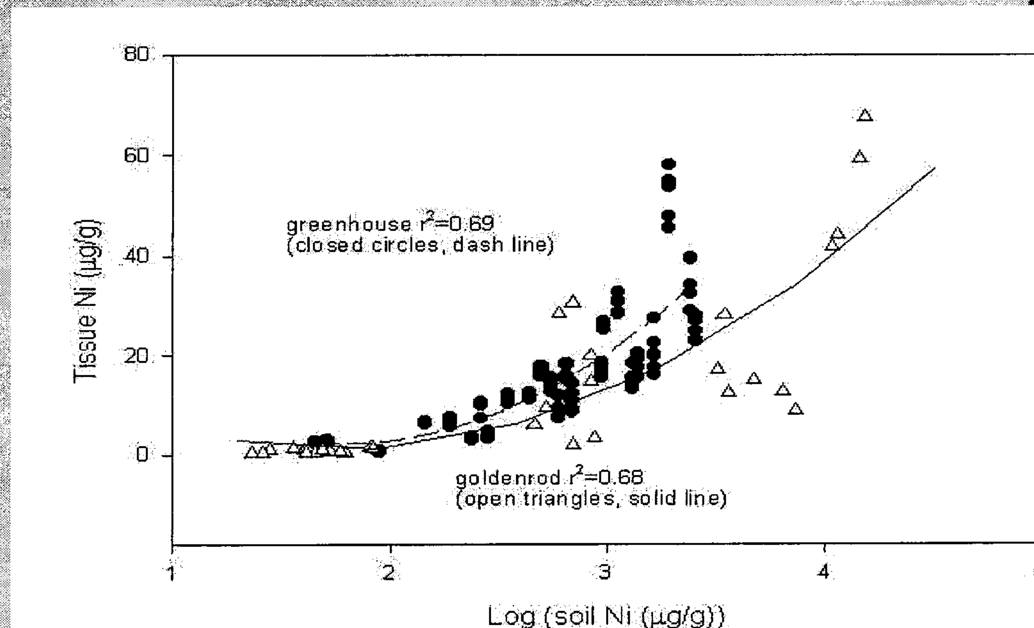
*derived from meta-analysis

Jacques Whitford

Sensitivity Analysis of Ni EC_{25} Tissue Levels

1) Comparison of GH 2001 oat tissue Ni concentrations to Field Biomonitoring 2001 goldenrod tissue Ni concentrations

- A similar r^2 relationship for Goldenrod Tissue Ni concentration and Oat Tissue Ni concentration as a function of log Soil Ni concentration was found
- Supports legitimacy of EC_{25} calculated from the GH 2001 Trials

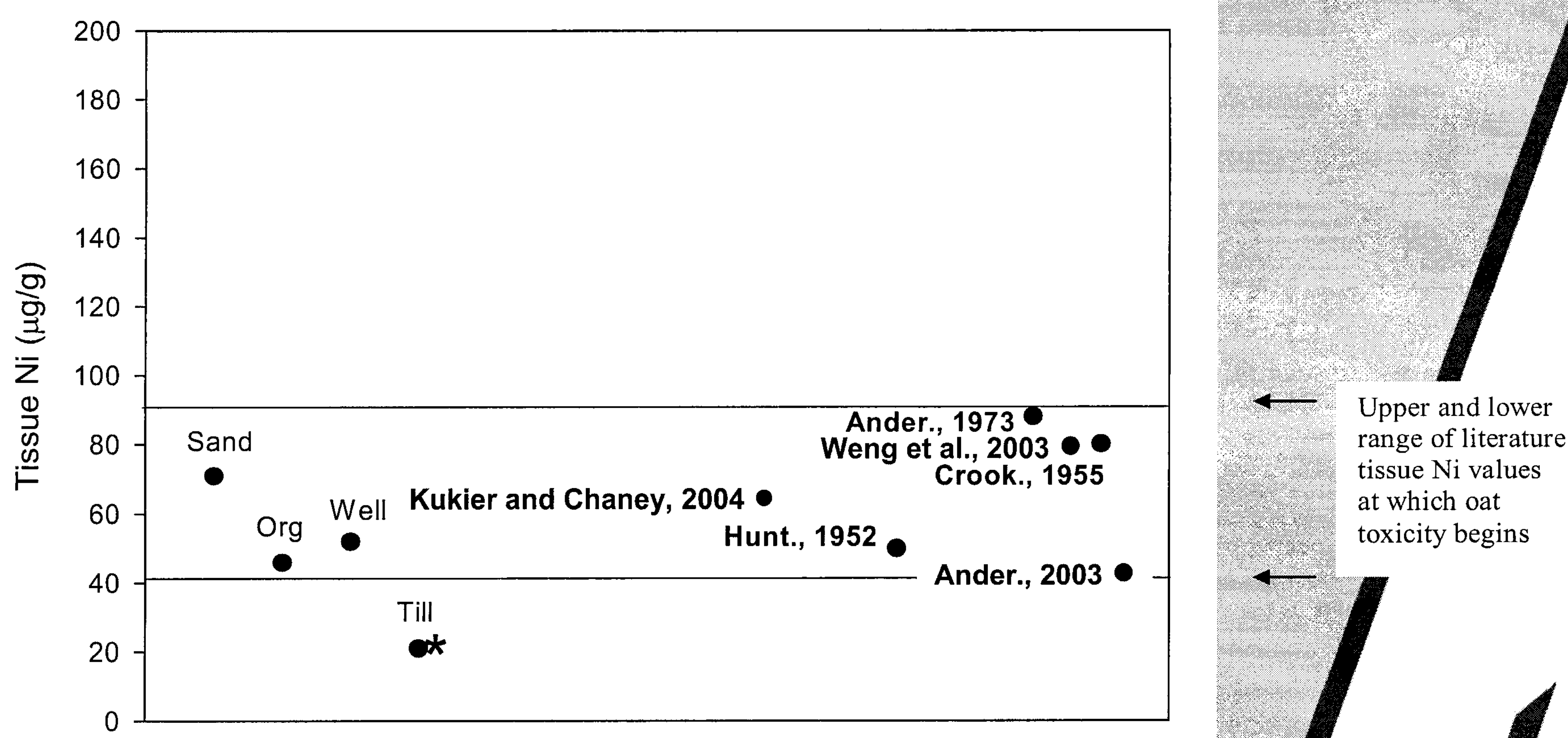


Regression of Oat- and Goldenrod-Tissue Ni Concentration as a Function of Log Soil Ni Concentration

Jacques Whitford

Sensitivity Analysis of Ni EC_{25} Tissue Levels

2) Comparison of GH 2001 Oat Tissue Ni concentrations to Literature-Reported Oat Tissue Ni Toxicity Thresholds



Crop Studies-determined oat tissue Ni EC_{25} thresholds are shown to be within the range of literature-reported oat toxicological thresholds for Ni

Jacques Whitford

Overall Findings of Sensitivity Analysis

Results from the sensitivity analysis provide strong scientific support for the legitimacy of the toxicity thresholds as calculated.

Jacques Whitford

Overall Findings
General Conclusions

Safe soil CoC thresholds have been derived, at and below which, the historically-deposited CoCs in Port Colborne soil present negligible risks (phytotoxicity) to crops.

Jacques Whitford


CROP STUDIES
PORT COLBORNE CBRA

PORT COLBORNE
COMMUNITY BASED RISK ASSESSMENT
ECOLOGICAL RISK ASSESSMENT - CROPS STUDIES
'FINAL REPORT'

"OPEN HOUSE and PRESENTATION"

- **Next Thursday**, representatives from Jacques Whitford Limited (Consultants to INCO) will hold an **Open House** dealing with the **"Final" Ecological Risk Assessment Report** related to **CROPS**; which forms part of the **Community Based Risk Assessment**. In 2003 and 2004 "Draft" of this report was the subject of considerable review both at the Technical Subcommittee, and, through a "public" comment and input process.
- The recently released "Final" version of the report (dated December 2004) is intended to address the "technical" and "public" comment/input received in 2003/2004.
- At the **"Open House"** and **"Presentation"**, the author (Jacques Whitford) will provide summary of the Report.

The session will be held at:

City Hall Council Chambers
on Thursday June 23, 2005
at 7.00 pm

The **Crops Studies** Report can be reviewed, and, a "Fact Sheet" respecting the report can be obtained by contacting Chuck Miller at City Hall, or, by attending the open house.

For further information, please contact:

Charles V. Miller
Strategic Projects
City of Port Colborne
66 Charlotte Street
Port Colborne, Ontario
(905) 835-2900 (ext. 303)
chuckmiller@portcolborne.com

Eric Veska - Lead Task Force Meeting RESCHEDULED, and, Crops Open House

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Date: 8/19/2005 1:03:46 PM
Subject: Lead Task Force Meeting RESCHEDULED, and, Crops Open House
CC: <marthatoscher@portcolborne.com>, <cao@portcolborne.com>, <janbeckett@portcolborne.com>, <City_Council% PORT__NOTES@portcolborne.com>, <stevenrivers@portcolborne.com>, <portcolborneleader@interpc.com>, <djohnso1@becon.org>, <mayor@portcolborne.com>, <lauralee@portcolborne.com>, <joanmiller83@hotmail.com>, <susanmabee@portcolborne.com>, <msturman@niagarathisweek.com>

Greetings...

This relates to next week's CBRA related scheduling...

1) Lead Task Force.......RESCHEDULED

As per my previous e-mails, a Lead Task Force meeting was scheduled for Wednesday August 24. That meeting has been RESCHEDULED to Thursday September 15 at 6.00 pm in Committee Room Three. Amongst other matters, the LTF will consider "Timing, Scope and Process" for the Committee to complete it's mandate...as requested by the Public Liaison Committee at last night's meeting.

2) Open House on the Crops Report, Thursday August 25 ✓

Dr. Watters has scheduled an "Open House" dealing with the "Crops Report" for Thursday August 25. Watters Environmental will be conducting the "Open House", which is particularly aimed at the farming community. The details are:

Thursday August 25, 2005
12.00 noon to 4.00 pm
7.00 pm to 9.00 pm
Council Chambers, City Hall

Cheers...

Chuck

Charles V. Miller
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Eric Veska - Confirmation of CBRA related meetings for next week.....

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Date: 11/11/2005 1:16:33 PM
Subject: Confirmation of CBRA related meetings for next week.....
CC: <marthatoscher@portcolborne.com>, <cao@portcolborne.com>, <janbeckett@portcolborne.com>, <City_Council% PORT__NOTES@portcolborne.com>, <stevenrivers@portcolborne.com>, <portcolborneleader@interpc.com>, <djohnso1@becon.org>, <mayor@portcolborne.com>, <lauralee@portcolborne.com>, <joanmiller83@hotmail.com>, <susanmabee@portcolborne.com>, <msturman@niagarathisweek.com>

Good afternoon...

This further to mine of October 31 which provided a revised schedule of CBRA related meetings for November....

This memo will confirm two meetings for next week as follows:

Wednesday November 16, 2005
TSC Meeting, 6.00 pm
Committee Room Three, City Hall
TSC review of "final" Crops Report (dated December 2004)

Thursday November 17, 2005
Regular PLC Meeting, 7.00 pm
Council Chambers, City Hall

I will circulate agenda for the PLC meeting early next week.

Have a good weekend,

Chuck

Charles V. Miller
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City of Port Colborne
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"Serving You to Create an Even Better Community"

CROP STUDIES

PORT COLBORNE CBRA

November 16, 2005

Presented by
Jacques Whitford Limited,
Consultants to Inco Ltd.

Conclusions and Recommendations

- Safe soil CoC thresholds have been derived for Port Colborne soils, at and below which, the historically-deposited CoCs in soil present negligible risks (phytotoxicity) to crops.
- At a maximum, EC_{25} values should be used for remediation based on MOE practice in criteria development and application.
- At a minimum, PNEC values for remediation could be used to lend another layer of conservatism.

Jacques Whitford

Presentation Outline

- Background
- Program Objective
- Study Design Requirements and Evaluation of Options
- Summary of Findings
- Sensitivity Analyses and Other Supporting Information
- Conclusions

Jacques Whitford

Background - Port Colborne Soils

- PC soils impacted by 80 years of historical emissions from the nickel refinery
- Deposition of Ni - and other CoC - particulates on the surface of soils (mixed by tilling to 15 cm depth)
- 4 major soils groups affected: Organic Muck, Welland Clay, Shallow Clay, Sand
- Impacted agricultural areas - only clays and organic soils, not sand
- Avg pH of 6.2 in clays and organic soils
- Avg pH of 6.9 in beach sands
- Speciation of Ni-particulates as >90% oxidic nickel and approx 10% as trace Ni in iron oxide

Jacques Whitford

Soil Nickel Speciation

SEM Identification of Oxidic Nickel in PC Soils*

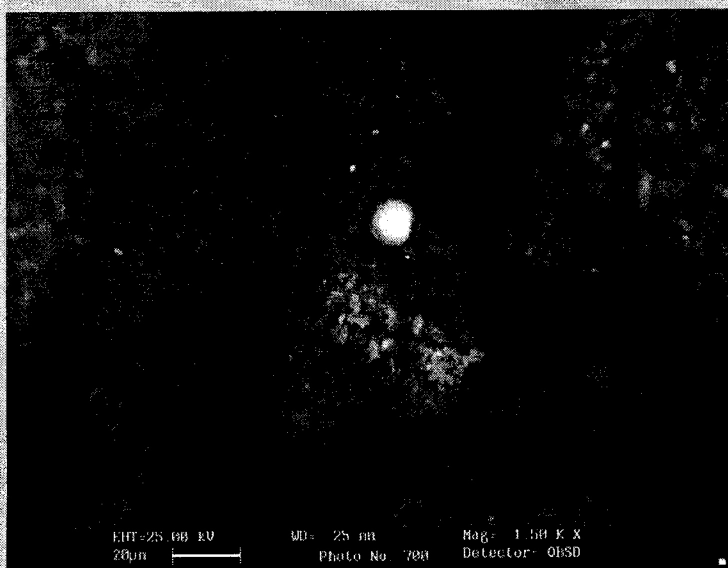


Photo 1: Welland High.

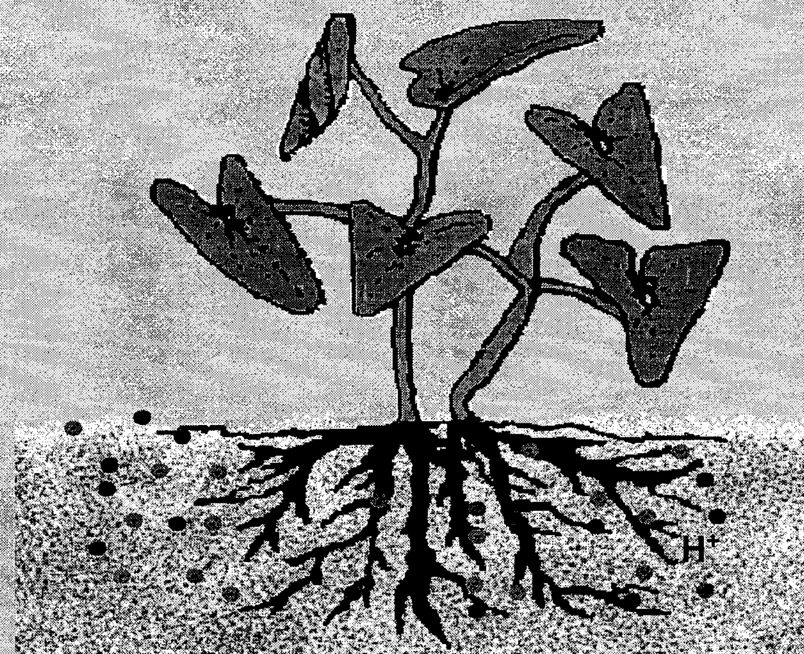
Photo 2: Till High.

*From SGS 2001

Jacques Whitford

Complexity of Ni Interaction

- Weathering, dissolution, mineralization affect insoluble oxidic Ni
- Aqueous Ni speciation
- Mineralization of Ni into Fe, Al, Mn - oxides
- Specific adsorption of Ni to the edges of clay minerals
- Cation exchange of Ni on the surfaces of clay minerals
- Specific adsorption of Ni to soil organic matter



Jacques Whitford

Program Objective

To derive safe soil CoC thresholds, at and below which, the historically-deposited CoCs in Port Colborne soil present negligible risks (phytotoxicity) to crops.

Study Design Requirements

- Identify test crops that are representative to farming in PC area, science and policy
- Use standard measurements of phytotoxicity (plant growth and/or plant metal accumulation)
- Ensure the changes in plant growth are due to the CoC effect, NOT other factors (change in soil pH, over/under watering, over/under fertilization)

Jacques Whitford

Possible Study Design Options

1. GH experiments using artificial soils and soluble Ni- and other CoC- salts (similar to Davis et al 1978)

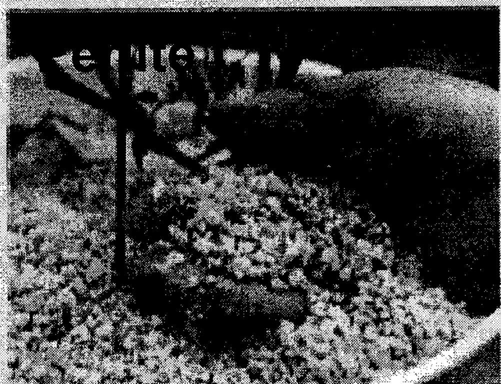
2. Field experiments on sites in Port Colborne with a range of varying Ni concentrations and representative averages in soil parameter values. Soil parameters include pH, CEC, organic carbon, clay content, Fe, Al, Mn, etc

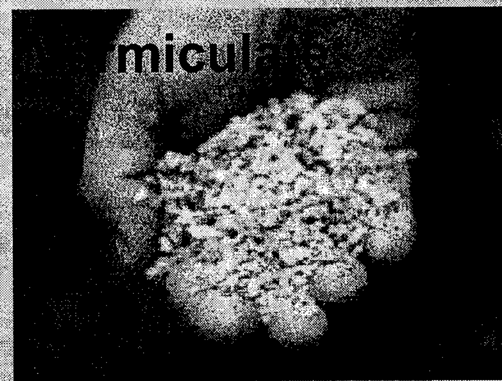
3. GH experiments using soils from Port Colborne, with a range of varying Ni concentrations and representative averages in soil parameter values

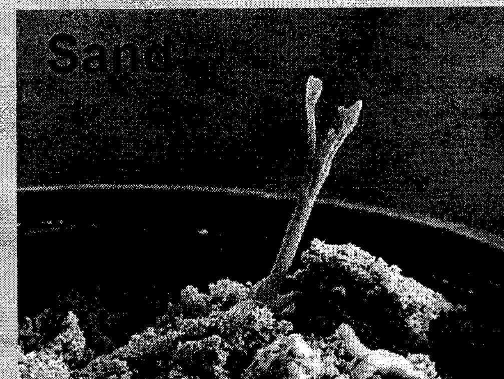
4. GH experiments involving blending of a highly Ni-impacted soil type from Port Colborne with a background soil of the same type from Port Colborne, keeping representative averages in soil parameter values the same in both the impacted and background soil pairs

Jacques Whitford

Evaluation of Design Option 1







Artificial Soils used in studies for phytotoxicity testing

- Use of artificial soils, in conjunction with soluble Ni salts does not represent the PC environment, and would lead to an over estimation of Ni uptake

- Scientific literature overwhelmingly illustrates this when comparison of salt- amended *vs.* "normal" soils are made (Cunningham et al., 1975a, 1975b; Dan, 2001)

Evaluation of Design Option 2

- Option 2 was not considered practical

- Soil Ni concentrations decrease with distance from refinery from which samples could be taken, BUT soil types vary from Organic Muck close to the refinery, to Welland Clay further away, and then to Shallow Clay at a further distance

- Access to some key agricultural fields restricted

- Interaction of CoCs to each of these soil types, with varying clay content and organic carbon content, is different

- Thus goal to evaluate phytotoxicity of plants on each soil type could not be met with Option 2

Jacques Whitford

Evaluation of Design Option 3

- Option 3 was considered and evaluated in Yr 2000
- Obstacles in obtaining reliable dose-response relationship:
  - Lined soil pots (anoxic conditions)
  - Limited volume capacity
  - Insufficient replicates and range in Ni concentrations
- Yr 2000 greenhouse results and findings were confounded by variable range in soil pH
- Findings of our evaluation of Option 3 in 2000 led to the design and implementation of Option 4 in Yr 2001 using the blended soils approach

Jacques Whitford

Year 2000: Greenhouse and Field Findings

	GH 2000 – Welland Clay		Field 2000 Clay 1 – Till Clay	
	Soil Ni, mg/Kg	Tissue Ni, mg/Kg	Soil Ni, mg/Kg	Tissue Ni, mg/Kg
Soybean	500	11	600	3
Oat	500	22	600	11

Observations: 1)Greenhouse phytotoxicity findings are overestimated compared to those measured in the field.
2) Ni accumulated in tissue in field soil with 600 mg Ni/Kg (ie.at 3(x) times greater than MOE generic value of 200 mg Ni/Kg) is well below literature-reported tissue Ni toxicity thresholds

Jacques Whitford

Evaluation of Design Option 4

- PC soils, consisting of a background control and a high Ni (CoC), for Sand, Till Clay, Welland Clay and Organic selected to ensure natural soil and CoC characteristics affecting crop growth and CoC phytoavailability present in GH tests

- Each soil type blended/homogenized in ratios to provide a range of Ni concentrations for each soil type, from control to high

- A key factor affecting Ni phytoavailability, pH was controlled by adjusting parent soil pH to the average of PC soils 6.2 prior to blending

- Blended soils with each CoC concentration were placed in GH pots in quintriplicate, and planted with oat (radish in Welland Clay only) seed, normal agricultural fertilizers were added to each pot

Jacques Whitford

Evaluation of Design Option 4

cont'd

- Seed germination 98% in all soils
- Ni phytotoxicity easily observed in the early stages of growth in plants growing in Sand and Welland Clay soils through banding chlorosis
- With the exception of the oat in Sand all experiments were conducted to maturity (long term exposure 28 days for Radish and 86 days for Oat)
- Mn deficiency symptoms "grey spec" in crop biomass occurred in Organic soils and High Clay in oats and radish
- Use of natural soils lends data to plot in typical dose response relationship subject to accepted statistical/mathematical scientific analysis methodology

Jacques Whitford

Evaluation of Design Option 4

cont'd

- Used Weight of the Evidence Method based on EC_{25} ,a commonly used procedure for deriving environmental soil guidelines for soil contact for agricultural land uses by:
  - MOE (Ontario Ministry of the Environment)
  - CCME (Canadian Council of Ministers of the Environment, 1999a)
  - OECD (Organization for Economic Co-operation and Development)
- EC_{25} - 25 % decrease in Dry Weight (DW) has been chosen as a point where the decrease in DW would be expected to be statistically significant; a level that would allow a scientifically valid conclusion about causality of DW reduction
- PNEC (predicted no-effects concentration) values also derived from upper confidence interval of Weibull curve. PNEC is more directly responsive to variability in data than EC_{25}.

Jacques Whitford

Summary of EC_{25} and PNEC Calculations Based on Soil Total Ni

Soil Type	EC_{25} (mg/kg)	PNEC (mg/kg)
	Ni	Ni
Sand	1350	750
Organic	>2400, 3400*	2350
Welland Clay	1880	1650
Till Clay	1950	1400

*derived from meta-analysis

Jacques Whitford

Sensitivity Analysis
Four Separate Methods:

1. Comparison of GH 2001 oat tissue Ni concentrations to Field Biomonitoring 2001 goldenrod tissue Ni concentrations
2. Comparison of GH 2001 oat tissue Ni concentrations to Literature-Reported oat tissue Ni Toxicity Thresholds
3. Effects of Blending Vs. Unblending on Ni-Bioavailability
4. Field observations of successfully maturing commercial crops

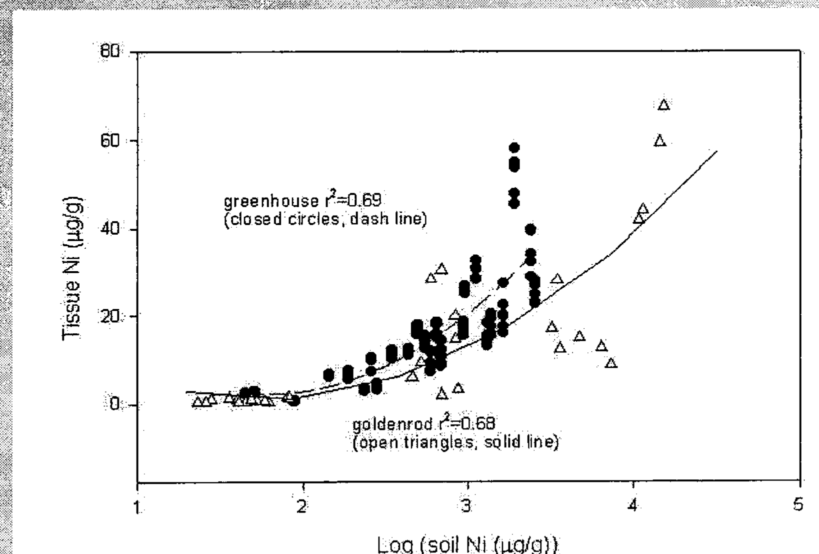
Jacques Whitford

Sensitivity Analysis of Ni EC_{25}

1) Comparison of GH 2001 Oat Tissue Ni concentrations to Field Biomonitoring 2001 Goldenrod Tissue Ni concentrations

- A similar r^2 relationship for Goldenrod Tissue Ni concentration and Oat Tissue Ni concentration as a function of log Soil Ni concentration was found

- Supports legitimacy of EC_{25} calculated from the GH 2001 Trials

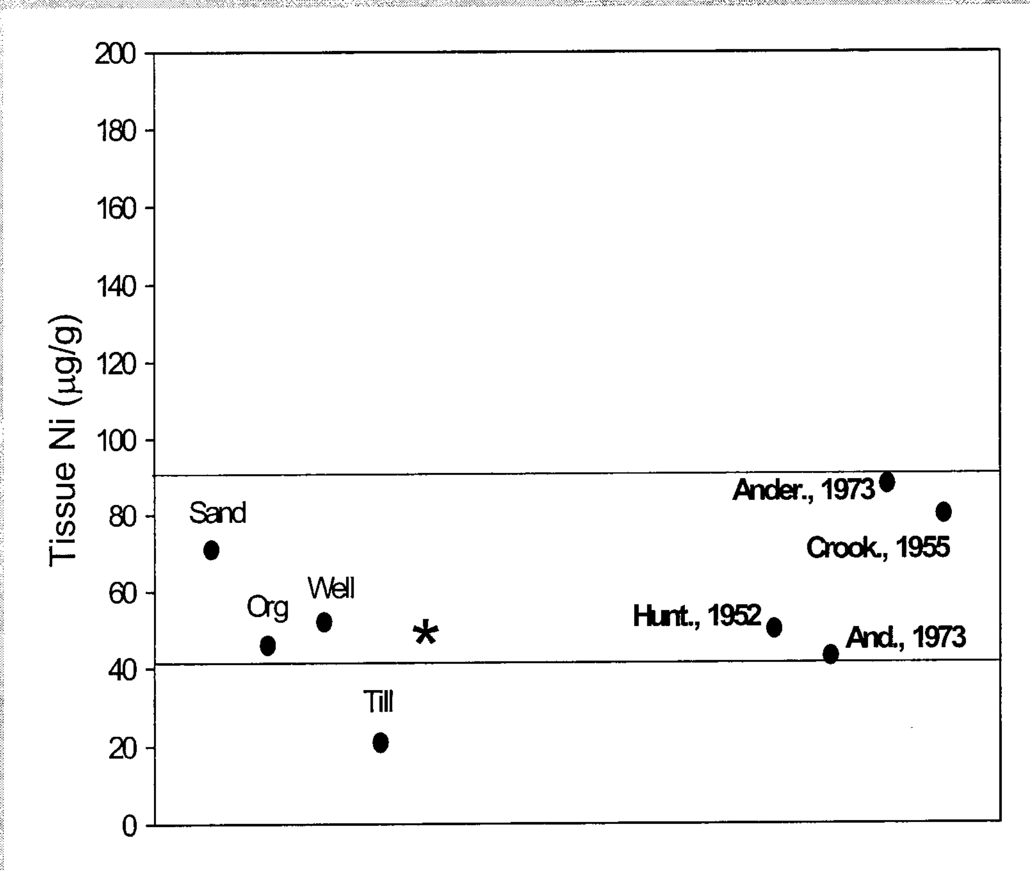


Regression of Oat- and Goldenrod-Tissue Ni Concentration as a Function of Log Soil Ni Concentration

Jacques Whitford

Sensitivity Analysis of Ni EC_{25}

2) Comparison of GH 2001 Oat Tissue Ni concentrations to Literature-Reported Oat Tissue Ni Toxicity Thresholds



* Soil properties (C%, CEC, Fe and Mn oxides) affected Ni bioavailability and induced mineral deficiencies

Upper and lower range of literature tissue Ni values at which oat toxicity begins

Crop Studies show that oat tissue Ni EC_{25} thresholds are within the range of literature-reported oat toxicological thresholds for Ni

Jacques Whitford

Sensitivity Analysis of Ni EC_{25}

3) Effects of Blending *Vs.* Unblending on Ni-Bioavailability

- Ni bioavailability affects outcome of determined Ni EC_{25}
- H_2O- and DPTA-extractable Ni data were used as indicators of bioavailability and compared between blended and unblended clay soils
- No significant differences
- Blending of soils did not result in decreased Ni bioavailability; thus GH 2001-determined Ni EC_{25} values are reliable
- Blending soils did not change the form or species of CoC present in PC soils

Jacques Whitford

4) Other Supporting Evidence

- Projection of Crude Ni EC_{25} values based on unblended Control and unblended High-Ni soils (ref: July 26, 2005 letter). Findings:
  - 907 mg/Kg Ni for Till Clay
  - 848 mg/Kg Ni for Welland Clay
- Field observations:
  - Oat tissue Ni 25 mg/Kg when grown on Welland Clay soil containing about 5000 mg/Kg Ni

Jacques Whitford

NOTICE OF MEETING

TECHNICAL SUBCOMMITTEE
of the
PUBLIC LIAISON COMMITTEE
for the
COMMUNITY BASED RISK ASSESSMENT

At **6.00 p.m.** on **Tuesday January 31, 2006** the Technical Subcommittee of the Public Liaison Committee will meet in the **Third Floor Committee Room, City Hall** to discuss certain matters relating to the Community Based Risk Assessment (CBRA).

The public is welcome to attend the meeting as observers. **The public can make submissions respecting the agenda item by submitting same in advance to Chuck Miller at City Hall (see below).**

The topic scheduled to be considered this Tuesday is the "Final" Ecological Risk Assessment Report related to Crops, as follows:

1) **Crops Studies Report, prepared by Jacques Whitford Environment, dated December 2004.**

The report can be reviewed, and additional information can be obtained, by contacting;

Charles V. Miller
Strategic Projects
City of Port Colborne
66 Charlotte Street
Port Colborne LEK 3C8
(905) 835-2900 (303)
chuckmiller@portcolborne.com

PORT COLBORNE

NOTICE OF MEETING

TECHNICAL SUBCOMMITTEE
of the
PUBLIC LIAISON COMMITTEE
for the
COMMUNITY BASED RISK ASSESSMENT

At **6:00 p.m.** on **Tuesday June 13, 2006** the Technical Subcommittee of the Public Liaison Committee will meet in the **Third Floor Committee Room, City Hall** to discuss certain matters relating to the Community Based Risk Assessment (CBRA) and the Community Health Assessment Project (CHAP).

The public is welcome to attend the meeting as observers. **The public can make submissions respecting the agenda items by submitting same in advance to Chuck Miller at City Hall (see below).**

The topics scheduled to be considered this Tuesday are;

1) **Continuing review of the "Final" "Crops Studies" Report dated December 2004 as prepared by Jacques Whitford, including "Addendum Report" dated January 2006, and, in light of the comments of the External Peer Reviewer (the most recent submission being memo report dated May 4, 2006).**

2) **Continuing review of the "Draft" CBRA "Integration" Report as tabled in January 2006.**

3) **Consideration of the proposed "CHAP" Study "D", being a Cancer incidence and mortality study.**

The relevant materials can be reviewed, and additional information can be obtained, by contacting;

Charles V. Miller
Strategic Projects
City of Port Colborne
66 Charlotte Street
Port Colborne L3K 3C8
(905) 835-2900 (303)
chuckmiller@portcolborne.com

PORT COLBORNE

NOTICE OF MEETING

PUBLIC LIAISON COMMITTEE
for the

COMMUNITY BASED RISK ASSESSMENT

Starting at **7:00 p.m.** on **Thursday June 22, 2006** the Public Liaison Committee will host an **"Open House"** in the **Council Chambers** at **City Hall** (address below). The "Open House" is intended to permit anyone interested to discuss matters related to the CBRA **"Crops Studies"** report with the peer reviewer of that report, **Dr. Murray McBride** from Cornell University.

The "Final" "Crops Studies Report" and "Addendum Report" (dated December 2004, and, January 2006) were prepared by Jacques Whitford and have been the subject of review at the Technical Subcommittee (for the CBRA) for some months. Dr. McBride provided the "peer review" of the report.

At 7:00 pm on Thursday, Dr. McBride will present a summary report of issues related to the "Crops Studies". That presentation will be followed by an open exchange (question and answers) with anyone interested.

The public is encouraged to attend the meeting.

The Crops Studies and related material, and additional information, can be obtained by contacting;

Charles V. Miller
Strategic Projects
City of Port Colborne
66 Charlotte Street
Port Colborne L3K 3C8
(905) 835-2900 (303)
chuckmiller@portcolborne.com

NOTICE

PORT COLBORNE
COMMUNITY BASED RISK ASSESSMENT
ECOLOGICAL RISK ASSESSMENT - CROPS

PORT COLBORNE

FINAL "CALL" FOR PUBLIC COMMENT

In December of 2004 the **"Final" Ecological Risk Assessment Report** related to **"Crops"**, and which forms part of the Community Based Risk Assessment (CBRA), was submitted to the Technical Subcommittee of the CBRA. The Report was prepared by **Jacques Whitford on behalf of Inco.**

The purpose of the study was to determine the concentrations of **Chemicals of Concern** that present an unacceptable risk to **Crops** in Port Colborne.

In 2003/2004 a "draft" of this Report was the subject of considerable review both by the Technical Subcommittee, and through a "public" comment and input process. The "Final" version of the Report (dated December 2004) is intended to address the comment/input received for the "draft" report.

The "Final" Report has been tabled and reviewed by the Technical Subcommittee of the Public Liaison Committee. That review and peer reviewer input has resulted in an **"addendum"** to the **"Final"** report, which addendum is dated January 2006. A copy of the reports and related material is available for review at the Port Colborne Public Library, or, at City Hall as explained below.

Public comment/input related to the "Final" Report and "Addendum" is welcomed. Public comment received will be addressed by Jacques Whitford through a further "addendum" to the report, and will form part of the consideration of the Public Liaison Committee.

The Public Liaison Committee is announcing a **final date for submission of "comments"** from the public related to the Report as follows:

FRIDAY JULY 14, 2006

Anyone wishing to submit comments, or seeking additional information about the Report, may do so through the office of:

Charles V. Miller
Strategic Projects
City of Port Colborne
66 Charlotte Street
Port Colborne, Ontario
L3K 3C8
(905) 835-2900 (303)
chuckmiller@portcolborne.com

PORT COLBORNE

NOTICE OF MEETING

TECHNICAL SUBCOMMITTEE
of the
PUBLIC LIAISON COMMITTEE
for the
COMMUNITY BASED RISK ASSESSMENT

Starting at **6:00 p.m.** on **Thursday September 14, 2006** the Technical Subcommittee of the Public Liaison Committee will meet in the **Third Floor Committee Room, City Hall** (address below) to discuss certain matters relating to the Community Based Risk Assessment (CBRA).

The public is welcome to attend the meeting as observers. **The public can make submission respecting the agenda items by submitting same in advance to Chuck Miller at City Hall (see below).**

The agenda items scheduled for Thursday are:

1) Jacques Whitford Limited will table it's review/response(s) to comments submitted and related to the "Final" "Crops Study" Ecological Risk Assessment Report, which report is dated December 2004.

2) Continued review of the "Human Health Risk Assessment Draft Report" prepared by Jacques Whitford Limited, for Inco, and dated May 2005.

The relevant material can be reviewed, and additional information can be obtained, by contacting;

Charles V. Miller
Strategic Projects
City of Port Colborne
66 Charlotte Street
Port Colborne L3K 3C8
(905) 835-2900 (303)
chuckmiller@portcolborne.com

NOTICE OF MEETING

PUBLIC LIAISON COMMITTEE
for the
COMMUNITY BASED RISK ASSESSMENT
for Soils Contaminated in the Port Colborne Area

Thursday September 21, 2006...7.00 pm
Council Chambers, City Hall

AGENDA

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1 **Approval of Agenda**

2 **Approval of Minutes**

-PLC Meeting of July 20, 2006
-PLC Meeting of August 17, 2006

3 **Delegations**

4 **"Final" Crops Studies and "Addendum"**

-Jacques Whitford will table "final"Crops Report and Addendum

4 **Updates of CBRA Activities**
- Status of CBRA Activities
- General CHAP Activities

5 **General Question and Answer Session**

6 **Next Meeting**

7 **Adjournment**

Persons wishing to be "delegates" to the Committee should register, in advance, with Chuck Miller at (905) 835-2900 ext. 303