REPORT

Commentary On Watters Environmental Group Inc. October 2008 Document - CBRA Crops Studies in Port Colborne, Ontario

VALE INCO LIMITED

PROJECT NO. ONT34657

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REPORT TO	VALE INCO LIMITED 187 Davis Street Port Colborne, Ontario L3K 5W2
FOR	Commentary on Watters Environmental Group Inc. October 2008 Document - CBRA Crop Studies in Port Colborne, Ontario

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EXECUTIVE SUMMARY

Watters Environmental Group Inc. (WEGI) peer reviewed the Crops December 2004 report which had been written by Jacques Whitford Limited (Jacques Whitford). WEGI's comments are incorporated in a letter document entitled: "Independent Consultant Peer Review Report for the Community Based Risk Assessment (CBRA) – Ecological Risk Assessment on Agricultural Crops in Port Colborne, Ontario" dated October 2008. Issues raised by WEGI in their October 2008 document pertained to uncertainties in Jacques Whitford's crops studies, studies of which led to the development of the proposed Port Colborne-specific CoC soil standards.

Jacques Whitford has provided herewithin commentary to each of the uncertainty issues raised by WEGI. All of the issues which were raised by WEGI have been resolved within this report.

In addition, Jacques Whitford have conducted supplemental calculations and logic checks, as well as provided additional insight on the phytotoxicity of CoCs in Port Colborne soils within this text, the results and findings of which have shown compelling evidence that the proposed Port Colborne-specific CoC soil standards are valid.

Jacques Whitford believes that the perceived gap between findings and interpretations as found in the December 2004 Final Crops Report and the issues raised by WEGI in their October 2008 document on these findings and interpretations have been considerably narrowed, if not completely eliminated within this report.

A scientific paper on the derivation of the same proposed Port Colborne-specific CoC soil standards as outlined in the December 2004 Final Crops Report was submitted to the Canadian Journal of Soil Science for publication. After rigorous review and scrutiny by the journal's scientific editors, this paper was accepted and published with the following reference.

Dan, T., Hale, B., Johnson, D., Conard, B., Stiebel, W.H., and Veska, E. *Toxicity Thresholds for Oat grown in Ni-impacted Agricultural Soils near Port Colborne, Ontario, Canada* . Can. J. Soil Science, May 2008.

Table of Contents

List of Tables

List of Figures

List of Appendices

1.0 INTRODUCTION

Jacques Whitford Limited (Jacques Whitford) produced a report entitled: "Port Colborne CBRA – Ecological Risk Assessment, Crops Studies" in December 2004. The focus of this report was on the crop phytotoxicity testing that had been carried out in years 2000 and 2001. This testing included both Greenhouse Trials and parallel Field Trials near a metals refinery (hereafter "Refinery") owned by Vale 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 nickel, arsenic, cobalt and copper. 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.

Watters Environmental Group Inc. (WEGI) peer reviewed the Jacques Whitford December 2004 report and incorporated their comments in a letter entitled: "Independent Consultant Peer Review Report for the Community Based Risk Assessment (CBRA) – Ecological Risk Assessment on Agricultural Crops in Port Colborne, Ontario" dated October 2008. A copy of the WEGI document is found in Appendix A of this text. WEGI claims in their October 2008 document that all previous comments by WEGI were focused mainly on highlighting technical, presentation and grammatical issues and that the comments expressed in their October 2008 document relate to an assessment of the uncertainties associated with Jacques Whitford/Inco-proposed Port Colborne-specific CoC soil standards. However, most if not all of WEGI's comments in their October 2008 document were already addressed in an earlier Jacques Whitford document entitled: "Port Colborne Community Based Risk Assessment Ecological Risk Assessment – Crops – Addendum Report" dated September 2006. Copies of this September 2006 report had been distributed to both to WEGI and other members of the Technical SubCommittee of the Public Liaison Committee, including the Ministry of the Environment (MOE). It is surprising to Jacques Whitford why none of WEGI's comments in their October 2008 review document did not acknowledge the earlier September 2006 document and the contents therein.

In any event, Jacques Whitford has provided herewithin commentary to each of the uncertainties raised by WEGI in their October 2008 document and the sections within this text where Jacques Whitford's commentary can be found, namely:

1. Uncertainties that arise from the difference in results obtained from the 2000 and 2001 greenhouse (GH) studies. Commentary found in Section 2 of this report;
2. Uncertainties relating to the use of blended soil in the 2001 GH studies. Commentary found in Section 3 of this report;

3. Uncertainties relating to a focus on nickel in the 2001 GH studies. Commentary found in Section 4 of this report;
4. Uncertainties relating to use of oats as indicator species in the 2001 GH studies. Commentary found in Section 5 of this report;
5. Uncertainties relating to use of shoot mass rather than crop yield as end-point of concern. Commentary found in Section 6 of this report;
6. Uncertainties relating to statistical treatment and establishment of dose-response curve in the 2001 GH studies. Commentary found in Section 7 of this report;
7. Uncertainties relating to pH adjustment through addition of calcium carbonate in the 2001 GH studies. Commentary found in Section 8 of this report; and
8. Sensitivity analyses. Commentary found in Section 9 of this report.

Note that the above order of the types of uncertainties which will be discussed in this report is not in the same order as that which appears in the WEGI October 2008 document. The reason for the revised order was to provide clarity to the reviewer of this document regarding the scientific process which had been followed in the Crop Studies from 2000 to 2001. The order of uncertainties by type which appear in the WEGI October 2008 document is confusing. For example, at the outset of the WEGI document (page 7 Appendix A), WEGI states that their review comments will only pertain to the 2001 studies, but further examination of subsequent pages 13, 18, 20, 21, 22 in the WEGI document (Appendix A), WEGI introduces unsupported speculations on the earlier fact-finding 2000 studies, followed by fabrication of misleading statements regarding the validity of the proposed Port Colborne-specific CoC soil standards that were based on the 2001 studies.

The order of the types of uncertainties as presented in this report is chronological from 2000 to 2001 and allows the reviewer of this report to better understand the scientific design of the crop studies and the sensitivity analyses that had been carried out by Jacques Whitford to validate the proposed Port Colborne-specific CoC soil standards.

2.0 UNCERTAINTIES IN 2000 AND 2001 GREENHOUSE STUDIES

This section addresses WEGI's comments in their section 3.7 of their October 2008 document in that the discrepancy between the 2000 and 2001 (blended soil) greenhouse experiments be resolved. Further, WEGI asks that Jacques Whitford be equally objective in explaining both the 2000 and 2001 greenhouse findings.

Jacques Whitford's response is provided below under six subsection headings as follows:

1. Designing a greenhouse experiment that will incorporate important factors in attaining defendable and representative Port Colborne-specific CoC soil standards (section 2.1);
2. Dose response evaluation based on greenhouse 2000 findings (section 2.2);
3. Comparison of greenhouse 2000 findings to field 2000 findings (section 2.3);
4. Incorporation of lessons learned from greenhouse 2000 experiments into scientific design of greenhouse 2001 experiments (section 2.4);
5. Dose response evaluation based on greenhouse 2001 findings (section 2.5); and
6. Comparison of greenhouse 2001 findings to field 2001 findings (section 2.6).

2.1 Designing a Greenhouse Experiment

There is no ideal greenhouse experiment that will capture all of the phytotoxic effects of CoCs that may be happening in the real environment (ie. the field). Though many phytotoxicity tests have been carried out by others and reported in the literature, none have successfully simulated the effects of CoCs in the real environment to a 100% level.

The ideal dose response experiment would be in a field setting with crops grown on sites in Port Colborne with impacted CoC soils at varying concentrations of CoCs and at constant values of all soil chemistry and physical parameters. That is, the only parameter that would vary is the CoC concentration keeping all other soil chemistry and physical parameters held constant. However, because of the limitations of actual site conditions in Port Colborne, this could not be considered. For example, while soil Ni concentrations in Port Colborne decrease with distance downwind from the refinery in a northeast direction through a cross section of agricultural lands growing various field crops, the soil types along this cross section also vary from Organic Muck soil with very high soil Ni concentrations close to the refinery, to Welland Clay soil with high to medium soil Ni concentrations still further away from the refinery, and then to Till Clay with relatively lower soil Ni concentrations at a further distance from the refinery. 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 inherent in each of these three soil types introduces too many varying parameters and thus would not yield a proper dose-response relationship representative of the field conditions. Another impracticality with such an undertaking is that it would be impossible to find a representative control site for this hypothetical field dose-response experiment.

To that end, it is important to design a proper greenhouse experiment to best simulate as best as possible the phytotoxicity effects of CoCs in the field. Three possible greenhouse study designs were examined by Jacques Whitford prior to and after the fact-finding 2000 greenhouse studies. Design options for Methods 1, 2, and 3 are provided below in the following subsections 2.1.1, 2.1.2 and 2.1.3, respectively.

2.1.1 *Design 1: Greenhouse experiments with crops grown on non-impacted Port Colborne soils spiked with soluble Ni- and other CoC- salts at increasing concentrations*

This design is somewhat similar to that referenced in earlier scientific literature (eg. Davis and Beckett, 1978) in which the 200 ug/g value of nickel was developed and adopted by the MOE as their soil generic standard.

Use of soluble Ni- and other CoC- salts (high bioavailability) on greenhouse samples taken from areas of non-impacted Port Colborne soils would not have properly simulated the insoluble oxidic forms of nickel (low bioavailability) that exist within the study area as a result of historical particulate deposition from the refinery. Design 1 would have led 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 fact when comparison of salt-amended *vs.* field-contaminated soils are made (Chaney et al., 2003). Design 1 was discarded as a viable option particularly as the Ni speciation of the Port Colborne soils suggest that Ni soil contamination was not soluble.

2.1.2 *Design 2: Greenhouse experiments with crops grown on representative soils collected from Port Colborne area for each of the three major soil types, with a range of varying soil Ni concentrations. Values of soil chemistry and physical parameters in selected soil samples may vary as this would not be controlled.*

Design 2 was used in the Year 2000 fact-finding Greenhouse Trials. Prior to 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 of each of the three major soil types. Evaluation of findings of Design 2 confirmed the initial concerns, that indeed there were noted variations in the values of soil chemistry and physical parameters, thus identifying deficiencies in the practical use of the experimental 2000 data. In particular, the findings of the 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 Design 2 which prevented the results from being used to develop reliable CoC dose-response relationships.

2.1.3 *Design 3: Greenhouse experiments with crops grown on representative soils of control and high Ni concentration sources collected from Port Colborne, pH adjusted to the average soil pH of Port Colborne agricultural soils within the area of impact, and blended to various ratio blends. In the process of blending, a consistency of representative averages in values of soil chemistry and physical parameters would be maintained.*

Design 3 was considered as the most appropriate and hence was implemented during the Year 2001 greenhouse trials. Dose-response curves were for prepared for the three major soil types (Welland Clay, Till Clay, Organic Muck) and Sand. Sand was used in this experiment as it closely resembles the media used in the scientific literature upon which the MOE had adopted the 200 ug/g number as their generic criteria. Several aspects that had been considered in the design of the 2001 greenhouse trials 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.
- The key factor affecting Ni phytoavailability is soil pH according to scientific literature (eg. Weng et. al. 2004). Soil pH was controlled by adjusting the soil pH of both the low nickel and high nickel soils to an average of pH 5.9 for the Organic Muck, pH 6.0 for the Till Clay, pH 6.1 for the Welland Clay and pH 7.2 for the Sand; ie. at soil pH values that best represents the average field soil pH for that soil type within the area(s) of impact. To increase or decrease soil pH, calcium carbonate or aluminum sulphate, respectively were added to the field soils. The exception was Sand, which did not receive any calcium carbonate or aluminum sulphate treatments.
- 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.

2.2 Dose Response Evaluation based on Greenhouse 2000 Findings

2.2.1 Design limiting factors of Greenhouse 2000 tests:

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:

- Multi clay types used in representing Clay soils. For example, samples of Clay Loam (till type of soil) at 34, 194 and 517 mg/Kg of soil nickel content and samples of Welland Clay (glaciolacustrine origin) at 3430 and 8280 mg/Kg of soil nickel content were used to construct the dose-response table for Clay. Soil chemistry and physical parameters for both Clay Loam and Welland Clay, as discovered later on in 2001, are not the same.
- Confounding 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 by 35% from pH 5.0 to 6.7 and in the Clay soil by 30 % from pH 5.4 to 7.3.
  - For example, % organic carbon in the Clay soil varied from 3.8 to 9.0 (140 % variance) and in the Organic soil from 23.4 to 33 (140 % variance).
- 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.

2.2.2 Additional stress factors in Greenhouse 2000 tests:

- 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*. 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).

2.2.3 Phytotoxicity Symptoms in Greenhouse 2000 tests:

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.

2.3 Comparison to Field 2000 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.

2.3.1 Ni accumulation in plant tissue in Field 2000 tests:

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. **Thus in the field, oat is more sensitive to the presence of nickel in the soil when compared to soybean and radishes.**

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 the Port Colborne Clay soil.

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 plants grown in 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. In comparison, soybean grown in Clay with a similar soil Ni concentration (actually 500 mg/kg soil Ni) in the greenhouse had tissue Ni concentration (ie. 11 mg/kg) of much greater than that found in the field; *clearly evidence of overestimation of Ni toxicity in the greenhouse*.

The same observation of overestimation of Ni toxicity in the greenhouse was noted for oat, which in Year 2000, accumulated approximately 22 mg/kg tissue Ni on clay with 500 mg/kg Ni in the greenhouse, whereas the same species grown in 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 which will be discuss later, would be greater than that expected in the field-grown crops, relative to soil Ni concentration.

2.3.2 Phytotoxicity Symptoms in Field 2000 tests:

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).

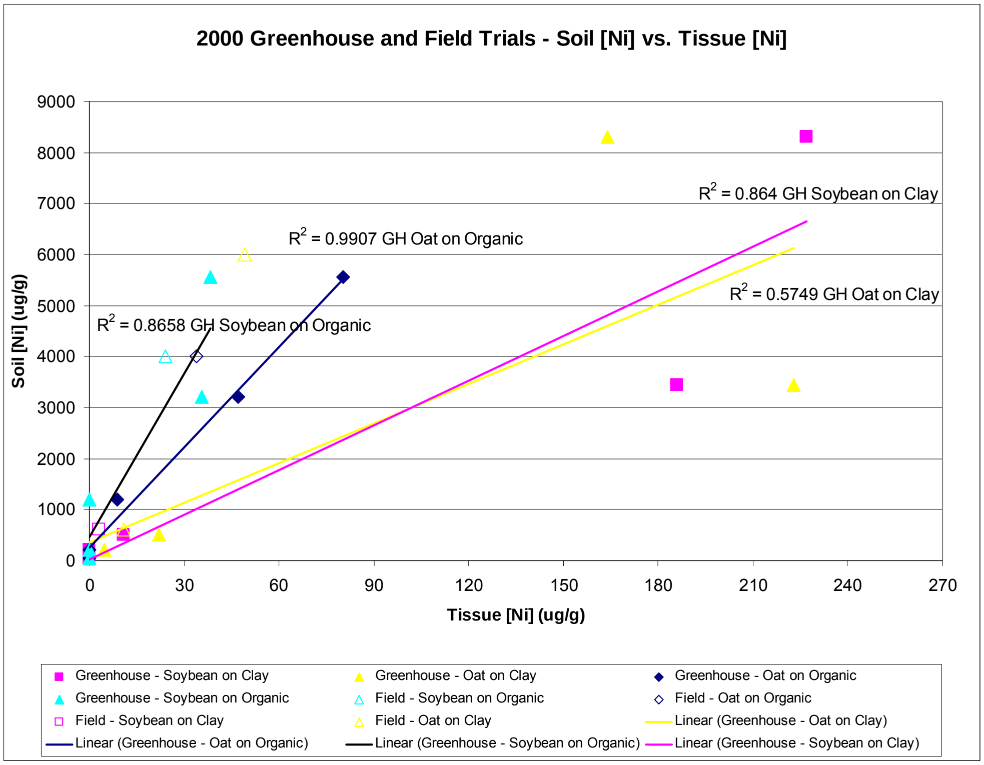
2.3.3 Comparison of Field 2000 tests to Previous Field Studies.

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, who carried out parallel Ni phytotoxicity test plots in Port Colborne. 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.

2.3.4 Integration of Greenhouse 2000 and Field 2000 Findings

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 that observed in the field-grown crops, relative to soil Ni concentration.** In summary, the greenhouse 2000 findings ***overstate*** the actual soil Ni phytotoxicity observed in the field.

2.3.5 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).

2.4 Incorporation of Lessons learned from Greenhouse 2000 Experiments into Scientific Design of Greenhouse 2001 Experiments

The results from the 2000 greenhouse and field studies 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);
- Organic Muck, Welland Clay and Till Clay are the predominant agricultural soil types within the area of impact in Port Colborne, and as such, these three soil types were used in the greenhouse trials. A fourth soil type, Sand, was also used in the trials for reasons of providing a comparative soil type to a similar medium used in the Davis and Beckett, 1978 study that led to the derivation of the 200 ugNi/g value.
- 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 a lower 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).

2.5 Dose Response Evaluation based on Greenhouse 2001 Findings

2.5.1 EC_{25} and PNEC based on Plant Growth – Greenhouse 2001

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 1.

Table 1 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 greenhouse-derived EC_{25}s and PNECs in Table 1 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 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 1 are overly conservative and thus considered safe for crops growing in Port Colborne soils**.

2.5.2 Phytotoxicity Symptoms – Greenhouse 2001

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 and 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).

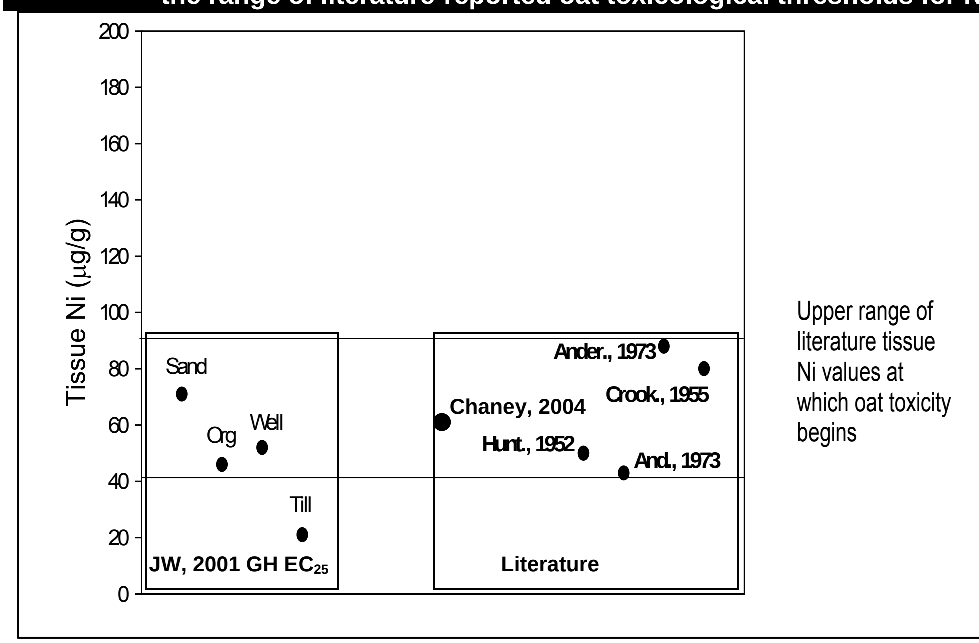
In Till Clay soils: In the Till Clay soils, chlorosis was recorded seven days after germination on the whole leaf surface, first in plants grown in soil contaminated with the highest levels of nickel in the unamended treatment. These symptoms were due to deficient Mn concentration as indicated by the Mn levels measured in the foliage. Similar symptoms were also observed in the plants grown on the amended soils, however the chlorosis was localized mainly towards the leaf tips.

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).

2.5.3 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 in Table 1 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

2.5.4 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.

2.6 Comparison to Field 2001 Findings

2.6.1 Phytotoxicity Symptoms of Field 2001

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 field 2001 results of the Clay 2 site 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. *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.*

2.6.2 Tissue nickel – Field 2001

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. *Soil pH, and not any other soil parameter, plays a significant role in the accumulation or the lack of accumulation of metals in plants.*

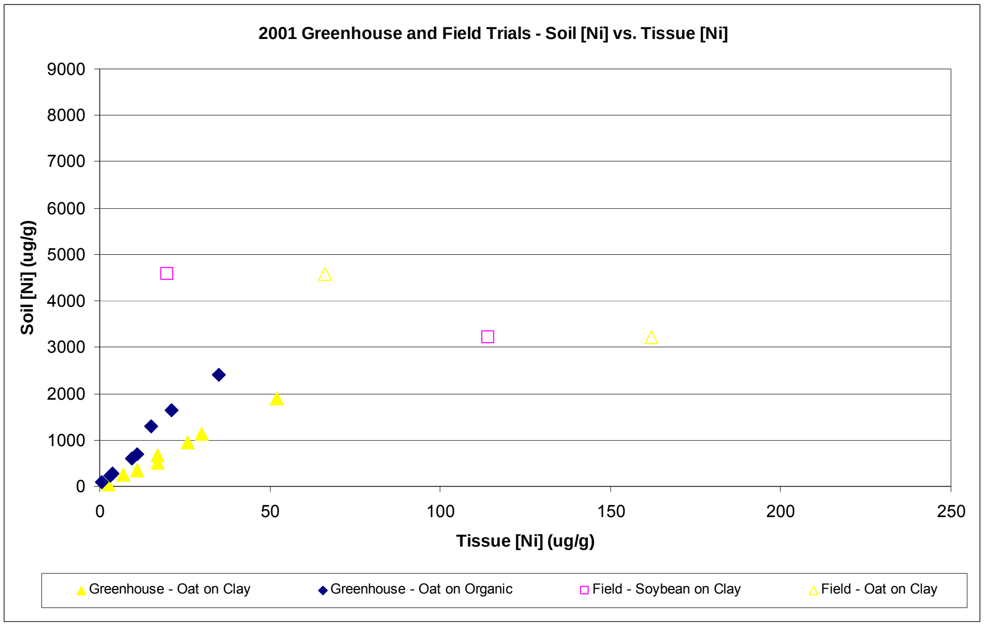
Weng et al. (2003) linked the separate effects of pH on sorption of Ni to soil and plant (i.e. increasing pH enhances shoot Ni accumulation from solution due to reduced competition for root uptake sites between H^+ and Ni^{2+}, balanced against the increased binding of Ni^{2+} to soil particles). Their 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 between predictions based on Weng et al. 2003 model using soluble Ni salts and field observed Ni toxicity of crops grown on insoluble Ni-containing soils of Port Colborne . 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. Second, it seems clear from this comparison that speciation of the Ni in the soil, in addition to the master variable (pH) 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.

2.6.3 Integration of Greenhouse 2001 and Field 2001 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, 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.



3.0 UNCERTAINTIES IN USE OF BLENDED SOIL IN THE 2001 GREENHOUSE STUDIES

WEGI takes issues on the use of blended soils in the 2001 greenhouse studies. It should be noted that the use of blended soils has been adopted by regulatory agencies in Canada and US and used as an approach that is recommended by Environment Canada (2005) for risk assessment (Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soils. EPS 1/RM/45).

3.1 Uncertainty of Blending Due to Variability in Confounding Factors

This section addresses WEGI's comments in their section 3.1.0 of their October 2008 document.

WEGI is of the opinion on pages 9 to 11 of their October 2008 document that soil parameters other than pH create confounding factors in the interpretation of dose response. There is overwhelming evidence in the scientific literature (eg. Weng et. al. 2004) that soil pH is the most important soil characteristic that modulates bioavailability of metals by affecting both the chemical speciation of metals in soil and the metal binding to the active sites on biota. Other than soil pH, the importance of confounding factors such as CEC are minimal. Soil pH is the most significant of all the possible confounding factors.

Weng et. al. 2004 found that the EC_{25} of total Ni is reduced with a decrease in soil pH independent of soil type and composition, ie. the lower the soil pH the lower EC_{25} of total Ni. Weng et. al showed that soil pH in the range of pH 4.7 to pH 7.0 explains up to a factor of 14 difference of nickel bioavailability in soils.

The varying pH dose response experiment conducted by Jacques Whitford (2004) where the soil Ni concentrations were held constant at 1900 mg/Kg in Welland Clay while soil pH varied from pH 5 to pH 7, resulted in a 300% decrease in biomass from plants grown at pH 7 compared to those at pH 5.

Hence soil pH is the most important soil parameter and for that very reason, was held constant in the greenhouse 2001 dose response trials, at average soil pH levels consistent with real soils within the study area.

WEGI's opinion on their interpretation on the strength of relationships between regressions for goldenrod and oat grown in a greenhouse with soil nickel is not justified. Jacques Whitford is of the view that the supporting evidence provided from the greenhouse studies of 2000 and 2001 provides solid legitimacy of the EC_{25} thresholds generated from plants grown in soil blends. Based on this premise, Jacques Whitford maintains that the variation in soil parameters in the goldenrod study that may have been confounded with soil Ni, did not have a large influence on the determination of EC_{25}.

WEGI also commented that greenhouse soils should not have been blended with too highly contaminated real soils (between 8,655 mg/Kg and 10,045 mg/Kg soil nickel) but instead with a more representative range of soils from the study area. Jacques Whitford did exactly take this approach in the greenhouse 2001 trials based on one of our lessons learned in the 2000 greenhouse trials. For example, the maximum soil concentrations used in the greenhouse 2001 trials were 1900 mg/Kg for Welland Clay, 2540 mg/Kg for Till Clay, 2400 mg/Kg for Organic Muck and 2310 mg/Kg for Sand.

3.2 Uncertainty of Blending Due to Variability in Available Nutrient

This section addresses WEGI's comments in their section 3.1.1 of their October 2008 document.

Within Section 3.1.1 of WEGI's October 2008 document, WEGI provides the same above title regarding nutrients but their comments within their section relate not to nutrients but instead to Total Organic Carbon (TOC) and Cation Exchange Capacity (CEC). To our knowledge, nutrients include macronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium and sulphur, as well as micronutrients such as boron, copper, iron, manganese, molybdenum, zinc and chlorine. TOC and CEC are not nutrients.

WEGI's comments centre around the variability in TOC and CEC levels in the soil blends for the Welland Clay and Till Clay. For both soil types, the greenhouse 2001 soils used in dose response were adjusted to a constant pH reflective of the average soil pH common within the affected agricultural area.

For Welland Clay, WEGI remarked on a 31% variability in TOC values (5.49 to 7.2%) amongst the Ni blends used in the greenhouse trials of 2001. But is this noted variability in TOC of greenhouse soils significant? Actual TOC values from 'real soils' from within the field plots of Welland Clay (referred to as Clay 2 and Clay 3 sites in the 2004 Crops Report) were found to range in TOC between 4.68% to 8.3%, which in our opinion is within the noted 5.49 to 7.2% TOC values amongst the greenhouse soil blends used in 2001. Thus the WEGI-noted variability in TOC values in the 2001 greenhouse soil blends reflect 'real soil conditions' and hence the generated EC_{25} values from the greenhouse dose response experiments on Welland Clay remain valid.

For Till Clay, WEGI remarked on a 300% variability in TOC (3.36 to 13.4%) amongst the soil blends used in the greenhouse trials of 2001. (Note that WEGI in their document had made a mistake in stating 14.5% TOC maximum instead of 13.4% in Table GH-35 of the 2004 Crops Report, and hence incorrectly stated a variability estimation of 400%). But again, is this noted variability in TOC significant? Examination of actual TOC values from 'real soils' from within the field plots of Till Clay (referred to as Clay 1 site in the 2004 Crops Report) were found to range in TOC between 5.0% to 10.2%, which in our opinion is within the noted 3.36 to 13.4% TOC values amongst the soil blends used in 2001. Thus the noted variability in TOC values reflect 'real soil conditions' and hence the generated EC_{25} values from the greenhouse dose response experiments on Till Clay remain valid.

WEGI also commented on variabilities in CEC of 30% variability for Welland Clay (4.5 to 6.2 meq/100 g) and 50% variability for Till Clay (5.0 to 9.5 meq/100 g). These stated variabilities in CEC appear of no consequence in view of the above discussion on the variabilities in TOC. Further, clays normally behave as weak acids (Stumm and Morgan, 1970), and because soil pH was held constant in the 2001 greenhouse trials for both Welland Clay and Till Clay, the significance of CEC as a soil parameter of concern is expected to be minimal.

Phosphorus is a nutrient but we note that WEGI did not comment on its variability in the soil blends. For the benefit of the reader, the variability in phosphorus levels was only 20% in the Welland Clay blends (682 to 878 mg/Kg) and even lower at less than 7% in the Till Clay blends (1311 to 1415 mg/Kg). Hence, the variability in nutrient levels had no impact on the generated EC_{25} values from the greenhouse dose response experiments on both the Welland Clay and Till Clay.

To Jacques Whitford, it appears odd that WEGI did not conduct a similar treatise as they did on the two clay types to examine the variability in soil parameters in the other two soil types, Organic Muck and Sand soils, that were used in blends of the 2001 greenhouse trials. Jacques Whitford's examination of variabilities in soil parameters in these other two soil types indicate low variabilities and thus the generated EC_{25} values of 3400 mg Ni/Kg and 1350 mg Ni/Kg for Organic Muck and Sand, remain valid.

WEGI has missed the point in their review of how EC_{25} was derived based on the phytotoxic level of Ni concentration in the above ground biomass. The EC_{25} is derived based on the physiological response of the plants accumulating nickel in the above ground biomass. Comparison of the oat greenhouse tissue Ni EC_{25} thresholds to the oat toxicity thresholds referenced in the scientific literature, clearly demonstrated in the 2004 Crops Report that the observed phytotoxicity in the 2001 greenhouse studies occurred for the most part within the concentration range observed in other studies (Hunter and Vergnano, 1952; Anderson *et al*., 1973). There were instances where comparison showed lower observed phytotoxicity than the literature data indicating the contribution of additional CoCs besides Ni to plant toxicity. That is to say that the literature threshold values for nickel concentration in tissue of plants can vary with total soil nickel concentration and other factors affecting its bioavailability.

The particular response observed in the Welland Clay soil and the fact that the EC_{25} are not lower in the amended versus the unamended soils is due to a particular response of the plants growing in the 673 mg/kg soil Ni concentration level which had shown a rather large accumulation of nickel (Appendix GH 1B, Table GH-28a) in tissue at 170 mg/Kg, a nickel tissue concentration not reflective of the dose response for this greenhouse experiment at lower and higher soil Ni concentrations as summarized from Table GH-28a for the reader below:

Soil Ni, mg/Kg	Tissue Ni, mg/Kg
45.3	2.4
188	6.8
347	11
498	17
673	***170***
956	26
1130	30
1900	52

Consequently when the Weibull regression was applied to the data, the fit was not as tight as in the case of the Till Clay, but was still significant.

Manganese induced deficiency in crops after applying limestone is a relatively well known fact to both farmers and the scientific community. This aspect could be related to tissue Mn deficiency in amended plants, as the tissue Mn sufficiency threshold for oat grown in the amended Welland Clay soil shows was that it was breached at lower soil total nickel and tissue nickel concentrations than in the unamended soil.

WEGI refers to the data obtained during the 2001 Greenhouse studies as showing "considerable spread" which in WEGI's opinion is reducing the certainty that can be placed on the results. WEGI'S comment is based on the false assumptions that the data shows large variation.

WEGI is reminded that variation in a dose response testing experiment is a desirable and expected effect (captures the response of a population and not an individual). Also more important is the fact that all data has undergone test for normality to ensure that all data distributions are normal and fulfilling the assumptions of normality in various statistical tests (Section 11, Binder 3 out of 3). Where necessary, data was transformed and outliers were removed prior to analyses. To conclude the "considerable spread" as referred to by WEGI is representative of the results obtained in the GH experiments that fall within a normally distributed population which one would expect when conducting a multitude of dose response tests (such as the ones conducted in the 2001 Greenhouse Studies).

3.3 Uncertainty of Blending Due to Variability in Soil Texture Characteristics within each Set of Soils

This section addresses WEGI's comments in their section 3.1.2 of their October 2008 document.

WEGI's comment on soil texture pertains primarily only to Till Clay used in the greenhouse 2001 blends, where they note that the clay content was 21.5% in the control Till Clay (Shallow Clay) source of location G2 on Drawing 3-2 of the 2004 Crops Report and 46.6% in the high concentration source of Till Clay (Shallow Clay) of location G2A on Drawing 3-1 of the 2004 Crops Report. The question is whether this observed discrepancy in clay content between the control source and the high concentration source is significant to the determination of EC25?

Definition of a *Till Clay* as provided in the 2004 report is a till soil with a clay to silty clay loam composition and a variable texture ranging from less than 30% clay on average if the soil grouping is a *Shallow Clay*, to somewhere between 20 to 40% clay on average if the soil grouping is a *Clay Loam*.

The actual measured (not average as reported in the above till clay definition) percent clay content in soils of test pits excavated by Jacques Whitford in areas of Till Clay (Shallow Clay) north and northeast of the refinery were found variable between 4% and 50% as documented in the Soil Characterization Report (Volume IV of the 2004 Crops Report), eg. 12% at TP-C, 6% at TP-F, TP-J at 9%, 50% at TP-K, 4% at TP-L and 26% at TP-M.

Thus the loamy nature of Till Clay in Port Colborne characteristic of a large variability in percent clay content made it impossible to select both a control source and a high Ni concentration source with minimum to no variability in clay content. The effect of this variability in percent clay content on EC_{25} had been discussed within the 2004 Crops Report.

Welland Clay is a true clay with a lacustrine deposition origin. In comparison, Till Clay is a loam material consisting of a combination of sand, silt and clay as a result of mixing from glacial action. The Welland Clay soil blends were developed from a control Welland Clay source [location G1 on Drawing 3-2 of the 2004 Crops Report] with a clay content of 40.1% and a high Ni concentration source of Welland Clay [location G1B on Drawing 3-1 of the 2004 Crops Report] with a clay content of 40.5%; ie. a variability between the control and high concentration sources of less than 1%.

WEGI's statement that Till Clay is the most important agricultural soil in the Port Colborne area is incorrect and misleading. Within the context of the major soil types in CoC-impacted agricultural areas surrounding the former nickel refinery where soil nickel concentrations exist above 500 mg/Kg (refer to Drawing 2-4 of the 2004 Crops Report), there are **three** major soil types, namely Organic Muck, Welland Clay and Till Clay, ie. not just one major soil type as claimed by WEGI.

Based on the above explanations, WEGI must accept the fact that the EC_{25} values of 1880 mg/Kg for agricultural Welland Clay soils and 1950 mg/Kg for agricultural Welland Clay soils are both valid.

To Jacques Whitford, it appears odd that WEGI did not conduct a similar treatise on soil texture variability on the other significant agricultural soil type, ie. Organic Muck. Jacques Whitford's examination of variabilities in soil texture in the Organic Muck soil blends indicate low variabilities and thus the generated EC_{25} value of 3490 mg Ni/Kg for Organic Muck remains valid.

As noted earlier, Sand was used in the greenhouse 2001 trials to determine a EC_{25} based on a methodology that would be similar to that which led to the MOE derivation of its 200 mg Ni/Kg generic value. The EC_{25} for Sand based on the 2001 greenhouse trials was found at 1350 mg Ni/kg, which should be considered acceptable to WEGI as 1) there was no pH adjustment to the Sand trials and 2) there was little to no variability in the soil parameters between the control and high concentration source samples and both kinds of samples were derived from dunes of beach sand (ie. well blended and homogenized by natural forces) along the north shores of Lake Erie, east and west of the Welland Canal.

There are no agricultural areas within Port Colborne that contain sand soils and WEGI is in error when they make the statement that “local sand soils are not routinely cultivated” (see page 5 of WEGI’s October 2008 document). The fact is that there are no agricultural areas with sand soils.

4.0 UNCERTAINTIES ON FOCUS OF NICKEL IN THE 2001 GREENHOUSE STUDIES

This section addresses WEGI's comments in their section 3.3 of their October 2008 document.

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 soil Ni concentrations, and at comparatively lower concentrations of As, Co and Cu in soil. Nickel concentrations in soil above MOE generic soil quality guideline (SQGs) level of 200 mg/Kg and originating from the refinery were found to impact an area of approximately 30 km^2. The greatest deposition of CoC particulates in surface soil was within one km northeast of the refinery as found evident by the 1000 ug/g soil Ni concentration contour line of Drawing 2-4 of the 2004 Crops report.

Scanning electron microscopy (SEM) analyses by SGS Lakefield (SGS Lakefield, 2002) identified nickel-bearing particulates in each of the submitted soil samples from this highly impacted area in Port Colborne. 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. *The presence of any other CoC such as Cu if present at all in the SEM samples were found scavenged with Ni-iron oxide/hydroxides in soil at concentrations less than 10%. Hence, the rationale as why the focus of these studies were on primarily nickel.*

Based on the elevated soil Ni concentrations relative to the other CoCs and the potential phytotoxicity of Ni, Ni was considered *a priori* the primary pollutant in the Port Colborne study area. This initial assessment was drawn from the results of an earlier phytotoxicity study by Frank *et al*., (1982) and verified by the results of the 2001 Greenhouse Trials that showed at the EC_{25} level, plant tissue phytotoxicity thresholds established in the literature were often approached for Ni but rarely by the remaining contaminants of concern (Cu, Co and As). However, this does not preclude the potential contributing phytotoxicity of the other CoCs either singly, interactively with Ni and/or with each other. Although EC_{25} calculations were based on Ni concentrations in soil and plant tissue, the approach of Jacques Whitford has been to consider these values site specific to Port Colborne contaminated soils and inclusive of the potential phytotoxicity of all CoCs. This interpretation is justified by the strong correlations (r>0.9) of soil total Cu, Co and As concentrations with soil total Ni concentration, which were consistent across soil types and range of Ni concentrations.

5.0 UNCERTAINTIES OF OATS AS INDICATOR SPECIES IN THE 2001 GREENHOUSE STUDIES

This section addresses WEGI's comments in their section 3.5 of their October 2008 document.

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 oat, radishes, soybean, corn and golden rod were evaluated by Jacques Whitford in the 2000 studies. Integration of phytotoxicity data from all plant species was done. *Oat was shown to be more sensitive than radishes, soybean, corn and golden rod to the site-specific conditions under both field and greenhouse conditions.* This was corroborated by findings by others such as Chaney et al 2004 and Chaney et al., 2003 on their site-specific studies in Port Colborne that had been carried out in parallel to those of Jacques Whitford's.

WEGI refers to the 2000 greenhouse studies where in their view, soy demonstrated a visible reduction in plant growth at 500 mg/Kg. WEGI is in error in making any meaningful interpretation of these data, for soy or for that matter, corn or oat. As discussed earlier, the clay soils used in the 2000 greenhouse trials do not represent a true dose response experiment as the 2000 experiment consisted of a mixture of the two major clay soil types in Port Colborne, ie. Welland Clay and Clay Loam (Till) and there existed large variabilites in soil parameters, eg. 30% in soil pH, 140% in TOC, 500% in the nutrient phosphorus, 220% in the nutrient potassium, etc. Table 2 below outlines the actual values and variabilities in the greenhouse 2000 trials on clay soils.

Table 2 Characteristics of Year 2000 Clay Soils used for Greenhouse Trials

	Concentration Level of Nickel in Clay Soils – Year 2000					Comment
	Control	Low	Medium	High	Very High	
Sample ID Locations, Dwgs 2-3, 2-4	GO1	GO1D	GO1C	GO1B	GO1A	
Clay Type, see Dwgs 2-3, 2-4	Clay Loam	Clay Loam	Clay Loam	Heavy Clay (Welland)	Heavy Clay (Welland)	Combination of Clay Loam and Heavy Clay is a design flaw. Any interpretation of this dose response is meaningless.
Clay Content, %	45.2	na	28.6	41.1	30.8	60% variance between max and min
Soil Ni, mg/Kg	34	194	517	3430	8280	Variable, but as expected
pH, unitless	7.3	6.7	5.5	5.4	5.8	30% variance of pH, the most significant soil parameter. This makes any interpretation of the 2000 dose response meaningless.
Organic Content, %	9.0	3.8	8.8	6.8	7.4	140% variance between max and min
Cation exchange capacity (CEC), meq100	Na	na	Na	Na	Na	-
Phosphorous, mg/Kg	73	66	12	14	29	500% variance between max and min
Potassium, mg/Kg	262	264	82	105	173	220% variance between max and min

Notes:
na -not analyzed

Of the above noted variabilities, soil type and soil pH were the most critical and in theory, should have been held at constant in the 2000 greenhouse experiments. Failure to have standardized the soil type and failure to have soil pH as a constant in each of the 2000 greenhouse soil pots invalidates any meaningful comparison of dose response of the 2000 Clay greenhouse trials.

WEGI's interpretation of soy, or for that matter any other crop at 500 mg/Kg soil Ni is meaningless. The soil pH at 500 mg/Kg soil Ni in Clay was 5.5 which is not consistent with the higher pH values of pH 7.3 and pH 6.7 in the lower soil Ni concentrations of 34 and 194 mg/Kg of the greenhouse 2000 soils as shown in

Table 2. As stated earlier in section 3.1 of this report, the varying pH dose response experiment conducted by Jacques Whitford (2004) where the soil Ni concentrations were held constant at 1900 mg/Kg in Welland Clay and the pH varied from pH 5 to pH 7 resulted in a 300% decrease in biomass from plants grown at pH 7 compared to those at pH 5. In like manner, the observations by WEGI of crops grown on 500 mg/Kg soil Ni at pH 5.5 would be at least 300% lower in biomass compared to the lower soil Ni clay concentrations at pH 7.3 and thus no meaningful interpretation on Ni phytotoxicity with these data can be made.

6.0 UNCERTAINTIES ON SHOOT MASS RATHER THAN CROP YIELD IN THE 2001 STUDIES

This section addresses WEGI's comments in their section 3.6 of their October 2008 document.

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.

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 equivalent to 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. There is virtually 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 weights differences 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 newer cultivars had higher mean harvest index than the older 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 lead protect against a smaller loss in marketable yield.

In addition, 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 (personal communication with 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.

7.0 UNCERTAINTIES ON STATISTICAL TREATMENT IN THE 2001 GREENHOUSE STUDIES

This section addresses WEGI's comments in their section 3.4 of their October 2008 document.

The Weibull is a continuous function that is particularly useful for biological functions, as it allows for growth stimulation at low doses, and is therefore appropriate for the characterization of plant response to essential elements, such as Ni (Taylor et al., 1991). A Weibull function ($y_i = \alpha e^{-(x_i/\omega)\lambda}$ where α is the theoretical yield in soil with background Ni concentration; ω is the soil Ni concentration at which Y is 37 % of its value at α; and λ is a fit parameter) was fit to the relative shoot biomass data for each soil, separately for its relationships to tissue and soil Ni concentrations. For the organic muck soil, the Weibull function did not fit the data well, so a linear function was used instead. The approach of using applying the best fit curve to the results of a dose response testing is a relatively common practice in the field of environmental toxicology and the use of it for the GH 2001 study has been validated by other peer reviewers and published in the Canadian Journal of Soil Science May, 2007).

The uncertainty related to the errors in precision in best curve fit and by extension, to the derivation of the EC25 values was determined based on the calculation of confidence intervals (CI). There are two calculations for 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.

8.0 UNCERTAINTIES ON PH ADJUSTMENT IN THE 2001 GREENHOUSE STUDIES

This section addresses WEGI's comments in their section 3.2 of their October 2008 document.

Many previously-conducted experiments and field tests with Ni rich soils had shown that adding limestone to raise soil pH could greatly reduce the potential phytotoxicity of natural or added Ni (e,g, Hunter and Vergnano, 1952; Crooke, 1956; Halstead, 1968). This simple remedy was tested for Port Colborne but the result did not agree with others studies. In the study of Kukier and Chaney (2000), it is reported that liming to reduce Ni^{2+} phytoavailability caused Mn deficiency of the Organic Muck soil from the Grotelaar farm area. The Ontario Ministry of Agriculture had advised farmers that liming these Lake Plain soils may induce Mn deficiency in susceptible crops (Baldwin and Johnston, 1986), but at the time of the studies of Frank et al. (1982) and Temple and Bisissar (1981), the interactions of pH with Ni and Mn availability to plants was not appreciated. Lake Plain soils are depleted of Mn during soil genesis (flooding causes reduction of Mn to the Mn^{2+} form, and leaching of the water can remove the dissolved Mn^{2+}). In subsequent testing, Kukier and Chaney (2001) added sufficient Mn to prevent Mn deficiency in the plants studied, and it was shown that both the Organic Muck soil and the Till Clay soil which caused Ni phytotoxicity at very low pH yielded normal plants if soil pH was raised (which caused lower concentration of Ni in the plants leaves). Because of the higher level of organic matter in the Organic Muck soil, Ni was less adverse at low pH than found for the Till Clay soil. But simple normal application of limestone plus Mn fertilizer allowed normal plant growth on all soils tested (Kukier and Chaney, 2000; 2001; 2004).

The study by Kukier and Chaney (2004) determined EC_{25}'s for a range of crop species, including corn and oat, using Heavy Clay soil from Port Colborne containing 2900 mg/kg of Ni, and amended with lime at two application rates. They determined that tissue Ni for oat at 25% reduction of shoot growth was 63 µg/g, which is similar to those observed in the Jacques Whitford's 2001 Greenhouse Study, for Sand and Heavy Clay, but three fold greater than the tissue Ni EC_{25} observed for Till Clay.

The observation of the different tissue Ni concentrations, at the EC_{25} within the 2001 Greenhouse experiments, is evidence suggesting that tissue Mn may have partially defined this threshold. Plants grown in organic soils, or mineral soils high in organic matter are more likely to be Mn deficient (OMAF, 2000), which explains the tissue Mn data for Sand vs Organic Muck. However, for the Till Clay, total organic carbon content increased with soil Ni concentration (from 6% to 16%), thus increasing the potential for Mn deficiency; the total organic carbon content of the Heavy Clay used for blending were similar, so the reason for the decline in tissue Mn concentration as soil Mn stayed constant in this soil type is not clear. Mn deficiency and Ni phytoxicity are likely not mechanistically related; these soils are chronically Mn deficient, and this limitation to growth adds to that which results from elevated Ni accumulation in the shoots (Kukier and Chaney, 2000).

One important observation is that of the tissue Fe concentration of the plants grown in Organic Muck (30 µg/g), Till Clay (29 µg/g) and Heavy Clay (36 µg/g) soils in the 2001 greenhouse study were below the sufficiency threshold for plant growth (60 µg/g) (Marschner, 1995), and were lower than the tissue Fe concentration observed for plants grown in Sand (53 µg/g). In none of the 2001 greenhouse soils was tissue Fe linked to Ni concentration in soils.

One of WEGI comments under their section 3.2 is that PNECs would be different in soils of different soil pH. Jacques Whitford concurs that the PNEC values are pH dependent. PNECs developed in the Crops 2004 study are applicable to soils with near-neutral pH which by design of the greenhouse experiments, reflect the representative soil pH within the agricultural community of Port Colborne. Table 3 below shows the specific soil pH range per soil type for the 2001 crop dose-response greenhouse experiments and corresponding applicable soil Ni EC_{25} and PNEC values.

Table 3 PNEC and EC25 values of Soil Ni Derived at Specific Soil pH Ranges for Soils within Port Colborne

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 agricultural areas of Port Colborne with soils above the upper limit of the experimental soil near neutral pH range ie. above pH 6.5, **higher PNECs** than those presented above in Table 3 would result.

Areas of agricultural areas of Port Colborne with soil pH values below the lower limit of the above-noted experimental soil near neutral pH range, or below pH 5.5, were not found in the soil characterization study that had been completed by Jacques Whitford (refer to soil pH map on Drawing 2-2 of the Crops Report (Jacques Whitford, 2004).

In practice, farming at soil pH values at or below pH 5.5 would not be desirable to a farmer. The Agronomy Guide for Field Crops (Ontario Ministry of Food and Rural Affairs, 2002, publication 811) recommends 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.

9.0 SENSITIVITY ANALYSES

This section addresses WEGI's comments in their section 4.0 of their October 2008 document.

WEGI states that there are many sources of uncertainty in the greenhouse studies of 2000 and 2001 and in their words, "these incompletely understood and unquantified variables result in considerable doubt that the suggested safe levels for CoCs in Port Colborne soils are, in fact, protective". There are no grounds for such a statement by WEGI based on the preceding discussion.

Fourteen sources of uncertainty in the derivation of PNEC values were identified by WEGI in their Table 1 of their report. Our comments to these uncertainties have been addressed in preceding discussion. For completeness, Jacques Whitford has provided below a summary of the fourteen WEGI-claimed uncertainties and our response:

1. Use of oat as a test species:
    a. Due to the specific visual phytotoxicity symptoms induced by Ni, oat offers a unique model system for studying this aspect in plant physiology.
    b. Oat model system has been well studied and characterized by other researchers offering an established etalon for comparison of findings (similar to the use of maples by the MOE for characterizing various chemical accumulation in tree foliage phytotoxicity effects).
    c. Oat was shown to be more sensitive than radishes, soybean, corn and golden rod to the site-specific conditions under both field and greenhouse conditions. This was corroborated by findings by others such as Chaney et al 2004 and Chaney et al., 2003 on site-specific studies in Port Colborne that were carried out in parallel to those of Jacques Whitford's.
2. Blending soils:
    a. Method of blending is recommended by Environment Canada (2005) when establishing effects of contaminated soils to vegetation.
    b. Replacement of nutrients in depleted soils: In the case of phosphorus (which is a macronutrient), the variability in phosphorus levels had no impact on the generated EC_{25} values from the greenhouse dose response experiments on both the Welland Clay and Till Clay

3. Blending of Soil: soil structure. WEGI's statement that Till Clay is the most important agricultural soil in the Port Colborne area is misleading and incorrect. There are **three** major soil types, namely Organic Muck, Welland Clay and Till Clay, ie. not just one major soil type as claimed by WEGI. Jacques Whitford's consistent examination of soil texture in the soil blends indicated low variabilities and thus the generated EC_{25} value for all four types of soils are valid.

4. Confounding factors: CEC and TOC: Jacques Whitford has taken into consideration all soil characteristics that could have a significant effect in the derivation of $EC_{25.}$

5. Confounding soil factors: The soils used in the blending process were not based on one location. As described in the report a large number of investigative samples were collected in order to identify the matching pairs.

6. Confounding soil factors: Organic Soils: The findings for the Organic Soil are in agreement with those documented in scientific literature by other independent researchers conducting phytotoxicology studies in Port Colborne (Kukier and Chaney, 2000 and 2004). It is also supported by field findings conducted in Port Colborne by Temple and Bisessar (1981) who found that for an Organic Muck soil from Port Colborne, growth of onion, potato, celery, cabbage and lettuce was reduced for some plant parts, at soil Ni concentrations in the range of 2000 to 3000 mg/kg; ie. similar to the 2001-generated values of 2350 mg/kg PNEC and 3400 mg/kg EC25.

7. Addition of lime to adjust pH: reduces the uptake of Ni. Liming of the soils was only used when the soil pH of the blended soils was not within the average soil pH conditions for a particular soil type in the area of impact and to provide consistency across the various treatments.

8. Addition of lime to adjust pH: need to provide factors for soils at different pH: The EC25 and PNEC values developed in the Crops 2004 study are applicable to soils with near-neutral pH which by design of the greenhouse experiments, reflect the representative soil pH within the agricultural community of Port Colborne. Hence, no need to have had other dose-response experiments at other soil pH levels as such levels are not be found in the Port Colborne area.

9. Mn deficiency: The effect of the Mn deficiency on the EC_{25} has been identified in our work. With regards to how Mn deficiency relates to agricultural crop production is outside the scope of this study. We are cognizant of separate independent studies carried out by the University of Guelph to address this very issue through agricultural crop field studies. Again, this is outside the scope of this study.

10. Focus on Ni: Although EC_{25} calculations were based on Ni concentrations in soil and plant tissue, the approach of Jacques Whitford has been to consider these values site specific to Port Colborne contaminated soils and inclusive of the potential phytotoxicity of all CoCs. This interpretation is justified by the strong correlations (r>0.9) of soil total Cu, Co and As concentrations with soil total Ni concentration, which were consistent across soil types and range of Ni concentration**.**

11. Statistics – Large Degree of Scatter in Data: All data has undergone test for normality to ensure that all data distributions are normal and fulfilling the assumptions of normality in various statistical tests (Section 11, Binder 3 out of 3). Where necessary, data was transformed and outliers were removed prior analyses. The results obtained in the GH experiments fall within a normally distributed population which one would expect when conducting a multitude of dose response tests (such as the ones conducted in the 2001 Greenhouse Studies).

12. Statistics- Use of different models to fit data: The approach of applying the best fit curve to the results of a dose response testing is a relatively common practice in the field of environmental toxicology and the use of it for the greenhouse 2001 study has been validated by other peer reviewers and published in the Canadian Journal of Soil Science May, 2008)

13. Statistics – Gap in data at moderate to high CoC concentrations at critical point in dose-response curve. The confidence intervals 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.

14. Use of shoot biomass as a surrogate for crop grain yield: 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.

10.0 DISCUSSION

Jacques Whitford has provided the reader with the following sections to put in context some of the uncertainties raised by WEGI on the greenhouse data, as well as to present scientific argument that supports the validity of the PNEC values generated from the greenhouse 2001 studies.

10.1 Conservatism of Using Greenhouse Test Results in Setting Soil Standards

The toxicity observed in the pot-grown soybean, and oat, in Jacques Whitford's greenhouse studies was found to be **more severe** than that observed in the field-grown crops, relative to soil Ni concentration.

Oat grown in the field in Year 2001, in the Clay 2 Test Site, a natural field soil of Welland Clay 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 2001 blended soils of Welland Clay *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).

	Total Soil Nickel, mg/Kg	**Tissue Nickel, mg/Kg**
Welland Clay – Field Environment	5000	58.2
Welland Clay – Field Environment	1900	52

The above indicates 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.

Thus the greenhouse findings **overstate** the actual soil Ni phytotoxicity observed in the field. Hence the greenhouse-generated PNEC values as reported in the 2004 Crops Report are overly conservative, and any uncertainty that may be related to the greenhouse trials is covered by this conservatism.

10.2 Logic Check using Non-Blended Greenhouse 2000 Data to Predict EC_{25} values

WEGI had concerns of deriving soil Ni EC_{25} values using the Greenhouse Year 2001 data because these data were based on 'blended' soils. An inference was made by WEGI in their October 2008 document that perhaps more realistic EC_{25} values could be obtained using the Greenhouse 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, an attempt was made by Jacques Whitford to address WEGI's request by back calculating or predicting soil Ni EC_{25} values in soil using the

Year 2000 greenhouse dose response data and adapting 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 'unblended' Welland Clay 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 Clay soil (Figure 4) and also on Organic soil (Figure 5). 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.

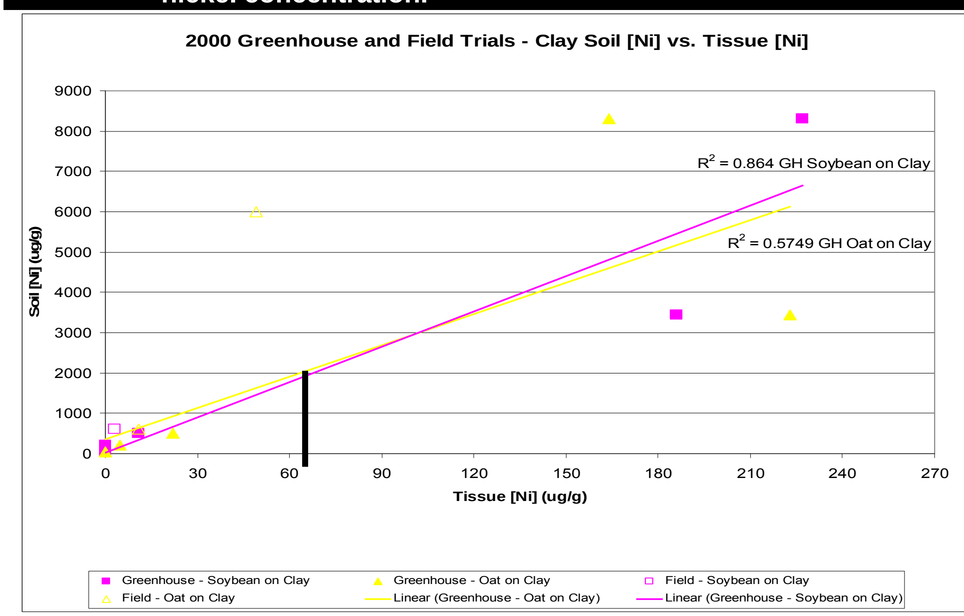
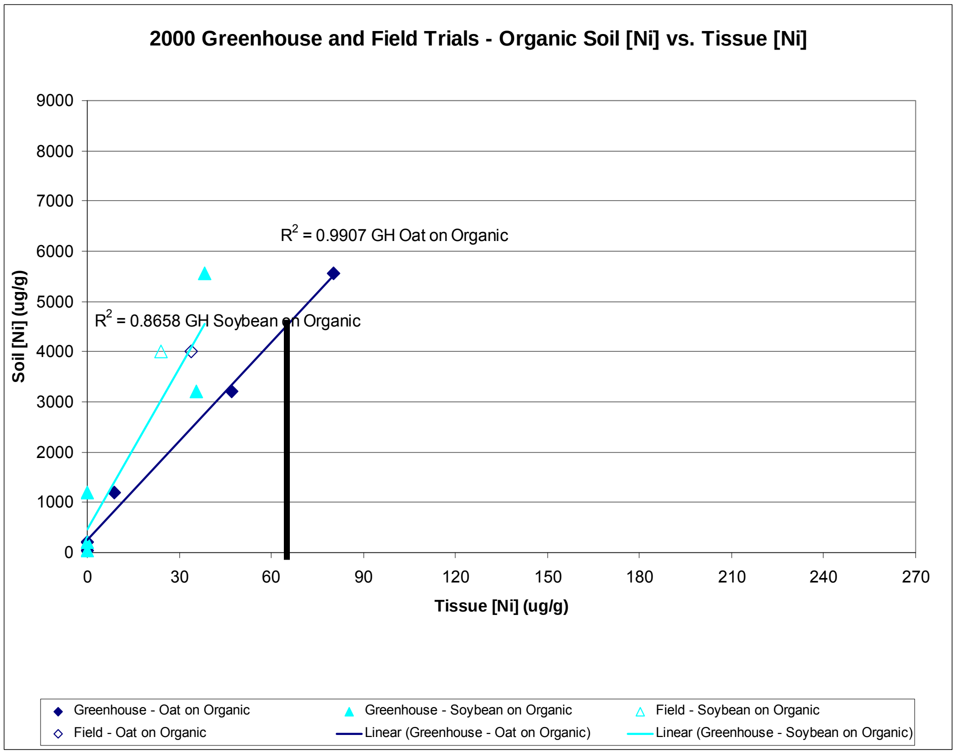


Figure 5 Year 2000 Crop Studies Estimated EC_{25} in Organic Soils using threshold tissue nickel concentration.



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 4.

Table 4 Predicted Ni EC_{25} using Year 2000 Greenhouse Data

Dose-Response Experiment	Ni EC_{25} in 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 4 show no significant differences in soil Ni EC_{25} values between the Year 2000 and Year 2001 dose response data for oat grown on Clay soil and Organic soil, when they are predicted from tissue Ni concentrations. Table 4 also shows that the predicted values of soil Ni EC_{25} for soybean are similar, if not greater than those of oat.

Also as shown in Table 4, 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.

10.3 Logic Check using the MOE 200 mg Ni /Kg Generic Criterion and Measured Ni Bioaccessibility Data to Derive PNEC

The MOE Generic Soil Criterion of 200 mg/kg was developed based on soluble nickel and not total (soluble and insoluble) nickel. To adjust the MOE soluble nickel soil criterion to an equivalent one based on total (soluble and insoluble) nickel soil concentration, nickel bioaccessibility of that soil needs to be included in its derivation. To that end, the MOE Generic Soil Criterion of 200 mg/kg (ie. based on soluble nickel) was divided by the bioaccessibility fraction of nickel measured in Port Colborne soils to derive adjusted (predicted) total nickel soil Ni criterion and to use these predicted values to compare with the CBRA greenhouse-derived total Ni soil PNEC values.

The MOE Soil Ni 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. This experiment used the *unaged medium* of quartz sand, which is in stark contrast of the CBRA greenhouse studies that used well developed and *aged soils*.

The quartz sand medium used by Davis et al. (1978) would be similar to mineral soils. Measurements of nickel bioaccessibility in mineral soils of Port Colborne were only done for Welland Clay and the Fill material of the Rodney Street residential area; information of which were extracted for this calculation from the MOE (2002) Rodney Street Report and the Jacques Whitford (2005) Human Health Risk Assessment Report.

Using the above-described equation of dividing the 200 mg/kg soluble nickel criterion by the measured bioaccessibility for mineral soils of the Welland Clay and Fill Material led to the calculation of PNEC values as shown in Table 5.

Good agreement between the derivated and measured total soil Ni PNEC values was found for Welland Clay, ie. 1429 mg/Kg versus 1650 mg/Kg.

Although a soil Ni PNEC could not be calculated for the Rodney Street Fill soil samples without greenhouse data, the derivated PNEC values for the Fill material ranged from 1212 mg/Kg based on the MOE stomach leach test, to between 1052 and 1429 mg/Kg based on the MOE SBRC Acid Extract test and between 2941 and 3703 mg/Kg based on the Jacques Whitford SBRC Acid Extract test. This range of these derivated PNEC values for the Fill material are within the reported range of PNEC values recommended for agricultural soils in Port Colborne (ie. 1650 mg/Kg for Welland Clay, 1400 mg/Kg for Till Clay and 2350 mg/Kg for Organic Muck.

Overall the findings from this logic check calculation illustrates that the Crops-derived PNEC values for the mineral soil types in Port Colborne are comparable to the MOE generic soil Ni criterion when the measured bioaccessibility of the soil from this area 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 5 Derivation of PNECs Using Laboratory-Determined Bioaccessibility Values

Soil Type	Method of Nickel Bioaccess -ibility and Source	% Nickel Bioaccess-ibility *mean* (range) Units - mg/kg	Derivated PNEC [Adjusted Generic Soil Clean-up Criterion 200/bioaccessibility fraction] (mg/kg)	Measured PNEC[e], JW 2004 (mg/kg)
Heavy {Welland} Clay	SBRC Acid Extract with glycine, JW 2002	*14*	*1429*	1650
Fill Material – mix of clay and organic	Simulated stomach leach, MOE 2002	*16.5 (11.8-23.3)*	*1212 (858 to1694)*	Dose Response on Fill Material not done. Assumed Fill PNEC for Fill in between: 1400 - Till Clay, 1650 - Heavy Clay 2350 - Organic Soil
	SBRC Acid Extract with glycine, MOE 2002	*14 (8-21)* *19 (11-28)*	*1429 (952 to 2500)* *1052 (1818-714)*	
	SBRC Acid Extract with glycine, MOE 2002	*6.8*	*2941*	

11.0 SUMMARY

Watters Environmental Group Inc. (WEGI) peer reviewed the Crops December 2004 report which had been written by Jacques Whitford Limited (Jacques Whitford) and incorporated their comments in a letter document entitled: "Independent Consultant Peer Review Report for the Community Based Risk Assessment (CBRA) – Ecological Risk Assessment on Agricultural Crops in Port Colborne, Ontario" dated October 2008. Issues raised by WEGI in their October 2008 document pertained to uncertainties in Jacques Whitford's crops studies, studies of which led to the development of the proposed Port Colborne-specific CoC soil standards.

Jacques Whitford has provided herewithin commentary to each of the uncertainty issues raised by WEGI. All of the issues which were raised by WEGI have been resolved within this report.

In addition, Jacques Whitford have conducted supplemental calculations and logic checks, as well as additional insight on the phytotoxicity of CoCs in Port Colborne soils within this text, the results and findings of which have shown compelling evidence that the proposed Port Colborne-specific CoC soil standards are valid.

Jacques Whitford believes that the perceived gap between findings and interpretations as found in the December 2004 Final Crops Report and the issues raised by WEGI in their October 2008 document on these findings and interpretations have been considerably narrowed, if not completely eliminated within this report.

Furthermore, a scientific paper on the derivation of the same proposed Port Colborne-specific CoC soil standards was submitted to the Canadian Journal of Soil Science for publication. After rigorous review and scrutiny by the journal's scientific editors, this paper was accepted and recently published. Details of this paper are as follows:

Dan, T., Hale, B., Johnson, D., Conard, B., Stiebel, W.H., and Veska, E. *Toxicity Thresholds for Oat grown in Ni-impacted Agricultural Soils near Port Colborne, Ontario, Canada* . Can. J. Soil Science, May 2008.

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APPENDIX A

WEGI Comments of October 2008