Developing Site-Specific Guidelines for Orchard Soils Based on Bioaccessibility – Can It Be Done?*

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Introduction

Horticultural land within the periurban fringe of NZ towns and cities increasingly is being developed for residential subdivision. Recent surveys have shown that concentrations of As, Cd, Cu, Pb, and ΣDDT (sum of DDT and its degradation products DDE and DDD) in such soils can exceed criteria protective of human health.[1] Soil ingestion is a key exposure pathway for non-volatile contaminants in soil. Currently in NZ, site-specific risk assessments and the derivation of soil guidelines protective of human health assume that all of the contaminant present in the soil is available for uptake and absorption by the human gastrointestinal tract. This assumption can overestimate health risks and has implications for the remediation of contaminated sites.[2] In comparison, the bioavailability of contaminants is considered when estimating exposure *via* dermal absorption and by ingestion of home-grown produce.[3] Dermal absorption factors and plant uptake factors are included in the calculations for estimating exposures via these routes.

Provided there are tools available to produce robust data on the bioavailability of contaminants in soil, it may be possible to derive site-specific guidelines that incorporate scientifically validated and refined risk scenarios. Site-specific guidelines could reduce the scope and costs of remediation on former horticultural land. A range of *in vivo* and *in vitro* methods have been developed to assess bioavailability of contaminants in soil and these are gaining increasing regulatory acceptance overseas.[4] Little is known about the oral bioavailability of contaminants from NZ soils, and differences in soil properties may mean that overseas data may not be directly applicable to NZ conditions. Herein, we provide an overview of methods to assess bioavailability of contaminants in soil via the oral route, and outline current barriers to using bioavailability in risk assessments for human exposure in NZ. In addition, the results of a preliminary investigation using the Solubility/Bioavailability Research Consortium (SBRC) *Stomach-Phase Extraction* in vitro *method* to estimate[5] the bioaccessible fraction of arsenic, cadmium, and lead in orchard soils are presented.

For human health risk assessments and the derivation of generic and site-specific soil guidelines, daily intakes from the relevant exposure pathways are estimated and compared with toxicological intakes. Intakes of carcinogenic substances are assessed against index doses derived from dose-response relationships, and intakes of threshold contaminants (non carcinogens and non-genotoxic carcinogens) are assessed against tolerable weekly (or daily) intakes.[6] Such toxicological intakes can be derived either from animal dosing trials with appropriate safety factors, or from epidemiological data from populations exposed to the contaminant of interest.[7] Toxicity parameters including tolerable daily intakes and index doses are generally calculated based on the *intake dose*.[5,6]

Definitions

The following definitions, adapted from Paustenbach,[8] are used herein:

> ***Oral Bioaccessibility*** of a substance is the fraction of that substance that is soluble in the gastrointestinal environment and is available for absorption through the gastrointestinal tract and into the bloodstream.
>
> ***Oral Bioavailability*** of a substance is defined as the fraction of an administered dose that reaches the bloodstream by absorption through the gastrointestinal tract.
>
> ***Relative Bioavailability*** - The relative bioavailability of a substance refers to comparative bioavailabilities of different forms of that substance or for different exposure media containing the substance.

Currently, the approach adopted in risk assessments for contaminated soil is to assume that the bioavailability of the contaminant in soil is equivalent to the bioavailability of the contaminant in the matrix used to derive the toxicity parameter.[9] Generally, in the studies used to derive toxicological intakes, the contaminant of interest was ingested with water or food rather than soil.[10] Often, in animal trials, trace elements have been reported as less bioavailable from soil than from other matrices such as food and drinking water[8,11-13] because contaminants can remain adsorbed to soil in the human gastrointestinal tract.[10] Factors that can reduce the oral bioavailability of contaminants in soil include the physicochemical properties, aging, chemical speciation, soil properties, particle size, and soil mineralogy.[5,14]

The following criteria have been proposed to identify when a site-specific bioavailability assessment can be undertaken:[5,11,15]

- Concentrations of contaminants only slightly exceed soil quality criteria.

- A limited number of contaminants exceed soil quality criteria.
- Soil ingestion is a key exposure pathway.
- The form of the contaminant is likely to have low relative bioavailability.
- The key contaminants are well aged in the soil.
- Remediation is costly or suitable techniques are unavailable.
- A large amount of land is involved.
- There is a risk of environmental degradation during soil remediation.

In vivo methods

In the absence of human studies or the availability of suitable epidemiological data, in vivo animal trials,[16] using rabbits, rats, primates and pigs,[5,17] have been used to measure the bioavailability of contaminants. Juvenile swine are commonly used to estimate the oral bioavailability of contaminants in soil for children, and to validate *in vitro* methods,[5,10,13] because they are comparable in size and have similar gastrointestinal physiology.[2,5] Testing protocols vary depending upon the contaminant of interest and the animal species involved.[5]

In animal studies, one group of animals in the trial is fed contaminated soil and the other given the contaminant of interest in a (usually more soluble) form that is comparable to the one used in studies to derive toxicity values. Concentrations of the contaminant of interest present in body tissues and/or excreta are measured at intervals after dosing, and the data are used to calculate a relative bioavailability factor.[5] However, animal trials are time consuming, expensive, and raise ethical concerns.[18] Additionally, concerns regarding the appropriateness of animal models as a surrogate measure for bioavailability in humans stem from differences in physiology and behaviour.[17]

In vitro methods

In vitro tests for measuring the bioavailability of contaminants have been developed to overcome the critical issues associated with animal testing.[4,18] One further advantage of *in vitro* testing is that the tests can be designed to simulate the processes and conditions occurring in the human gastrointestinal tract.[18] *In vitro* methods measure the bioaccessible fraction of contaminants in soil, *i.e*. that proportion of the contaminant that is desorbed from the soil in the human gastrointestinal tract and is potentially available for absorption. *In vitro* methods are suitable only for estimating the bioavailability of contaminants in soil if dissolution of the contaminant of interest is the rate-limiting step for absorption.[4]

In vitro methods are generally based on the paediatric gastrointestinal tract.[5] Soil is extracted at body temperature (37°C) with a simulated gastric fluid prepared from HCl and containing selected enzymes and amino acids. Summaries of the various simulated gastric extraction techniques are presented by Wragg and Cave,[19] Oomen *et al*.,[20] and Grøn and Anderson.[11] Points of difference between simulated gastric extraction methods include solid to solution ratios, the inclusion of food to simulate fed or fasting conditions (dough or dairy products), and the inclusion of a second extraction stage to simulate processes occurring in the intestines.

The bioaccessibility factors determined for some trace elements correlate with relative bioavailability factors obtained from animal feeding trials.[2,5] It should be noted that the correlation between the results from the *in vitro* and the *in vivo* methods may not be a one-to-one relationship.[8] However, not all *in vitro* methods have been validated against animal trials[18] and the bioaccessible fraction does not always correlate with the relative bioavailability measured in an *in vivo* trial.[17] While *in vitro* methods have been developed also for organic contaminants including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), validation data for these contaminants have not been published.[16] *In vitro* methods for organic contaminants will prove more difficult to validate using animal trials as the organic compounds also can be metabolised and/or degraded by microorganisms in the intestine.[21]

To date, *in vitro* methods have not been used routinely to estimate the bioaccessibility of trace elements in NZ soils, nor have they been validated against animal trials. However, under the Toxic Substances Amendment Regulations (1999), a comparable simulated gastric acid extraction method is used to screen children's graphic materials, *i.e*. paints and crayons, for toxic levels of selected trace elements.

Examples where *in vitro* testing methods have been used overseas include assessment of the bioaccessibility of naturally high concentrations of trace elements in soil, measurement of the bioaccessibility of arsenic in soil, derivation of site-specific soil criteria for arsenic, and in monitoring the effectiveness of *in situ* stabilization techniques.[22] *In vitro* testing methods also have been used to measure the bioaccessibility of contaminants in matrices other than soil including dust, children's toys, and food.[23]

Case Study: Bioaccessibility of Arsenic, Cadmium and Lead in New Zealand orchard soils

Lead arsenate ($PbHAsO_4$) was widely used as a pesticide in NZ orchards until the 1960s and Cd is a contaminant in fertilisers.[24] Several investigations have shown that NZ orchard soils can contain As, Cd and Pb in concentrations that exceed the levels protective of human health (Table 1).[1,25] These elevated concentrations are of concern when former orchards are converted into residential subdivisions. Contaminated orchard soils meet the criteria detailed above for bioavailability assessments due to the large amount of land potentially involved, the limited remediation options for these elements in soils, and the potential for the remediation activities to have an adverse effect on the environment.

Table 1. Range of selected trace element concentrations (mg/kg) from NZ orchard soils.

Region	As	Cd	Pb	Ref.
Auckland	2-34	0.1-1.1	11-178	1
Tasman	3-48	0.3-1.0	15-243	1
Waikato	4-58	0.8-1.5	14-251	1
Hawkes Bay	4-43	0.05-0.5	16-341	25

Methodology

A modified version of the SBRC's *Standard Operating Procedure for Stomach-Phase Extraction*[5] was used to determine the bioaccessibility of As, Cd and Pb from ten orchard soils. For gastric extractions, the soils were sieved to <250 μm to represent the fraction of soil likely to adhere to children's hands and be ingested.[5] Briefly, for the gastric extraction, 1 g of <250 μm soil was extracted using 100 mL of simulated gastric fluid composed of 0.4 M glycine adjusted to pH 1.5 with c.HCl. The resulting slurries were shaken on an orbital mixing incubator for 1 h at 37ºC, filtered, and analysed for trace elements by ICP-MS.

Results

The percentage of bioaccessible fractions for As, Cd and Pb ranged from 12–45%, 64–100% and 56–83%, respectively. The mean percentage bioaccessible fraction followed the order: Cd > Pb > As and was consistent with order of extraction of metals from soils using the comparable *Simple Bioaccessibility Extraction Test* method developed by the UK Geological Survey.[20] The range of %bioaccessible fraction for metals obtained indicates that the bioaccessibility of these contaminants in orchard soils varies on a site-specific basis. For As and Pb there was a significant correlation (Fig. 1) between the [Fe] and the %bioaccessible fraction, indicating that soil characteristics are a controlling factor for the bioaccessibility of As and Pb. This also suggests that significant portions of these contaminants are present in a chemically speciated form associated with the iron content of the soil - specifically the amorphous iron-hydroxide phase.

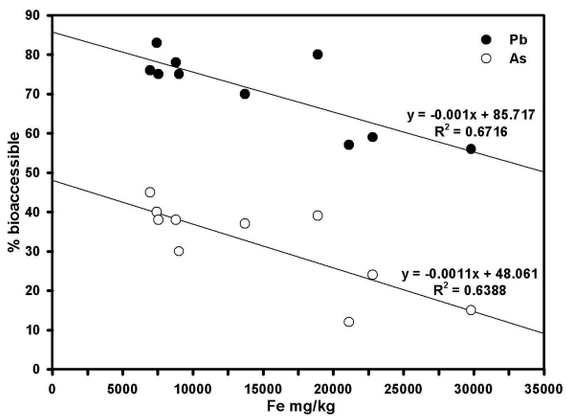

Fig. 1. Relationships between the bioaccessible fraction (%) of As and Pb and the [Fe] (mg/kg) of the <250 μm soil fraction.

Barriers to Using Bioaccessibility Data in Risk Assessments

While the preliminary results presented in Fig. 1 indicate that bioaccessibility of trace elements is likely to vary between orchard sites, there are several barriers to introducing *in vitro* testing as part of risk assessments in NZ in the short term.[2,10,19,26] These barriers include:

1. Lack of international consensus on the appropriate test method(s) to determine bioaccessibility due to:
   - variability of results between test methods, soil types, and test laboratories;
   - concerns of the relevance of *in vitro* tests to human exposures to contaminants in soil, and the scientific validity of the tests;
   - limited method validation including human data and few reference materials to support inter-laboratory validation of methods.
2. Lack of policy to support the use of bioaccessibility adjustments including:
   - guidance on how to incorporate bioaccessibility/bioavailability adjustments into risk assessment.
3. Lack of information on the bioaccessibility of contaminants in food.
4. Questions regarding the appropriateness of adjusting the currently available toxicological intakes.
5. Limited information on the long-term stability of bioaccessibility measurements.
6. Lack of awareness of some end users of the limitations of *in vitro* test methods.
7. Regulatory acceptance.

It is possible that bioaccessibility could be incorporated into risk assessments in the future provided that the issues identified above can be resolved, and international consensus reached. Bioaccessibility of contaminants in soil is an active area of research. There are several international collaborations underway aimed to improve understanding of the scientific validity of *in vitro* bioaccessibility testing, and to identify standard test methods. These include Bioavailability Research Canada (BARC), the Bioavailability Research Group Europe (BARGE), and the Solubility/Bioavailability Research Consortium (US). In addition, the International Standards Organisation (ISO) has recently published a standard for bioaccessibility - *Soil Quality: Assessment of human exposure from ingestion of soil and soil material; guidance on the application and selection of physiologically-based extraction methods for the estimation of the human bioaccessibility/bioavailability of metals in soil* (ISO/TS 17294:2007).

The validity and acceptability of bioaccessibility testing in NZ has yet to be subjected to governmental evaluation. Moreover, NZ has not participated in the international collaborative projects for validating and standardizing *in vitro* test methods for contaminants in soil. The Ministry for the Environment is currently developing *a nationally consistent NZ risk-based methodology for deriving soil*

contaminants for human health.[27] The question of whether or not bioaccessibility-based adjustments are able to be readily accommodated within this methodology will need to be considered by the Ministry's Technical Reference Group as part of this work.

Summary

Internationally, *in vitro* methods are being used to estimate the bioaccessible fraction of contaminants in soil. These chemical extraction methods simulate conditions and processes occurring in the human gastrointestinal tract and provide a surrogate measure of bioavailability. An *in vitro* method was used to estimate the bioaccessible fraction of arsenic, cadmium, and lead in orchard soils and gave values that ranged from 12–45% for As, 64–100% for Cd, and 56–83% for Pb. These results indicate that the oral bioaccessibility of these metals can vary on a site-specific basis and that it may be feasible, under some circumstances, to derive site-specific guidelines to protect human health. However, there are significant barriers to using bioaccessibility data in risk assessments, including questions regarding the relevance of the *in vitro* testing, and a lack of guidance on how to incorporate bioaccessibility values into risk assessments.

Acknowledgements

We are grateful to Environment Waikato for funding the sample analyses, landowners for providing access for sampling, and Hill Laboratories for the gastric extractions and trace element analyses. Drs. Jo-Anne Cavanagh (Landcare Research), Graeme Proffitt (Pattle Delamore Partners) and Lou Gallagher (ESR) are thanked for useful discussions and Peter Cressey (ESR) provided helpful comments on the draft; ESR funded manuscript preparation. Financial assistance to SKG (Waikato University) is acknowledged.

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