British Geological Survey
NATURAL ENVIRONMENT RESEARCH COUNCIL

Inter-laboratory Trial of a Unified Bioaccessibility Procedure

Chemical & Biological Hazards Programme

Open Report OR/07/027

BRITISH GEOLOGICAL SURVEY

CHEMICAL & BIOLOGICAL HAZARDS PROGRAMME

OPEN REPORT OR/07/027

Inter-laboratory Trial of a Unified Bioaccessibility Procedure

J Wragg, M Cave, H Taylor, N Basta, E Brandon, S Casteel, C Gron, A Oomen, T Van de Wiele

Keywords

Bioaccessibility, In vitro, In vivo, Arsenic, Cadmium, Lead.

Front cover

BioAccessibility Research Group of Europe logo.

Bibliographical reference

WRAGG, J, CAVE, M, TAYLOR, H,BASTA, N, BRANDON, E, CASTEEL, S, GRON, C, OOMEN, A, VAN DE WIELE, T. 2009. Inter-laboratory Trial of a Unified Bioaccessibility Procedure. *British Geological Survey Open Report*, OR/07/027. 90pp.

Keyworth, Nottingham British Geological Survey 2009

BRITISH GEOLOGICAL SURVEY

The full range of our publications is available from BGS shops at Nottingham, Edinburgh, London and Cardiff (Welsh publications only) see contact details below or shop online at www.geologyshop.com

The London Information Office also maintains a reference collection of BGS publications, including maps, for consultation.

We publish an annual catalogue of our maps and other publications; this catalogue is available online or from any of the BGS shops.

The British Geological Survey carries out the geological survey of Great Britain and Northern Ireland (the latter as an agency service for the government of Northern Ireland), and of the surrounding continental shelf, as well as basic research projects. It also undertakes programmes of technical aid in geology in developing countries.

The British Geological Survey is a component body of the Natural Environment Research Council.

British Geological Survey offices

BGS Central Enquiries Desk

Tel 0115 936 3143 Fax 0115 936 3276

email enquires@bgs.ac.uk

Kingsley Dunham Centre, Keyworth, Nottingham NG12 5GG

Tel 0115 936 3241 Fax 0115 936 3488

email sales@bgs.ac.uk

Murchison House, West Mains Road, Edinburgh EH9 3LA

Tel 0131 667 1000 Fax 0131 668 2683

email scotsales@bgs.ac.uk

London Information Office at the Natural History Museum (Earth Galleries), Exhibition Road, South Kensington, London SW7 2DE

Tel 020 7589 4090 Fax 020 7584 8270

Tel 020 7942 5344/45 email bgslondon@bgs.ac.uk

Columbus House, Greenmeadow Springs, Tongwynlais, Cardiff CF15 7NE

Tel 029 2052 1962 Fax 029 2052 1963

Forde House, Park Five Business Centre, Harrier Way, Sowton EX2 7HU

Tel 01392 445271 Fax 01392 445371

Maclean Building, Crowmarsh Gifford, Wallingford OX10 8BB

Tel 01491 838800 Fax 01491 692345

Geological Survey of Northern Ireland, Colby House, Stranmillis Court, Belfast BT9 5BF

Tel 028 9038 8462 Fax 028 9038 8461

www.bgs.ac.uk/gsni/

Parent Body

Natural Environment Research Council, Polaris House, North Star Avenue, Swindon SN2 1EU

Tel 01793 411500 Fax 01793 411501

www.nerc.ac.uk

Website www.bgs.ac.uk

Shop online at www.geologyshop.com

Foreword

This report is the published product of a study by the British Geological Survey (BGS) carried out in conjunction with the Bioaccessibility Research Group of Europe (BARGE). The study covers the factual findings from an inter-laboratory trial on the measurement of the bioaccessibility of As, Cd and Pb in soils.

Acknowledgements

In addition to the BGS authors of this report, all members of the BARGE group contributed to the production of this report.

A large number of individuals from the participating research institutes have contributed their time, advice, laboratory skills and samples to the project. This assistance has been received at all stages of the study. BARGE members have helped to review draft versions of the in-vitro methodology and draft chapters of this report. Of the many individuals who have contributed to the project we would particularly like to thank the following:

Ms Ashley Campbell

Mrs Kim House

Mrs Leigh Easton

Mr Julien Caboche

Mr Shane Whitacre

Mrs Kay Green

Contents

FIGURES

TABLES

Summary

This report describes the factual results of a unified bioaccessibility protocol undertaken as a part of the Environmental Geochemistry and Health project of the Environment and Health Programme of the British Geological Survey.

In 2005 the Bioaccessibility Research Group of Europe (BARGE) undertook a joint decision to progress research in the field of *in vitro* bioaccessibility method development for the harmonisation of the use of bioaccessibility in human health risk assessment of contaminant soils in Europe. The progression took the form of combining their collective efforts to evaluate one *in vitro* method already in existence. The chosen method was that previously published by researchers at the Dutch Institute of Public Health, the RIVM, as this was considered to be most representative of the physiochemical conditions in the human gastrointestinal (GI) tract. Modifications to the RIVM methodology to ensure adequate conservatism, and that the *in vitro* test was robust and applicable to the local geological conditions in a range of different countries, were considered and tested prior to the preparation of a standard operating procedure (SOP) and detailed at the start of the full evaluation exercise.

Evaluation of the unified bioaccessibility method (UBM) was undertaken by means of an international inter-laboratory comparison exercise. The BARGE members with affiliated laboratories who participated in the exercise were; the Dutch Institute of Public Health (RIVM), DHI soil and water (Denmark), INERIS (France), the University of Ghent (Belgium), Ohio State University (USA), the Royal Military College of Canada (RMC) and the British Geological Survey (BGS). The BGS undertook the role, in the evaluation exercise, as the lead laboratory, and the subsequent analytical requirement for the measurement of the bioaccessible element content in each extract was undertaken by the BGS Analytical Geochemistry Laboratories (AGL).

The inter-laboratory study used soils and related materials with associated *in vivo* swine data, donated by Professor Nick Basta of the School of Environment Natural Resources at Ohio State University and by Professor Stan Casteel of the College of Veterinary Medicine at the University of Missouri. The materials under investigation include slag materials, soils, river sediments and house dusts containing the primary contaminants of concern, arsenic (As), cadmium (Cd) and lead (Pb).

Main analytical performance characteristics of the bioaccessibility measurement (repeatability and reproducibility) were determined in the collaborative study using the procedure described in the ISO standard 5725-2.

1 Introduction

In 2005 the Bioaccessibility Research Group of Europe (BARGE) undertook a joint decision to progress research in the field of *in vitro* bioaccessibility method development for the harmonisation of the use of bioaccessibility in human health risk assessment of contaminated soils in Europe. The progression took the form of combining their collective efforts to evaluate one *in vitro* method already in existence. The chosen method was that previously published by researchers at the Dutch Institute of Public Health, the RIVM (Oomen, 2000; Oomen et al., 2002) as this was considered to be most representative of the physiochemical conditions in the human gastrointestinal (GI) tract. Modifications to the RIVM methodology to ensure adequate conservatism, that the *in vitro* test was robust and applicable to the local geological conditions in a range of different countries, were considered and tested prior to the preparation of a standard operating procedure (SOP), detailed in Appendix 1, and the start of the full evaluation exercise. Modifications to the RIVM *in vitro* methodology are detailed in table 1.

Table 1 Summary of modifications to the RIVM in vitro method

Parameter	Modification
Saliva pH	Reduction of pH to 6.3 ± 0.5
Stomach pH	Reduction of the stomach pH to 0.9-1.0
Duodenal pH	Reduction to pH 7.4 ± 0.2
Stomach & Saliva pH	Addition of pH checking step to ensure the pH is 1.2
Stomach & Intestine pH	Increase of mixed pH to 6.3
Stomach Residence time	Reduction from 2 to 1 hr
Stomach Sampling Phase	Addition of an extra pH check and a sampling phase to collect a 'stomach' sample
Intestine Residence time	Increase from 2 to 4 hr
Intestine Phase final pH	Inclusion of a pH check stage at the end of the intestine phase
Uric acid product code	Merck code 100817 specified by RIVM replaced by Merck-Prolabo code 20745.134. The product is of the same quality.
Duodenal fluid matrix	Increase in the amount of $NaHCO_3$ used in the fluid, to account for the lower stomach pH and negate the use of 1M NaOH for pH correction. The increase in the $NaHCO_3$ content was tested in the lead laboratory.
Bile fluid matrix	Increase in the volume of 37 % HCl.

Evaluation of the unified bioaccessibility method (UBM) was undertaken by means of an international inter-laboratory exercise. The BARGE members with affiliated laboratories who participated in the inter laboratory trial were; the Dutch Institute of Public Health (RIVM), DHI

soil and water (Denmark), INERIS (France), the University of Ghent (Belgium), Ohio State University (USA), the Royal Military College of Canada (RMC) and the British Geological Survey (BGS). The BGS undertook the role of the lead laboratory in the evaluation exercise, and the subsequent analytical requirement for the measurement of the bioaccessible element content in each extract was undertaken by the BGS Analytical Geochemistry Laboratories (AGL). The AGL are accredited to ISO 17025 by the United Kingdom Accreditation Service (UKAS) and are registered as UKAS Testing Laboratory number 1816. Although the procedures described in this document for measurement purposes are not accredited for the sample matrix under investigation, the samples were treated according to the requirements of the UKAS accreditation in all other respects, particularly with respect to sample handling, quality control and reporting of data. A number of the participating laboratories undertook 'in-house' analysis of separate aliquots of the bioaccessibility extraction fluids. Where this data has been used, the analytical methodology has been described. The unified *in vitro* methodology and the resulting data are described in this report.

2 Materials and Methods

The inter-laboratory trial was made possible through the donation of materials with associated *in vivo* swine data by Professor Nick Basta of the School of Environment Natural Resources at Ohio State University and by Professor Stan Casteel of the College of Veterinary Medicine at the University of Missouri. The materials under investigation include slag materials, soils, river sediments and house dusts containing the primary contaminants of concern, arsenic (As), cadmium (Cd) and lead (Pb). A limited amount of information is available in the peer reviewed literature for a number of the materials under study including previous investigations of the *in vitro* bioaccessibility and the *in vivo* bioavailability, mineralogical information and information relating to the total contaminant content (Basta et al., 2007, Rodriguez et al., 2003; Schroder et al., 2003; Schroder et al., 2004). Where information on the source of the material contaminants and subsequent testing data was not readily available in the literature, this was provided by the donor of the individual samples. In addition to *in vivo* tested soils as a primary source of contaminated material, the inter-laboratory trial also included the two National Institute of Standards and Technology (NIST) standard reference materials (SRMs), 2710 and 2711, which have been studied by various workers in relation to their bioaccessible contaminant contents (Ellickson et al., 2001; Cave et al., 2003; Schroder et al., 2004) and the recently prepared BGS arsenic bioaccessibility guidance soil, BGS 102. Tables 2 and 3 summarise the materials studied

in the inter-laboratory trial, including the material type, references to published information and the total and relative bioavailable data available.

Table 2 Summary of materials under investigation

Sample Name	Material Type	Primary Element(s)	Supplier
As 1 – As 5	Calcine Soils (Rodriguez et al., 1999; 2003)	As	Basta
As 6 – As 10	Iron Slag Soils (Rodriguez et al., 1999; 2003)	As	Basta
AR 1 & AR 2	Aberjona River Sediments	As	Casteel
ETM 1	Pt. Mugu Soil 1B	Cd	Casteel
ETM 2	CO-SCS Soil	Cd	Casteel
ETM 3	OK-SS Soil	Cd	Casteel
ETM 4	Dugway Soil #4	Cd	Casteel
NB Cd 1	Nick Basta Cd Study sample 1, Blackwell Soil (Schroder et al., 2003)	Cd	Basta
NBR-255B-04	Nick Basta Pb-Cd studies samples 5 and 4, Jasper Yard soil (Schroder et al., 2003; Schroder et al., 2004)	Cd, Pb	Basta
NBR-256-04	Nick Basta Pb-Cd studies samples 12 and 6, Murray Slag (Schroder et al., 2003; Schroder et al., 2004)	Cd, Pb	Basta
NBR-261-04	Nick Basta Pb-Cd studies samples 2 and 2, Butte NPL (Schroder et al., 2003; Schroder et al., 2004)	Cd, Pb	Basta
NBR-267-04	Nick Basta Pb-Cd studies samples 13 and 7, Murray Soil (Schroder et al., 2003; Schroder et al., 2004)	Cd, Pb	Basta
NB Pb 11	Nick Basta Pb study sample 11, Murray Slag Soil (Schroder et al., 2004)	Pb	Basta
NB Pb 9	Nick Basta Pb study sample 9, Leadville Slag Soil (Schroder et al., 2004)	Pb	Basta
B & V 1A &1B	Composite Soils	Pb	Casteel
B & V 2 TM 1	House Dust	Pb	Casteel
B & V TM 2	Composite Soil	Pb	Casteel
DNR5 1	0.5 % Phosphate-treated soil	Pb	Casteel
DNR5 2	1 % Phosphate-treated Soil	Pb	Casteel
MSE 2	Soil	Pb	Casteel
NIST 2710 & 2711	Standard Reference Materials	As, Cd, Pb	NIST
BGS 102	Ironstone soil guidance material	As	BGS

Table 3 Published total (mg kg^{-1}) and relative bioavailable (RBA, %) As, Cd and Pb data for the soils under investigation

Soil	Total As	RBA As	Total Cd	RBA Cd	Total Pb	RBA Pb
As 1	1130	8.62	n/a	n/a	n/a	n/a
As 2	17500	4.07	n/a	n/a	n/a	n/a
As 3	13500	7.88	n/a	n/a	n/a	n/a
As 4	11500	22.8	n/a	n/a	n/a	n/a
As 5	6250	n/a	n/a	n/a	n/a	n/a
As 6	405	38.7	n/a	n/a	n/a	n/a
As 7	450	43.0	n/a	n/a	n/a	n/a
As 8	1180	39.1	n/a	n/a	n/a	n/a
As 9	5020	32.9	n/a	n/a	n/a	n/a
As 10	4650	21.9	n/a	n/a	n/a	n/a
AR 1	676	37.0	n/a	n/a	n/a	n/a
AR 2	313	51.0	n/a	n/a	n/a	n/a
ETM 1	n/a	n/a	4109	60.0	n/a	n/a
ETM 2	n/a	n/a	452	89.0	n/a	n/a
ETM 3	n/a	n/a	102	79.0	n/a	n/a
ETM 4	n/a	n/a	46.8	18.0	n/a	n/a
NB Cd1	n/a	n/a	465	55.0	n/a	n/a
NBR-255B-04	n/a	n/a	188	54.0	4050	90.0
NBR-256-04	n/a	n/a	30.0	10.0	11500	53.0
NBR-261-04	n/a	n/a	43.0	30.0	8600	22.0
NBR-267-04	n/a	n/a	26.0	57.0	3500	71.0
NBPb 11	n/a	n/a	n/a	n/a	7895	17.0
NBPb 9	n/a	n/a	n/a	n/a	10600	20.0
B & V 1A	n/a	n/a	n/a	n/a	1650	102
B & V 1B	n/a	n/a	n/a	n/a	1630	75.0
B & V 2 TM1	n/a	n/a	n/a	n/a	2283	52.0
B & V 2 TM2	n/a	n/a	n/a	n/a	2306	97.0
DNR5 1	n/a	n/a	n/a	n/a	2833	99.0
DNR5 2	n/a	n/a	n/a	n/a	4233	76.0
MSE2	n/a	n/a	n/a	n/a	2021	82.0

3 Unified Bioaccessibility Method

3.1 IN VITRO EXTRACTION

Prior to undertaking the inter-laboratory trial, the lead laboratory issued instructions to all participating laboratories which included a list of materials to be extracted and the number of duplicate blank extractions expected. The *in vitro* bioaccessibility extraction consisted of three

stages the mouth, stomach and intestine cavities which produced two individual extracts per test sample for analysis. The sample known as 'stomach' phase consisted of an extraction solution removed from the system after simulation of the mouth followed by the stomach compartments and the sample known as 'stomach & intestine' represented the extraction solution removed after simulation of the mouth, stomach and intestine phases of the system. The *in vitro* extraction procedure was conducted according to the UBM SOP detailed in Appendix 1. To ensure standardisation of the procedure and reduction in uncertainty estimates, all equipment and reagents were sourced according to Tables 1 and 2 of the SOP (Appendix 1). Where a contributing laboratory was unable to obtain suitable reagents, the lead laboratory satisfied the requirement by supplying said laboratory. In order to facilitate the analytical requirements of the inter-laboratory trial, the lead laboratory provided Sarstedt polypropylene tubes with screw top lids (catalogue number 60.541.545, 101 x 16.5mm, with a capacity of 13 ml). The tubes were selected for a number of reasons including:

- their ability to withstand a 70°C hot-block digestion for 3 hours (tested by the lead laboratory);
- the leak-free status of the screw tops, a requirement because of the long distance transportation required for some samples;
- the tubes were the correct size to fit the dedicated auto-sampler associated with the BGS Inductively Couple Plasma Atomic Emission Spectrometer (ICP-AES), the method chosen for the measurement of contaminant bioaccessibility.

This enabled the samples to be digested (see section 3.4) and analysed (see section 4) thereby avoiding analyte loss during sample transfer stages and reduction in the staff time required to carry out the bioaccessibility determinations.

The lead laboratory provided individual 100 mg l^{-1} spiking solutions of As, Cd and Pb to all laboratories with instructions for the inclusion of a mixed spiking solution in the evaluation of the UBM *in vitro* test.

The laboratory from Ohio State University saved an aliquot of each individual extract and carried out their 'in-house' analysis for comparison as described in section 4.3.

Two of the contributing laboratories (RIVM and RMC) extracted an additional aliquot of the As contaminated test materials provided by Professor Nick Basta to evaluate the UBM at a second solid:liquid ratio, 1:1000, as is standard in the RIVM protocol. The RIVM provided the lead laboratory with extraction solutions for digestion and analysis of As, Cd and Pb, in line with the main inter-laboratory exercise. The RMC carried out the analysis 'in-house' and provided the

lead laboratory with the resulting As data. A description of the analytical methodology undertaken by RMC is described in section 4.4.

In addition to the standard operating conditions the BGS carried out the 'stomach & intestine' phase of the UBM at a reduced intestine phase residence time in order to investigate the potential for reducing the extraction time required for the procedure.

On receipt from the individual participating laboratories all batches of sample extracts were stored at 1-8°C prior to further analytical preparation and analysis. Each batch of samples was individually recorded or 'booked in' to the BGS laboratory analysis system and given uniquely identifiable sample numbers for traceability. All stages of sample preparation and analysis, data checking and reporting were tracked and monitored to ensure that the quality of the data produced was maintained and that data were reported within the required timescale.

On completion of the analysis the data was reported to the contributing laboratory in the form of a 'QA return' and checked for inconsistencies and errors prior to the writing of this report.

3.2 QUALITY CONTROL SAMPLES

At the request of the lead laboratory, duplicate extractions of each test material were carried out; however this was not possible for all laboratories. Duplicates were included within the inter-laboratory trial to gain an insight into the within-laboratory repeatability. Within each batch of sample extractions a number of reference materials, blank and spiking quality control (QC) samples were also extracted and the subsequent extracts submitted for analysis. The reference material QC samples were either the NIST 2710 or 2711 reference material (or both where possible) and the BGS 102 As bioaccessibility guidance soil. The blank QCs consisted of the individual 'stomach' or 'stomach & intestine' matrix being taken through the entire UBM *in vitro* procedure, prior to submission for analysis, to account for As, Cd and Pb contamination from the chemicals and the extraction equipment in use. Finally, spiking solutions consisting of a mixture of As, Cd and Pb were extracted with no test material present to check the percentage (%) recovery of the extraction method, i.e. that no analyte was adsorbed to the extraction tubes or lost during the extraction procedure. Instructions for the spiking procedure, provided to each participating laboratory in order that the solution concentration after preservation was 0.1 mg l^{-1} (within the calibration range of the ICP-AES used for detection purposes) and to ensure consistency of application within the laboratories, were as follows;

- 'Stomach' Extract: Add 0.25 ml of each 100 mg l^{-1} solution provided by BGS to the extraction vessel and carry out the UBM with no soil present.

- 'Stomach & Intestine' Extract: Add 0.6 ml of each 100 mg l^{-1} solution provided by BGS to the extraction vessel and carry out the UBM with no soil present.

3.3 PRESERVATION

For preservation purposes it was requested that all sample extracts, were diluted by pipette to a ratio of 1:10 in 0.1 M HNO_3 prior to analysis by ICP-AES. As the bioaccessible metal content of BGS 102 is relatively low compared to the *in vivo* validation soils, contributing laboratories were requested to provide an additional 10.0 ml aliquot of unpreserved and therefore undiluted extract for analysis. Where undiluted extract material for BGS 102 was provided the analysis was undertaken by ICP-AES, where only preserved sample was provided the analysis was undertaken using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) because of the improved detection limit capability of this instrumentation.

3.4 BIOACCESSIBILITY DIGESTION

Concerns over sample degradation resulting from delays in sample transit, temperature fluctuations and possible sample scheduling problems prompted the adoption of the previously used BARGE technique of the acid digestion of samples prior to analysis (Van de Wiele et al., 2007). Samples were digested in the polypropylene screw cap centrifuge tubes supplied to the participating laboratories on arrival at the lead laboratory in order to re-solubilise any analytes sorbed to the surface of the containers during transit and storage. In addition this process ensured that all samples were of the same acidic matrix for analysis, regardless of minor operational differences applied in the individual laboratories. The acid digestion was carried out according to the following procedure;

- Within a given batch of samples, both the tubes and caps were labelled 1……X+1 in order that after uncapping, to enable each cap to be matched to its original tube, avoiding any opportunity for cross contamination.
- To each digestion tube 1.0 ml of AristaR grade ® c.HNO_3 and 1.0 ml of 70 % $^{v}/_{v}$ H_2O_2 were added by auto-pipette.
- Each tube was loosely capped, and the tubes placed in a temperature controlled hot-block for 3 hours at 70 °C
- After 3 hours, the samples were left to cool, removed and capped tightly.
- All samples were stored at 1 – 8 °C prior to analysis.

4 Instrumental Analysis

4.1 INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY (ICP-AES)

The extracted elements were determined directly by a Varian/Vista AX CCD simultaneous instrument with dedicated Varian SPS-5 Auto-sampler and PC running the latest version of ICP Expert software supplied by the instrument manufacturer. The instrument views the plasma along its axis, was equipped with a free running air-cooled 40.68 MHz RF generator and a high resolution Echelle polychromator with a Vista Chip^{TM} image mapped Charge Coupled Device (CCD) solid-state detector which covered a wavelength range of 167 to 785 nm. Each sample was introduced with 1% caesium chloride via a peristaltic pump into a glass concentric slurry nebuliser connected to a cyclonic action spray chamber. The operating conditions of the ICP-AES are summarised in Table 4.

Table 4 Varian Vista Standard Operating Conditions (Cave and Wragg, 2002)

Parameter	Typical Operating Conditions
Forward Power	1.3 kW
Coolant Gas Flow	18 l min^{-1}
Auxiliary Gas Flow	1.5 l min^{-1}
Nebuliser Gas Flow	0.75 l min^{-1}
Integration Time	Average of 3 x 10 s measurements
Pump Speed	15 rpm for 1.01 mm bore sample intake tube
Sample Uptake Rate	1 ml min^{-1}
Rinse Time	10 s
Uptake Delay	30 s
Stabilisation Delay	15 s

Analysis was carried out on c.2.5 ml of the UBM digested ‘stomach’ or ‘stomach & intestine’ extraction solution. Arsenic, Cd and Pb were determined after calibration using a minimum of 5 mixed element standards in a 1% HNO_3 matrix. The calibration standards covered concentrations up to 100 mg l^{-1} for As and Pb and 10 mg l^{-1} for Cd. The instrument was re-calibrated to analyse the samples from each contributing laboratory and after not more than 125 unknown samples. Two quality control standards, high and low, were analysed after each calibration, after no more than ten unknown solutions during the run and at the end of each run to check for drift. As the UBM bioaccessibility matrix had been diluted to a ratio of 1:10 with

0.1M HNO_3 prior to shipping, and broken down by mixed HNO_3/H_2O_2 digestion on arrival at the BGS (see section 3.4) no further matching to the 1% HNO_3 matrix of calibration or QC standards was deemed necessary. All reported measurements, as mg l^{-1}, were based on the average of three 10 second replicate analyses. All element concentrations quoted in this report have been converted into mg kg^{-1} extracted from the solid.

4.2 INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

ICP-MS was used to determine the As, Cd and Pb concentrations in the extracts of preserved BGS 102 where only a preserved aliquot was supplied to the lead laboratory. The analysis was carried out using a Thermo Elemental ExCell quadrupole ICP-MS instrument (serial number 123) in combination with a Cetac ASX-510 autosampler. A summary of typical instrument operating conditions for the analysis of aqueous solutions is shown in Table 5.

Table 5 Summary of ICP-MS instrumental and analytical parameters

Parameter	Typical operating conditions
Forward power	1350 W
Coolant gas flow	13 l min^{-1}
Auxiliary gas flow	0.8 – 0.9 l min^{-1}
Nebuliser gas flow	0.95 l min^{-1}
Integration time	3 x 30 sec
Nebuliser	Glass Expansion concentric
Spray chamber	Impact bead
Spray chamber temperature	3°C
Mode of acquisition	Scanning
Type of detector	Split dynode electron multiplier
Detector mode	Pulse counting

The instrument was calibrated at the beginning of each analytical run using standards prepared from certified Claritas PPT® (Spex CertiPreP) multi-element solutions in the range 0 to 50 µg l^{-1}. In addition, 10 µg l^{-1} mixed element standards were inserted at regular intervals throughout the analysis run and used to correct for any drift in instrument sensitivity. Indium and rhenium were added to all solutions via a T-piece connection and used as internal standards to correct for any matrix suppression. Multi-element QC standards containing As, Cd and Pb were analysed after at most every 20 samples. All of the samples were diluted by a factor of two with 1% nitric acid prior to analysis, as the volume of solution was limited for some samples.

4.3 OHIO 'IN-HOUSE' ICP-AES ANALYSIS

A high resolution Varian Vista MPX Simultaneous ICP-AES with SPS-5 Auto Sampler was used by Ohio State University to analyse a second aliquot of their own bioaccessibility extracts for As, Cd and Pb. The instrument was calibrated using at least four calibration standards (including the calibration blank), and the linear calibration of each element of interest was required to meet the minimum r^2 criteria of 0.995. An initial calibration verification (ICV) QC standard (SPEX CertiPrep Group LPC standard 1) was analysed as supplied immediately after instrument calibration. Continuing calibration verification (CCV) QC standards, prepared by diluting the ICV QC standard by a factor of 10, were run after every 10 unknown samples. An yttrium internal standard was used to correct for Cd suppression caused by salt effects. All calibration standards, check standards and Fisher QC standards were prepared in a 0.01 M NaCl solution in order to approximate the sample matrix. Counts of the internal standard in blank extraction solutions ('stomach' and 'stomach & intestine') and the NaCl solution were within 10%.

4.4 RMC 'IN-HOUSE' ICP-MS ANALYSIS OF 1:1000 EXTRACTS

The UBM extracts for the soil:solution ration of 1:1000, produced by RMC, were analysed according to analytical procedures routinely adopted by RMC. The bioaccessible As content of each extract was measured (at mass to charge (m/z) ratio 75) using a Thermo X7-X series ICP-MS with quadrupole detector fitted with a standard ICP torch. The instrument was calibrated across the range 0 to 1000 µg l^{-1} using a minimum of five multi-element standards and re-calibrated after no more than 48 unknown samples. Quality control samples, at 25% and 75% of the calibration range, were analysed at the beginning and end of each analytical run and after no more than 12 unknown samples. All standards, QC samples and bioaccessibility extracts were matrix matched in 2% nitric acid prior to undertaking the analysis. Corrections for plasma drift and matrix effects were assisted by the inclusion of indium (m/z 115) and scandium (m/z 45) as internal standards.

4.5 QUALITY CONTROL

Each participating laboratory supplied a minimum of three blank extraction samples from each of the UBM phases, under the standard (1:100) extraction conditions, for analysis. All of the blank extracts for the stomach phase returned values below the reporting limit for As (<6.75 mg kg^{-1}), with the exception of one sample from one laboratory where the Cd value was within 2 times the reporting limit (<0.90 mg kg^{-1}), and two samples for a second laboratory where the Pb values were within three times the reporting limit for Pb (<0.225 mg kg^{-1}). For the 'stomach & intestine phase' all of the blank extraction samples returned values less than the intestinal reporting limit for As (<17.6 mg kg^{-1}), Cd (<2.34 mg kg^{-1}) and Pb (<5.85 mg kg^{-1}). All of the

test sample extraction data for As, Cd and Pb were above the reporting limits for both the 'stomach' and 'stomach & intestine' phases. Where ICP-AES values for test sample extractions were returned below the reporting limit, the samples were re-analysed by ICP-MS for its increased sensitivity and reciprocal lower reporting limits. The data from the extraction blanks provides a good indication that the reagents or equipment used in the UBM methodology did not contribute As, Cd or Pb to the sample data.

For the additional UBM extraction carried out at the increased soil:solution ratio of 1:1000, the RIVM provided the BGS laboratories with two blank extracts and the RMC provided data for two blank extracts for the 'stomach' and the 'stomach & intestine' phases. In all cases the resulting data for As (RIVM and RMC), Cd and Pb (RIVM only) was below the reporting limit of their methods.

All of the participating laboratories provided a minimum of two mixed element spike extracts for each phase of the UBM, under the standard (1:100) extraction conditions, for analysis. Exceptions to this were the University of Ghent, who were unable to provide any extraction spikes, and the RIVM laboratory that provided separate, Cd and Pb spike extracts for each phase. Table 6 summarises the mean As, Cd and Pb spike concentration values in each of the two UBM phases across the participating laboratories and the associated standard deviation for the As, Cd and Pb spike extractions for each laboratory. The As recovery for the spike samples extracted by Ohio and analysed by the BGS was erroneously low in comparison to those provided by the other participating laboratories and Ohio's own 'in-house' analysis of an aliquot of the same extraction samples. As a result, all further Ohio sample data quoted in this report is that determined by Ohio and not by the BGS. A summary of the range of mean As, Cd and Pb spiking concentrations is shown in Figure 1. Figure 1 shows that there is a wider spread of data in the 'stomach & intestine' phase of the UBM for the As, Cd and Pb spiking solution extractions. Table 6 shows that for the 'stomach' phase the As, Cd and Pb % recovery was 100 ± 15 % for all participating laboratories. For the 'stomach & intestine' phase of the UBM, Table 6 shows the As recovery data to be 100 ± 10% for four laboratories and 100 ± 20 % for the remaining two, Cd recovery data to be 100 ± 10% for five out of the six laboratories who returned spiking solutions, with one laboratory returning data 100 ± 20%, and Pb recovery data to be poor by comparison. The percentage spike recovery for Pb in the 'stomach & intestine' phase ranges from c.55 to 105% (Table 6), the variability is thought to be a function of the solubility of Pb at neutral pH and within laboratory variability.

For the extractions carried out at the increased soil:solution ratio, As spike recoveries of 100 and 99% for the 'stomach' and 'stomach & intestine' phases respectively were returned.

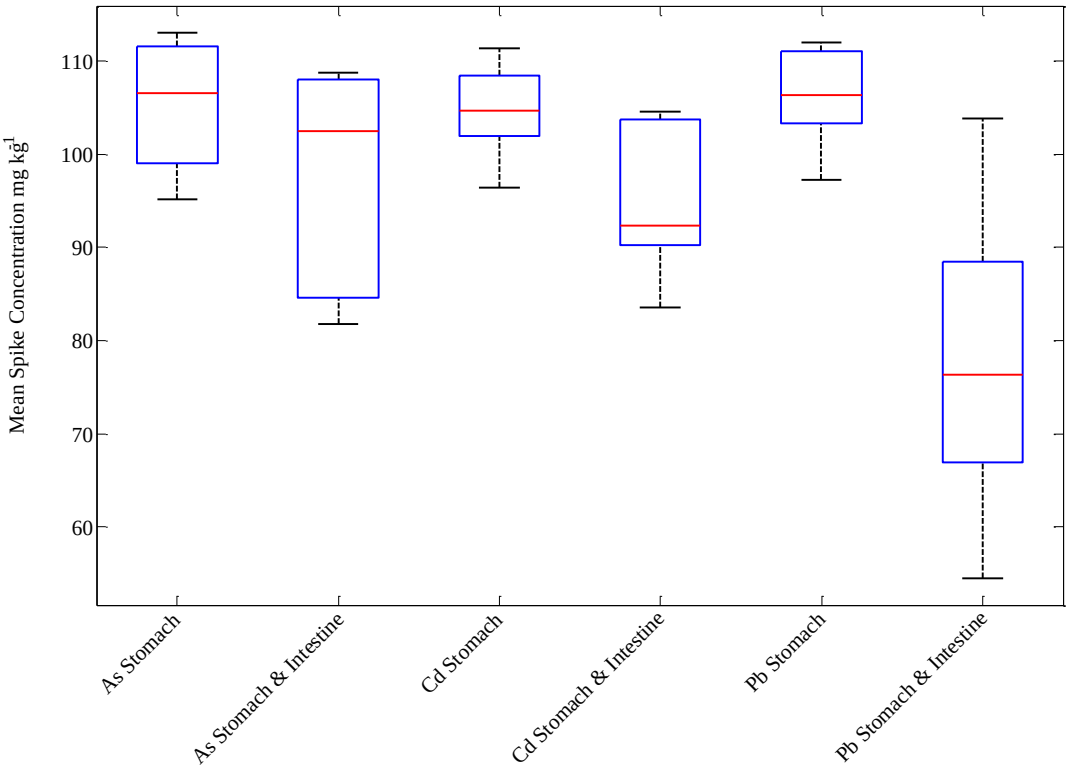


Figure 1 Summary of mean As, Cd and Pb spiking concentrations in both phases of the UBM

Table 6 Summary of Spike Data for 'Stomach' and 'Stomach & Intestine' Phases

Laboratory	BGS	DHI	INERIS	OHIO	RIVM	RMC
Mean Stomach As (mg kg^{-1}) and recovery (%)	104	109	112	113	95.2	99.1
Stomach As SD (mg kg^{-1})	7.26	4.37	4.39	2.37	1.62	2.60
Mean Stomach & Intestine As (mg kg^{-1}) and recovery (%)	102	103	109	108	94.0	82.0
Stomach &Intestine As SD (mg kg^{-1})	5.40	2.71	3.90	0.296	0.00	7.19
Mean Stomach Cd (mg kg^{-1}) and recovery (%)	102	106	108	111	96.5	104
Stomach Cd SD (mg kg^{-1})	3.27	0.148	5.93	0.372	0.403	3.54
Mean Stomach & Intestine Cd (mg kg^{-1}) and recovery (%)	92.0	90.2	104	105	93.0	84.0
Stomach & Intestine Cd SD (mg kg^{-1})	0.800	0.881	4.30	2.35	1.00	7.74
Mean Stomach Pb (mg kg^{-1}) and recovery (%)	104	108	111	112	97.3	103
Stomach Pb SD (mg kg^{-1})	2.37	1.68	5.84	1.07	6.83	1.05
Mean Stomach & Intestine Pb (mg kg^{-1}) and recovery (%)	71.0	54.5	88.0	104	81.0	67.0
Stomach & Intestine Pb SD (mg kg^{-1})	3.70	2.28	38.0	0.688	0.00	7.30

SD denotes standard deviation

5 Statistical Data Analysis

The main analytical performance characteristics of the bioaccessibility measurement (repeatability and reproducibility) were determined in the collaborative study using the procedure described in ISO Standard 5725-2 (ISO).

5.1 STATISTICAL METHOD

The raw data from the analysis of the samples produced by the inter-laboratory trial were arranged into Forms A, B and C as described in the standard and summarised in Appendices 2-45.

5.2 MANDEL STATISTICS

The Mandel statistics and plots were calculated from the summary tables using the MATLAB® programming language. The blue and red lines on the plots represent the 90^{th} and 99^{th} percent confidence limits respectively on the calculated 'h' and 'k' statistics (from tables in ISO Standard 5725-2). The 'h' statistic plots the between-laboratory consistency and the 'k' statistic plots the within-laboratory consistency.

Examination of the h and k plots may indicate that specific laboratories exhibit patterns of results that are markedly different from the others in the study. This is indicated by consistently high or low within-cell variation and/or extreme cell means across many levels.

Various patterns can appear in the h plots. All laboratories can have both positive and negative h values for different soil samples. Individual laboratories may tend to give either all positive or all negative h values, and the number of laboratories giving negative values is approximately equal to those giving positive values. Neither of these patterns is unusual or requires investigation, although the second of these patterns may suggest that a common source of laboratory bias exists. On the other hand, if all the h values for one laboratory are of one sign and the h values for the other laboratories are all of the other sign, then this should be investigated. Likewise, if the h values for a laboratory are extreme and appear to depend on the soil sample being tested in some systematic way, then the reason should be sought.

If one laboratory stands out on the k plot as having many large values, then the reason this should be investigated: this indicates that it has a poorer repeatability than the other laboratories. A laboratory could give rise to consistently small k values because of such factors as excessive rounding of its data or an insensitive measurement scale.

5.3 OUTLIER TESTS

Outlier testing using Grubbs' test (Grubbs, 1950) and Cochran's test (Snedecor and Cochran, 1980) were carried out in the R statistical programming language (R Development Core Team, 2007). The 'stragglers' and 'outliers' identified by these tests are shown in Tables B and C in the Appendices. The outlier removal rules specified in ISO Standard 5725-2 were applied.

5.4 ADDITIONAL STATISTICS

The final repeatability and reproducibility calculations were carried out in MS Excel™. In addition to the ISO 5725-2 statistical calculations, the final bioaccessibility data is plotted against *in vivo* bioavailability data to show the relationship between the *in vitro* and the *in vivo* measurements. Linear trends were calculated using Theil's robust method (Glaister, 2005) which does not assume that all the errors are in the y direction or that the x or y errors are normally distributed, and it is not affected by the presence of outlying results. Confidence intervals on the linear trend were calculated using a Monte Carlo simulation (10000 trials) based on the between-laboratory mean and its associated standard deviation of the bioaccessibility measurement calculated using ISO Standard 5725-2 statistics.

For the comparison between the 3 and 4 hour intestine residence times a paired 't' and a non-parametric bootstrap test were used to assess if the effect of time was significant.

6 Results

6.1 UBM AT THE STANDARD SOIL: SOLUTION RATIO (1:100)

6.1.1 As soils

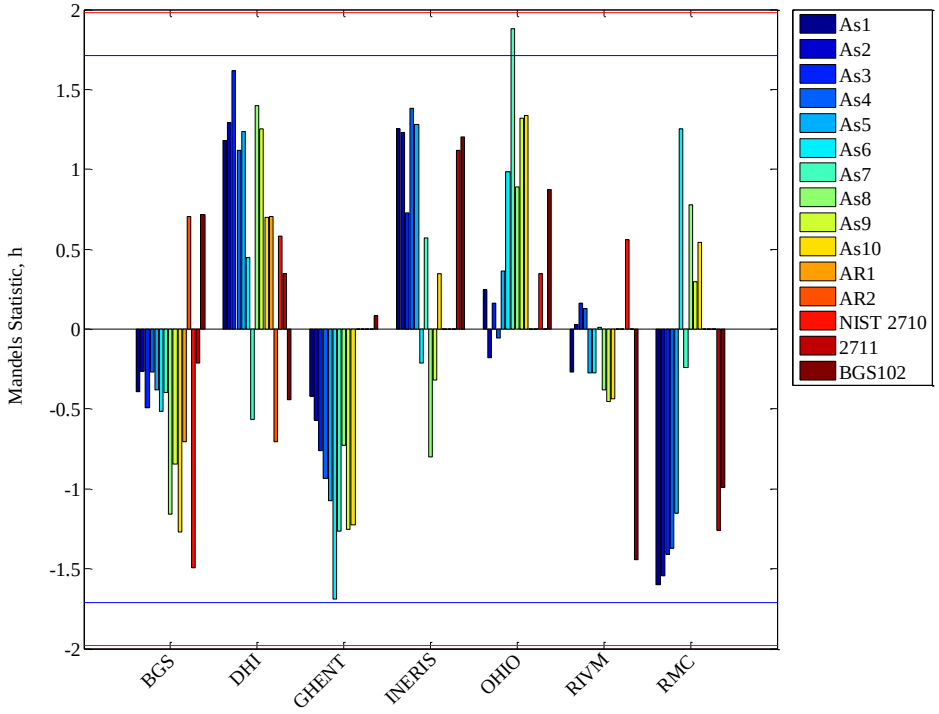


Figure 2 Mandels Statistic h, 'stomach' data for As soils

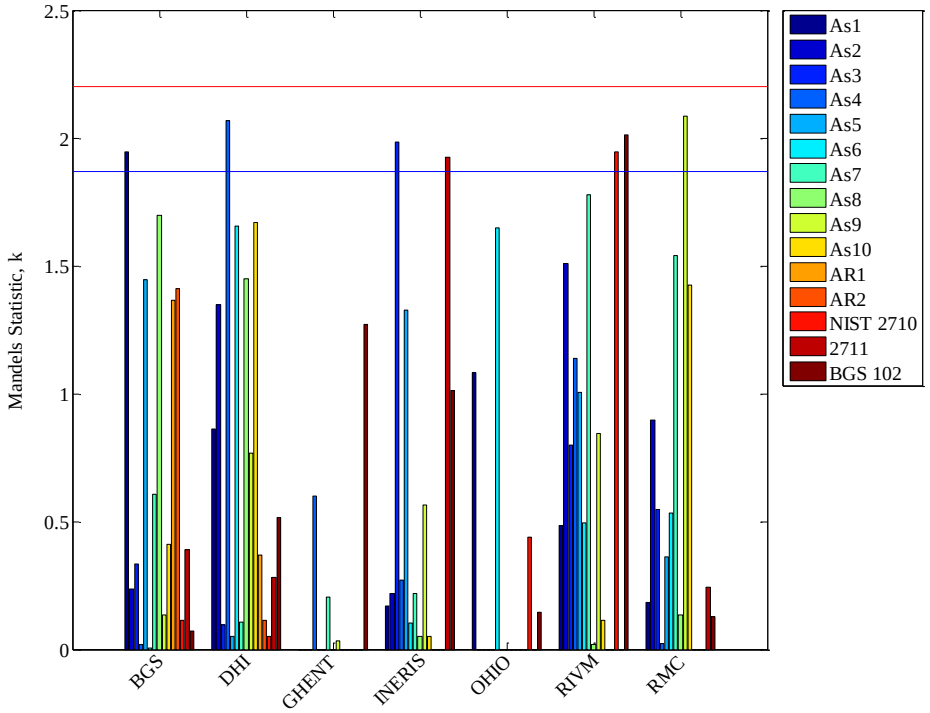


Figure 3 Mandels Statistic k, 'stomach' data for As soils

Table 7 Summary ISO Statistics for As soils 'stomach' data (mg kg^{-1})

Sample Name	**As 1**	**As 2**	**As 3**	**As 4**	**As 5**	**As 6**	**As 7**	**As 8**	**As 9**	**As 10**	**AR 1**	**AR 2**	**NIST 2710**	**NIST 2711**	**BGS 102**
Number of Data points	7	7	7	7	7	7	7	7	6	7	2	2	4	4	7
Overall mean	176	200	316	544	388	93.3	110	306	1137	781	68.7	76.6	323	55.1	4.52
Overall median	165	191	331	534	364	85.4	96.8	219	906	872	68.7	76.6	343	55.5	4.60
Repeatability variance	24.4	56.3	377	1171	152	197	39.5	422	1449	4483	6.29	202	319	3.16	1.46
Between laboratory variance	1637	2302	8306	28075	7359	1287	3079	51986	552701	65826	13.7	858	1681	33.6	0.169
Reproducibility variance	1662	2359	8682	29246	7511	1483	3118	52408	554150	70310	20.0	1060	2000	36.7	1.63
Repeatability SD	4.94	7.50	19.4	34.2	12.3	14.0	6.29	20.5	38.1	67.0	2.51	14.2	17.9	1.78	1.21
Between laboratory SD	40.5	48.0	91.1	168	85.8	35.9	55.5	228	743	257	3.70	29.3	41.0	5.80	0.411
Reproducibility SD	40.8	48.6	93.2	171	86.7	38.5	55.8	229	744	265	4.47	32.6	44.7	6.06	1.28
Reproducibility MAD	27.3	24.7	78.0	192.4	89.7	32.0	23.27	230	590	267	3.77	28.5	6.50	5.14	0.975
SD/mean	0.232	0.243	0.295	0.314	0.224	0.413	0.506	0.747	0.655	0.340	0.065	0.425	0.138	0.110	0.282
% RSD	23.2	24.3	29.5	31.4	22.4	41.3	50.6	74.7	65.5	34.0	6.50	42.5	13.8	11.0	28.2
% RSD MAD	16.5	12.9	23.6	36.0	24.6	37.4	24.0	105	65.1	30.6	5.49	37.2	1.90	9.26	21.2

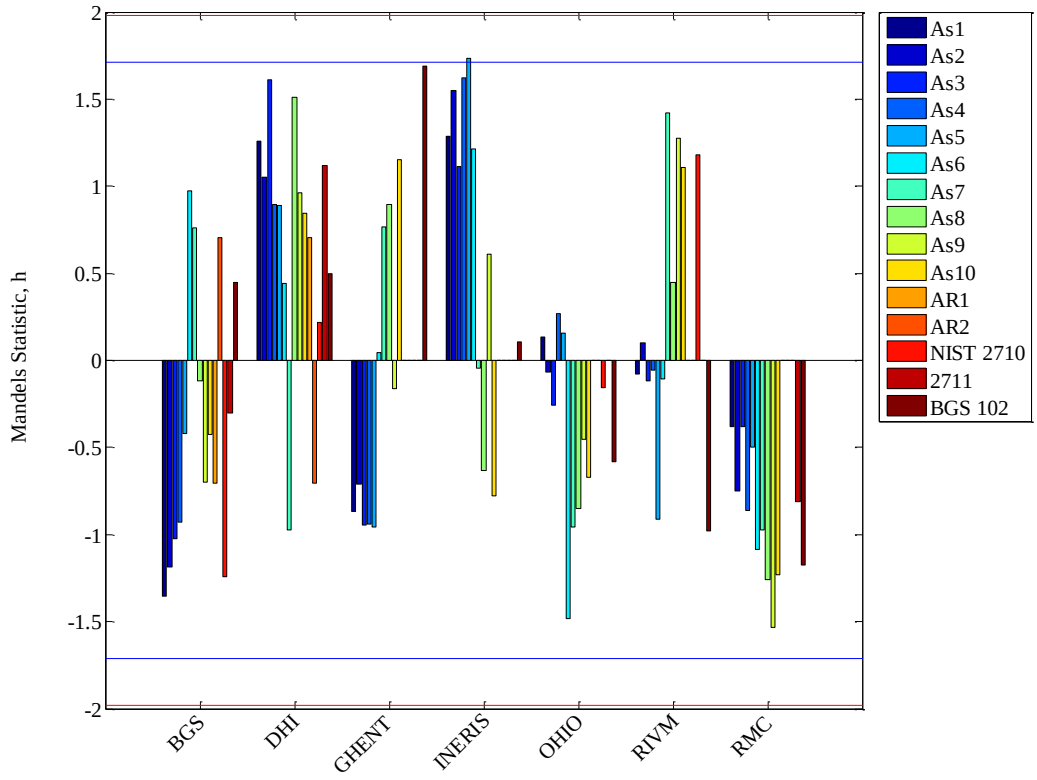


Figure 4 Mandels Statistic h, 'stomach & intestine' data for As soils

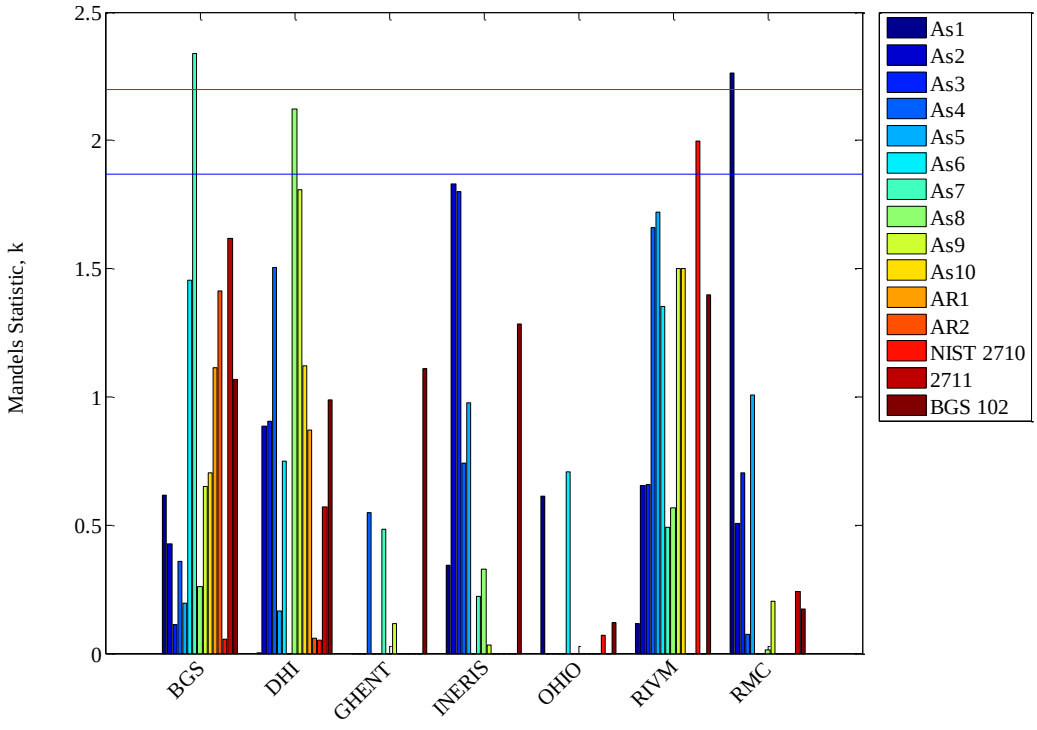


Figure 5 Mandels Statistic k, 'stomach & intestine' data for As soils

Table 8 Summary ISO Statistics for As soils 'stomach & intestine' data (mg kg^{-1})

Sample Name	**As 1**	**As 2**	**As 3**	**As 4**	**As 5**	**As 6**	**As 7**	**As 8**	**As 9**	**As 10**	**AR 1**	**AR 2**	**NIST 2710**	**NIST 2711**	**BGS 102**
Number of Data points	7	7	7	7	7	7	6	7	7	7	2	2	3	3	7
Overall mean	188	209	318	543	330	30.9	27.3	58.0	354	271	41.9	55.2	264	45.6	5.38
Overall median	185	206	298	535	302	31.8	19.3	53.4	318	181	41.9	55.2	270	42.7	5.58
Repeatability variance	142	189	484	251	126	5.45	3.87	216	1916	332	14.7	3.95	2.80	15.2	3.69
Between laboratory variance	1013	2232	5513	19597	4454	405	496	1415	47685	45339	5.35	421	329	85.3	2.03
Reproducibility variance	1155	2421	5997	19848	4579	411	500	1631	49601	45671	20.0	425	332	101	5.71
Repeatability SD	11.9	13.8	22.0	15.8	11.2	2.33	1.97	14.7	43.8	18.2	3.83	1.99	1.67	3.90	1.92
Between laboratory SD	31.8	47.2	74.2	140	66.7	20.1	22.3	37.6	218	213	2.31	20.5	18.1	9.24	1.42
Reproducibility SD	34.0	49.2	77.4	141	67.7	20.3	22.4	40.4	223	214	4.48	20.6	18.2	10.0	2.39
Reproducibility MAD	33.8	42.9	67.6	159	46.8	24.4	13.3	37.1	222	224	1.30	18.9	11.7	6.37	1.76
SD/mean	0.181	0.235	0.244	0.259	0.205	0.655	0.818	0.696	0.629	0.788	0.107	0.374	0.069	0.220	0.445
% RSD	18.1	23.5	24.4	25.9	20.5	65.5	81.8	69.6	62.9	78.8	10.7	37.4	6.89	22.0	44.5
% RSD MAD	18.3	20.8	22.7	29.6	15.5	76.9	69.2	69.4	69.9	124	3.10	34.3	4.33	14.9	31.5

6.1.2 Cd soils

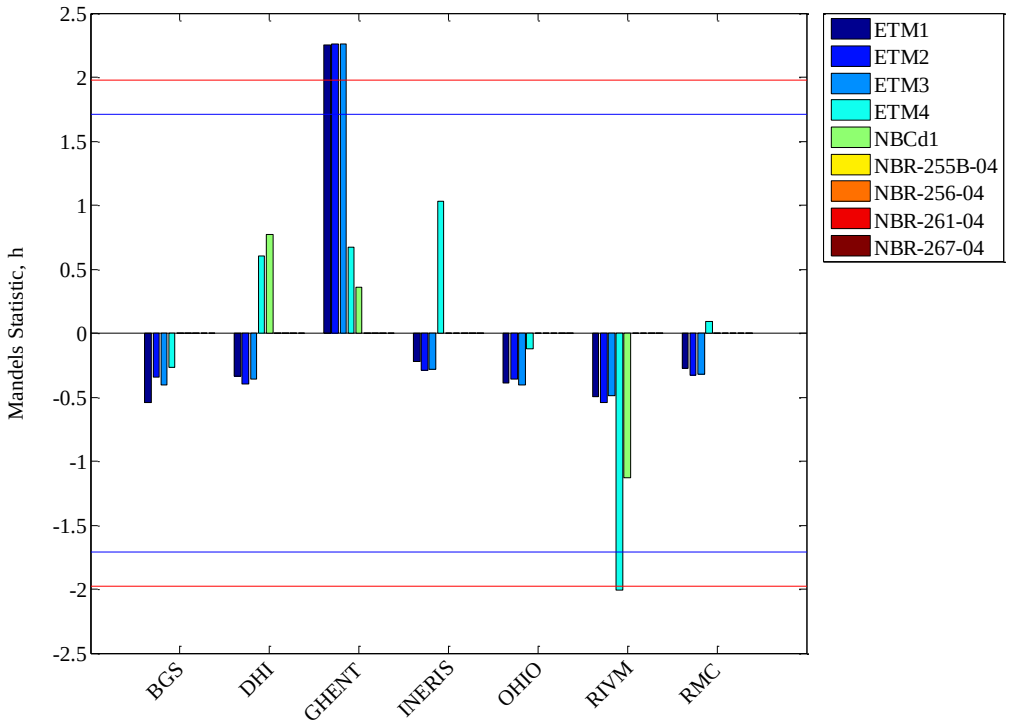


Figure 6 Mandels Statistic h, 'stomach' data for Cd soils

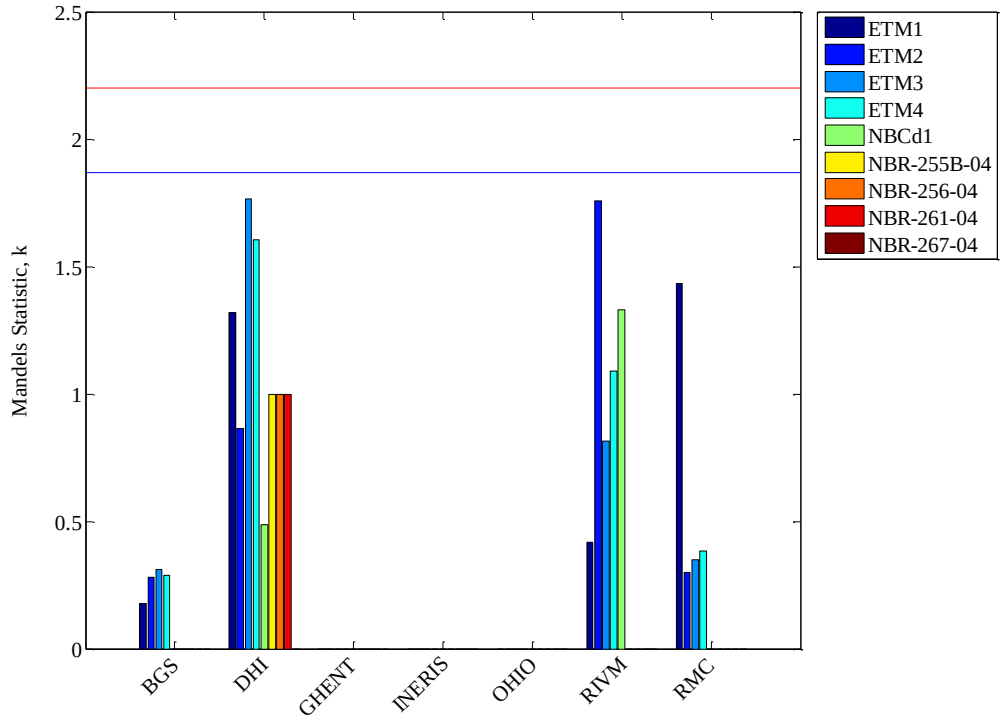


Figure 7 Mandels Statistic k, 'stomach' data for Cd soils

Table 9 Summary ISO Statistics for Cd soils 'stomach' data (mg kg^{-1})

Sample Name	ETM 1	ETM 2	ETM 3	ETM 4	NB Cd 1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	NIST 2710	NIST 2711	BGS 102
Number of Data points	6	6	6	7	3	1	1	1	1	4	4	7
Overall mean	3480	379	82.8	16.5	309	89.2	13.4	10.2	16.5	14.8	33.8	0.281
Overall median	3496	386	82.7	16.6	312	89.2	13.4	10.2	16.5	15.1	34.9	0.25
Repeatability variance	13907	159	13.4	0.049	296	12.0	0.168	0.039	0.000	0.747	0.758	0.007
Between laboratory variance	18651	476	9.21	1.88	93.5	n/d	n/d	n/d	n/d	0.442	9.00	0.022
Reproducibility variance	32558	634	22.6	1.93	389	n/d	n/d	n/d	n/d	1.19	9.76	0.029
Repeatability SD	118	12.6	3.66	0.220	17.2	3.46	0.410	0.198	0.000	0.864	0.870	0.084
Between laboratory SD	137	21.8	3.03	1.37	9.67	n/d	n/d	n/d	n/d	0.665	3.00	0.147
Reproducibility SD	180	25.2	4.75	1.39	19.7	n/d	n/d	n/d	n/d	1.09	3.12	0.170
Reproducibility MAD	183	11.7	2.99	0.91	3.90	n/d	n/d	n/d	n/d	0.585	0.975	0.104
SD/mean	0.052	0.066	0.057	0.084	0.064	0.000	0.000	0.000	0.000	0.074	0.092	0.603
% RSD	5.18	6.64	5.74	8.44	6.38	0.000	0.000	0.000	0.000	7.36	9.24	60.3
% RSD MAD	5.22	3.03	3.62	5.48	1.25	0.000	0.000	0.000	0.000	3.89	2.79	41.6

n/d denotes statistic not calculated

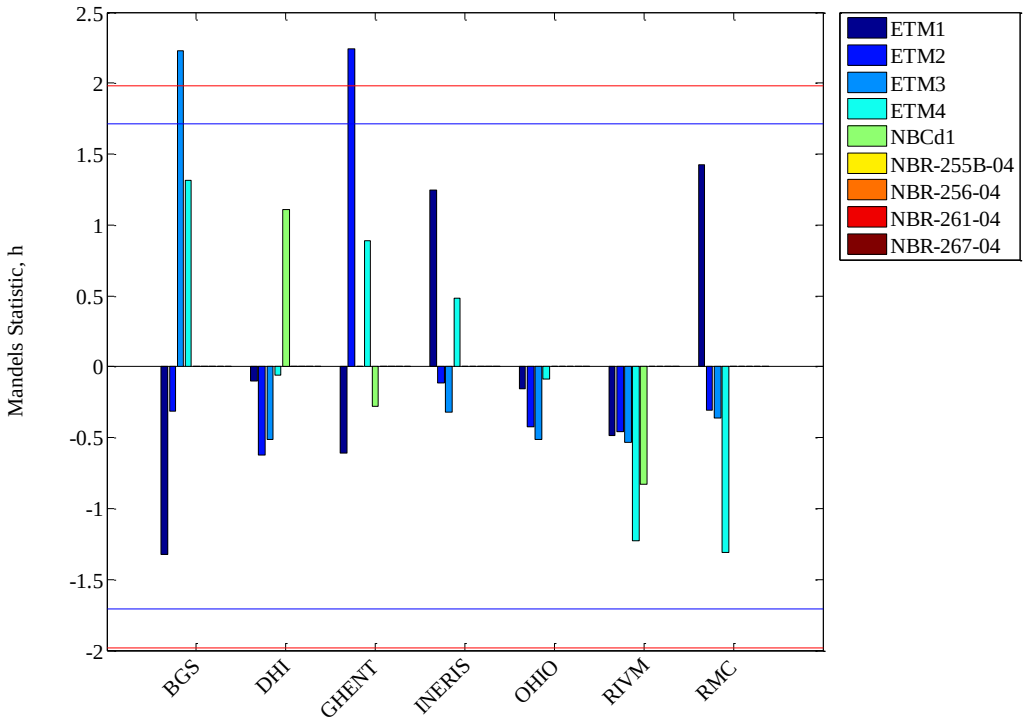


Figure 8 Mandels Statistic h, 'stomach & intestine' data for Cd soils

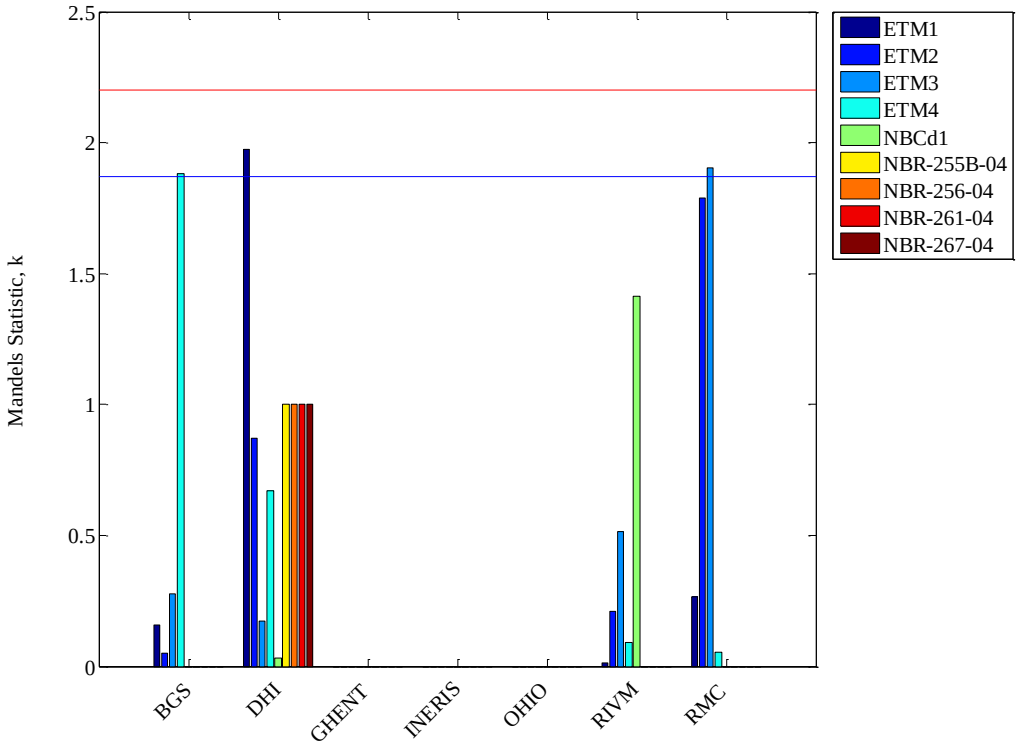


Figure 9 Mandels Statistic k, 'stomach & intestine' data for Cd soils

Table 10 Summary ISO Statistics for Cd soils 'stomach & intestine' data (mg kg^{-1})

Sample Name	ETM 1	ETM 2	ETM 3	ETM 4	NB Cd 1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	NIST 2710	NIST 2711	BGS 102
Number of Data points	6	6	6	7	3	1	1	1	1	4	3	7
Overall mean	1171	232	74.0	10.2	221	68.6	10.1	4.99	9.04	7.86	16.2	0.593
Overall median	905	234	63.3	10	219	68.6	10.1	4.99	9.04	7.73	18.8	0.64
Repeatability variance	323	209	67.8	8.37	109	1.14	0.039	0.274	0.005	2.90	1.36	0.076
Between laboratory variance	751165	3394	1183	9.55	2.63	n/d	n/d	n/d	n/d	0.360	20.9	0.203
Reproducibility variance	751489	3604	1251	17.9	112	n/d	n/d	n/d	n/d	3.26	22.3	0.2786
Repeatability SD	18	14.5	8.24	2.89	10.5	1.07	0.198	0.523	0.071	1.70	1.16	0.275
Between laboratory SD	867	58.3	34.4	3.09	1.62	n/d	n/d	n/d	n/d	0.600	4.57	0.450
Reproducibility SD	867	60.0	35.4	4.23	10.6	n/d	n/d	n/d	n/d	1.81	4.72	0.528
Reproducibility MAD	668	33.8	19.8	4.55	5.20	n/d	n/d	n/d	n/d	14.82	5.642	1.26
SD/mean	0.740	0.259	0.478	0.414	0.048	0.000	0.000	0.000	0.000	0.230	0.292	0.890
% RSD	74.0	25.9	47.8	41.4	4.79	0.000	0.000	0.000	0.000	23.0	29.2	89.0
% RSD MAD	73.8	14.4	31.2	45.5	2.37	0.000	0.000	0.000	0.000	192	30.0	197

n/d denotes statistic not calculated

6.1.3 Pb soils

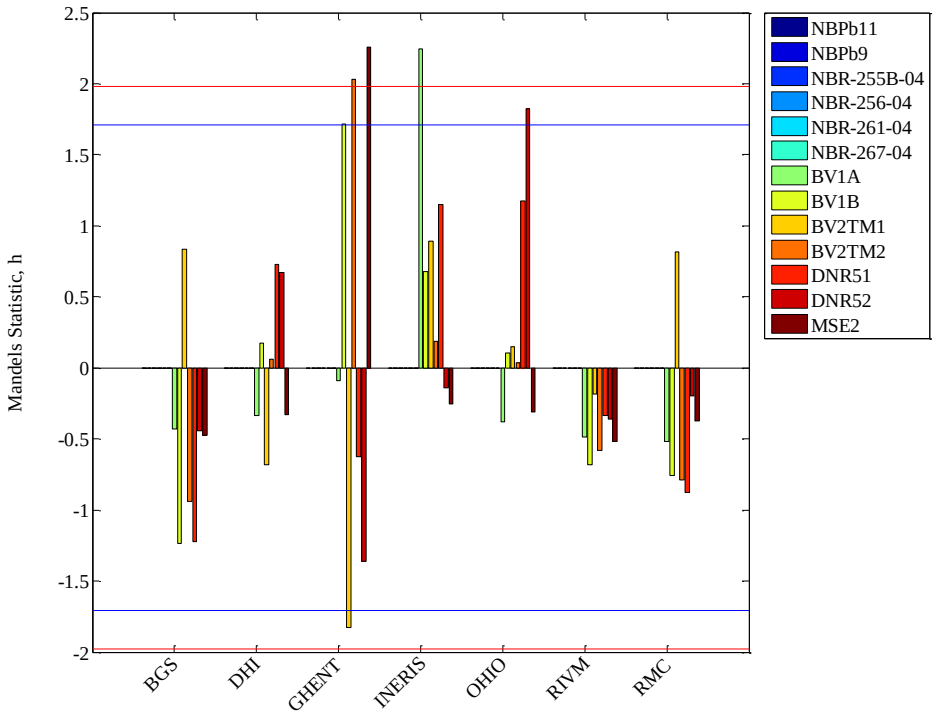


Figure 10 Mandels Statistic h, 'stomach' data for Pb soils

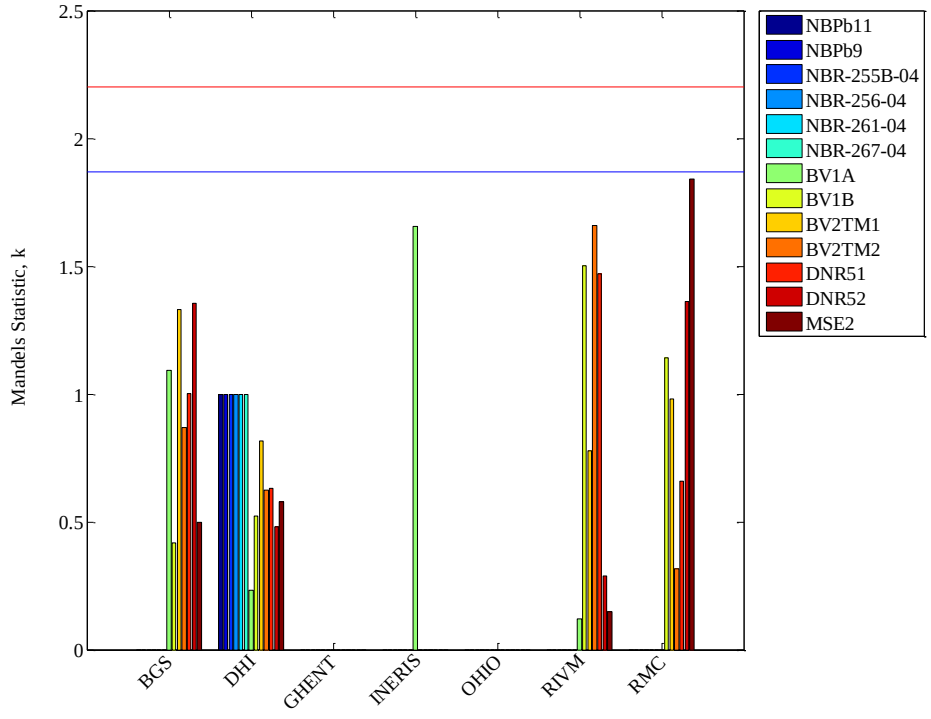


Figure 11 Mandels Statistic k, 'stomach' data for Pb soils

Table 11 Summary ISO Statistics for Pb soils 'stomach' data (mg kg^{-1})

Sample Name	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2TM1	B & V 2TM2	DNR5-1	DNR5-2	MSE 2	NIST 2710	NIST 2711	BGS 102
Number of Data points	1	1	1	1	1	1	6	7	7	7	7	7	6	4	3	6
Overall mean	46.4	96.8	467	788	881	112	1843	1364	1021	1953	1654	1926	1709	3785	958	12.8
Overall median	46.4	96.8	467	788	881	112	1789	1396	1084	1970	1462	1771	1742	3954	960	12.9
Repeatability variance	n/d	n/d	n/d	n/d	n/d	n/d	50270	1634	2106	538	2401	728	5206	44042	686	2.90
Between laboratory variance	n/d	n/d	n/d	n/d	n/d	n/d	40645	92275	176341	202966	330305	619775	12185	175709	122	32.9
Reproducibility variance	n/d	n/d	n/d	n/d	n/d	n/d	90915	93909	178448	203504	332706	620502	17391	219751	807	35.8
Repeatability SD	n/d	n/d	n/d	n/d	n/d	n/d	224	40.4	45.9	23.2	49	27	72.2	210	26.2	1.70
Between laboratory SD	n/d	n/d	n/d	n/d	n/d	n/d	202	304	420	451	575	787	110	419	11.0	5.74
Reproducibility SD	n/d	n/d	n/d	n/d	n/d	n/d	302	306	422	451	577	788	132	469	28.4	5.98
Reproducibility MAD	n/d	n/d	n/d	n/d	n/d	n/d	164	311	390	360	664	251	102	155	24.7	1.95
SD/mean	n/d	n/d	n/d	n/d	n/d	n/d	0.164	0.225	0.414	0.231	0.349	0.409	0.077	0.124	0.030	0.468
% RSD	n/d	n/d	n/d	n/d	n/d	n/d	16.4	22.5	41.4	23.1	34.9	40.9	7.72	12.4	2.96	46.8
% RSD MAD	n/d	n/d	n/d	n/d	n/d	n/d	9.16	22.3	36.0	18.3	45.4	14.2	5.86	3.91	2.57	15.1

n/d denotes statistic not calculated

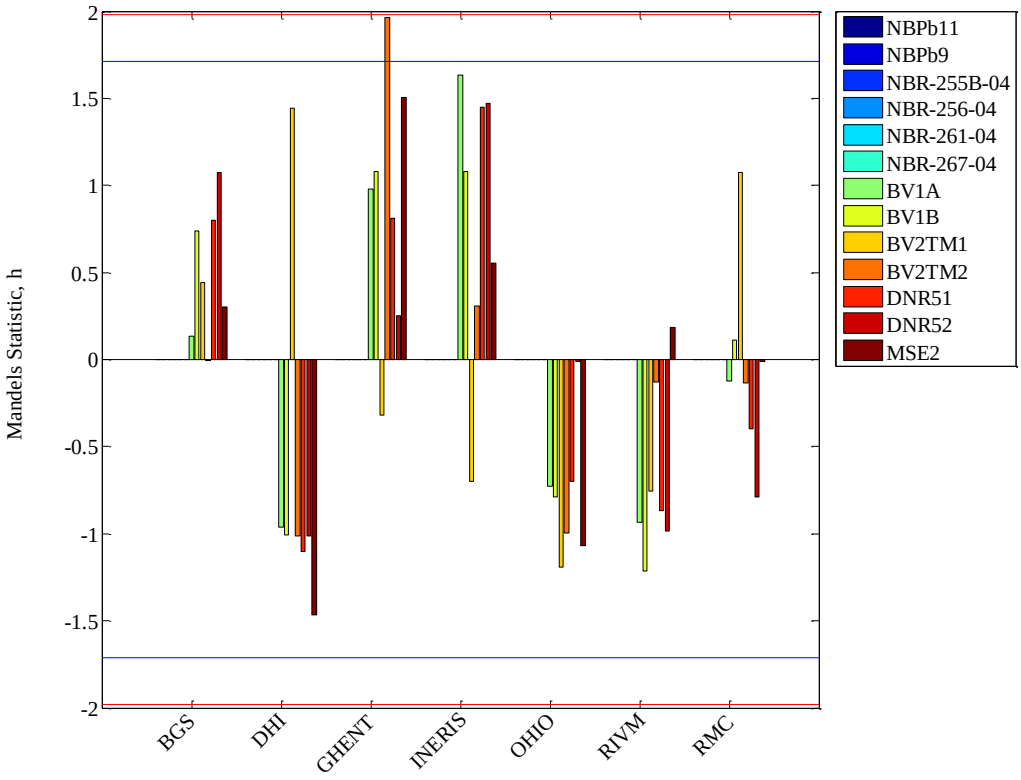


Figure 12 Mandels Statistic h, 'stomach & intestine' data for Pb soils

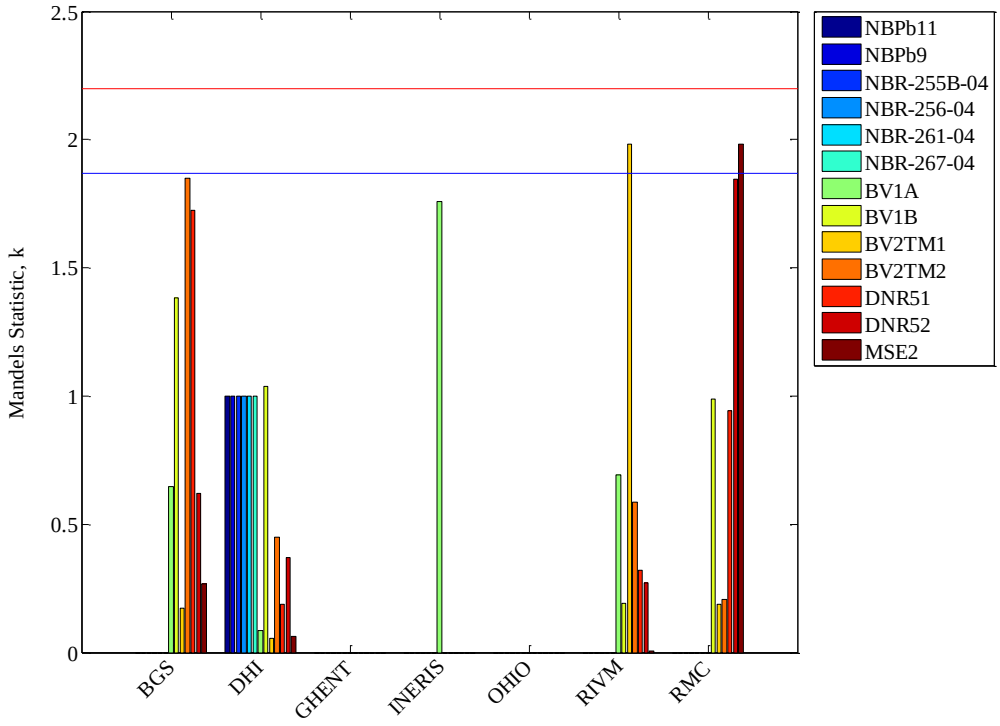


Figure 13 Mandels Statistic k, 'stomach & intestine' data for Pb soils

Table 12 Summary ISO Statistics for Pb soils 'stomach & intestine' data (mg kg^{-1})

Sample Name	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2TM1	B & V 2TM2	DNR5-1	DNR5-2	MSE 2	NIST 2710	NIST 2711	BGS 102
Number of Data points	1	1	1	1	1	1	7	7	6	7	7	7	6	4	4	7
Overall mean	61	83.1	325	656	5.04	105	960	460	308	863	555	423	710	1138	101	3.11
Overall median	61	83.1	325	656	5.04	105	839	499	301	795	379	418	820	1202	78.1	1.87
Repeatability variance	n/d	n/d	n/d	n/d	n/d	n/d	1532	3335	9.47	20708	27829	647	527	252756	1701	18.5
Between laboratory variance	n/d	n/d	n/d	n/d	n/d	n/d	957800	121602	11802	277522	183095	151425	249734	576621	7427	0.621
Reproducibility variance	n/d	n/d	n/d	n/d	n/d	n/d	959332	124937	11811	298230	210924	152073	250262	829377	9129	19.1
Repeatability SD	n/d	n/d	n/d	n/d	n/d	n/d	39	58	3.08	144	167	25	23	503	41.2	4.30
Between laboratory SD	n/d	n/d	n/d	n/d	n/d	n/d	979	349	109	527	428	389	500	759	86.2	0.788
Reproducibility SD	n/d	n/d	n/d	n/d	n/d	n/d	979	353	109	546	459	390	500	911	95.5	4.38
Reproducibility MAD	n/d	n/d	n/d	n/d	n/d	n/d	900	442	138	304	407	492	482	804	71.63	0.351
SD/mean	n/d	n/d	n/d	n/d	n/d	n/d	1.02	0.769	0.353	0.633	0.828	0.923	0.705	0.800	0.946	1.41
% RSD	n/d	n/d	n/d	n/d	n/d	n/d	102	76.9	35.3	63.3	82.8	92.3	70.5	80.0	94.6	141
% RSD MAD	n/d	n/d	n/d	n/d	n/d	n/d	107	88.6	45.8	38.3	107	118	58.8	66.9	91.7	18.8

n/d denotes statistic not calculated

6.2 UBM AT THE 1:1000 SOIL:SOLUTION RATIO

As only two laboratories (RIVM and RMC) carried out the 1:1000 ratio test no Mandel plots have been produced.

Table 13 Summary ISO Statistics for As soils 'stomach' data at 1:1000 ratio (mg kg^{-1})

Sample Name	**As 1**	**As 2**	**As 3**	**As 4**	**As 5**	**As 6**	**As 7**	**As 8**	**As 9**	**As 10**	**NIST 2710**
Number of Data points	2	2	2	2	1	2	2	2	2	2	2
Overall mean	209	240	328	606	534	171	209	457	1768	1067	335
Overall median	209	240	328	606	534	171	209	457	1768	1067	335
Repeatability variance	118	403	195	26.3	373	63.9	39.2	240	1021	1261	680
Between laboratory variance	183	21.0	515	251	n/d	28.5	3852	425	330	3882	255
Reproducibility variance	301	424	710	278	n/d	92.5	3892	664	1351	5143	935
Repeatability SD	10.9	20.1	14.0	5.13	19.3	7.99	6.26	15.5	32.0	35.5	26.1
Between laboratory SD	13.5	4.58	22.7	15.9	n/d	5.34	62.1	20.6	18.2	62.3	16.0
Reproducibility SD	17.3	20.6	26.6	16.7	n/d	9.62	62.4	25.8	36.8	71.7	30.6
Reproducibility MAD	272	311	426	787	n/d	222	272	593	2298	1386	435
SD/mean	0.083	0.086	0.081	0.028	n/d	0.056	0.298	0.056	0.021	0.067	0.091
% RSD	8.30	8.60	8.14	2.75	n/d	5.64	29.8	5.65	2.08	6.72	9.14
% RSD MAD	130	130	130	130	n/d	130	130	130	130	130	130

n/d denotes statistic not calculated

Table 14 Summary ISO Statistics for As soils 'stomach & intestine' data at 1:1000 ratio (mg kg^{-1})

Sample Name	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
Number of Data points	2	2	2	2	1	2	2	2	2	2	2
Overall mean	210	223	354	626	611	218	199	361	1437	961	316
Overall median	210	223	354	626	611	218	199	361	1437	961	316
Repeatability variance	6.53	322	6646	12.3	68.4	225	230	1219	2159	512	1754
Between laboratory variance	2761	1582	1208	30497	n/d	713	91.1	1887	10807	212	809
Reproducibility variance	2767	1904	7854	30510	n/d	938	322	3106	12966	723	2563
Repeatability SD	2.55	18.0	81.5	3.51	8.27	15.0	15.2	34.9	46.5	22.6	41.9
Between laboratory SD	52.5	39.8	34.8	175	n/d	26.7	9.5	43.4	104	14.6	28.4
Reproducibility SD	52.6	43.6	88.6	175	n/d	30.6	17.9	55.7	114	26.9	50.6
Reproducibility MAD	272	290	460	813	n/d	283	259	470	1867	1249	411
SD/mean	0.251	0.196	0.250	0.279	n/d	0.141	0.090	0.154	0.079	0.028	0.160
% RSD	25.1	19.6	25.0	27.9	n/d	14.1	9.01	15.4	7.93	2.80	16.0
% RSD MAD	130	130	130	130	n/d	130	130	130	130	130	130

n/d denotes statistic not calculated

6.3 UBM UNDER REDUCED 'STOMACH & INTESTINE' PHASE RESIDENCE TIME

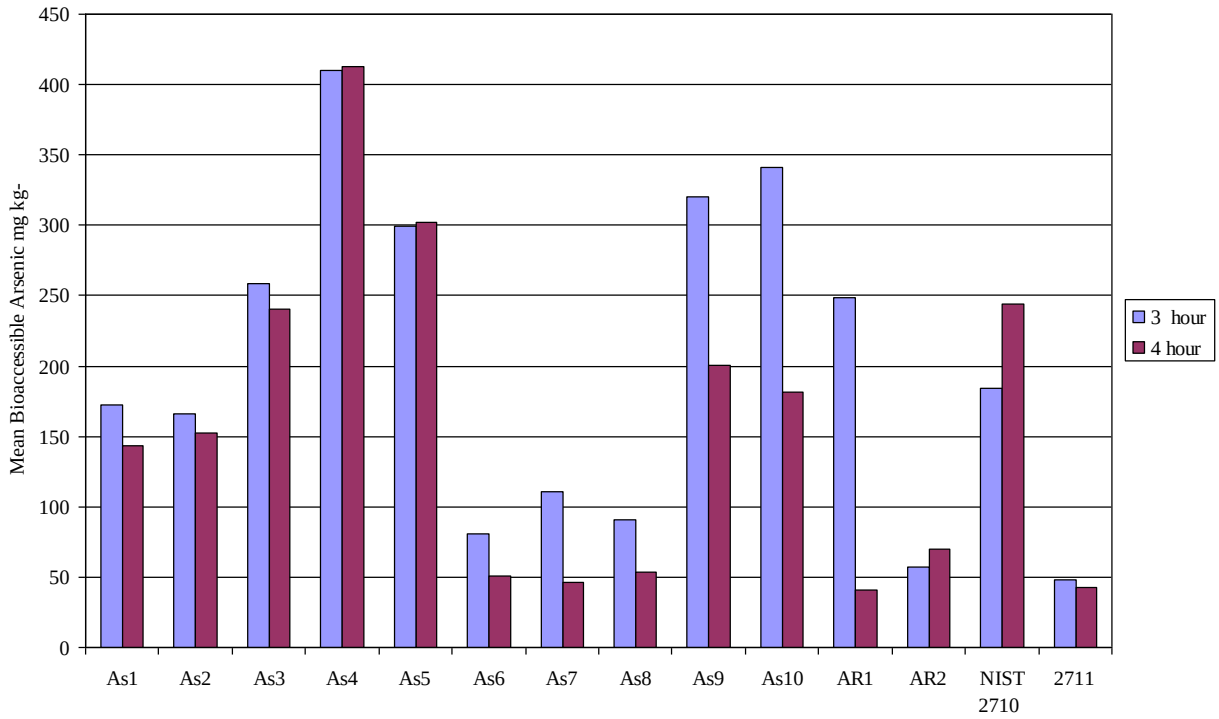


Figure 14 Comparison of the 3 and 4 hour 'stomach & intestine' residence time data for As

Figure 14 shows the individual bioaccessibility data for each of the arsenic soils at the 3 and 4 hour residence times. The results are an average of two measurements at each time period and were carried out by the BGS laboratories only.

A two tailed t test gives a p-value of 0.043 showing that there is a significant difference between the two time periods at the 95% confidence level. The 95% confidence interval for mean difference is 1.6027 to 85.3512 mg kg^{-1}.

The non-parametric bootstrap test (10000 runs) of the ratio of the 4 h value to the 3 h value of the paired means gives 95% confidence interval of 1.55 to 1.68 confirming that the 4 h value is significantly lower than the 3 h value.

6.4 VIVO-VITRO DATA COMPARISON

The bioavailable versus bioaccessible data plots have been produced for As, Cd and Pb in the 'stomach' and 'stomach & intestine' phases for the As, Cd and Pb soils (Figures 15-20). In addition, plots for the bioavailable and bioaccessible As in the As soils for the 'stomach' and 'stomach & intestine' phases have been produced for the 1:1000 solid to liquid ratio (Figures 21 and 22). The statistics used to calculate error bars and the estimated linear relationships are described in Section 5.1. The area and colour of the points gives an indication of the total

element concentration in each soil which can be referenced to the calibrated colour bar in each plot.

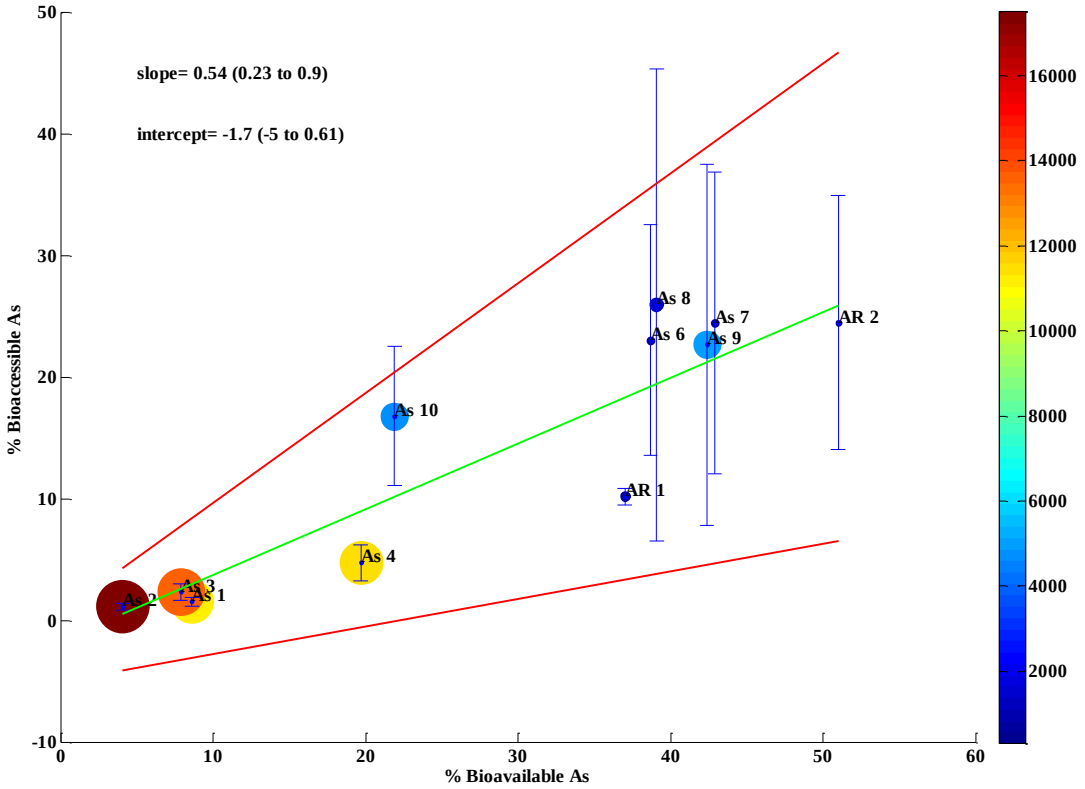


Figure 15 Bioavailability vs Bioaccessibility for As in the As soils in the 'stomach' phase

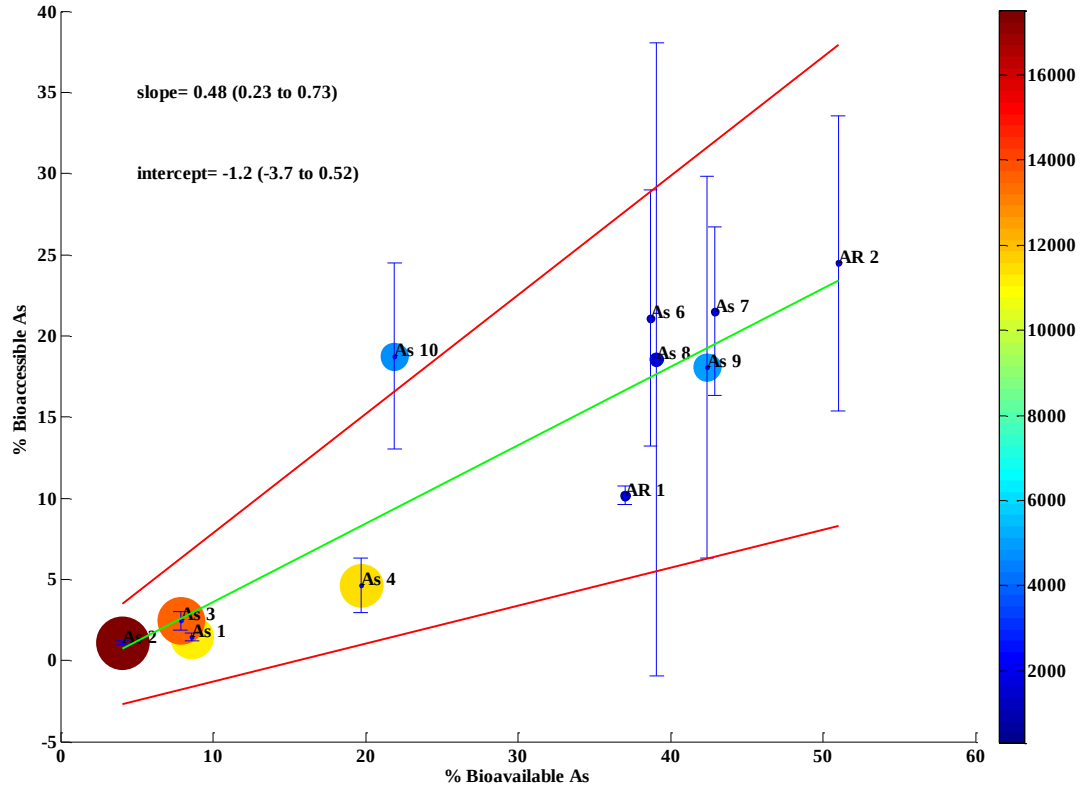


Figure 16 Bioavailability vs Bioaccessibility for As in the As soils in the 'stomach & intestine' phase

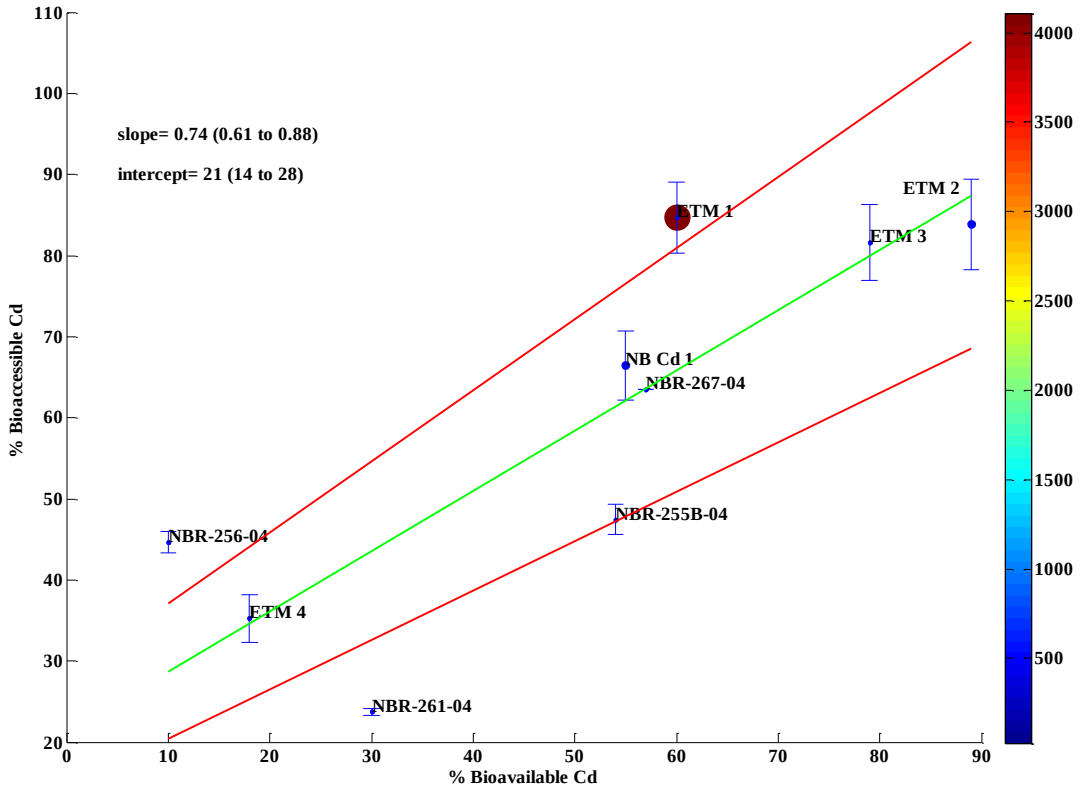


Figure 17 Bioavailability vs Bioaccessibility for Cd in the Cd soils in the 'stomach' phase

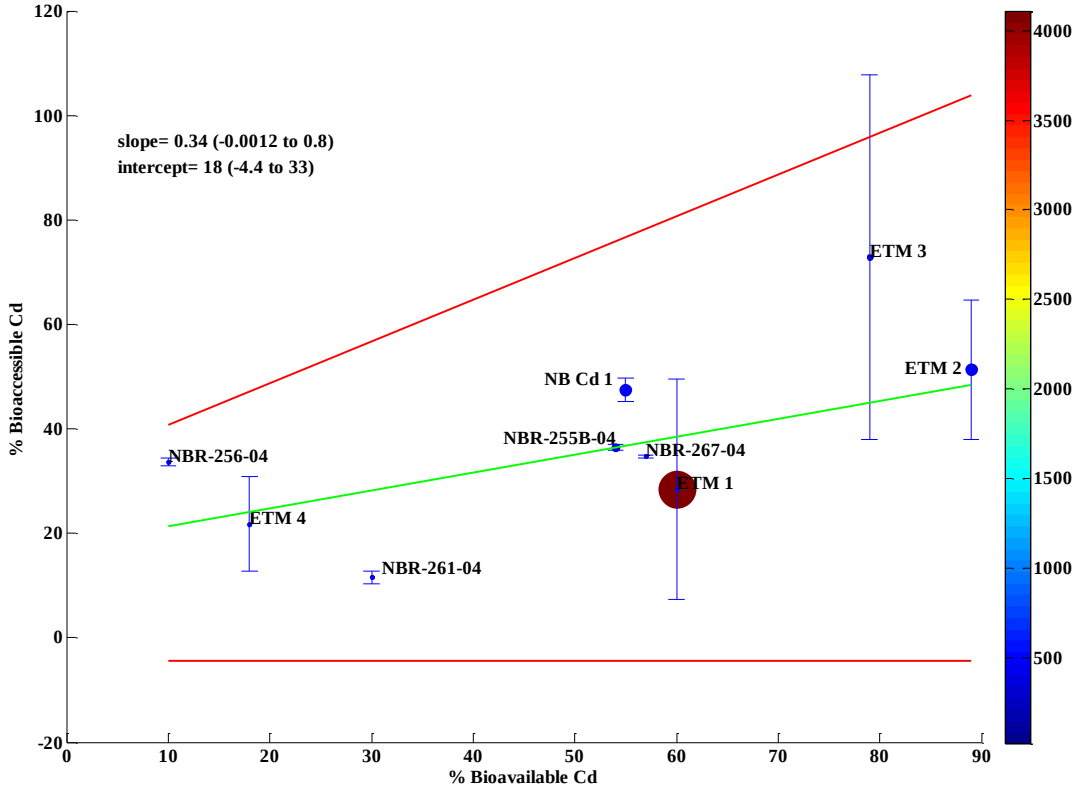


Figure 18 Bioavailability vs Bioaccessibility for Cd in the Cd soils in the 'stomach & intestine' phase

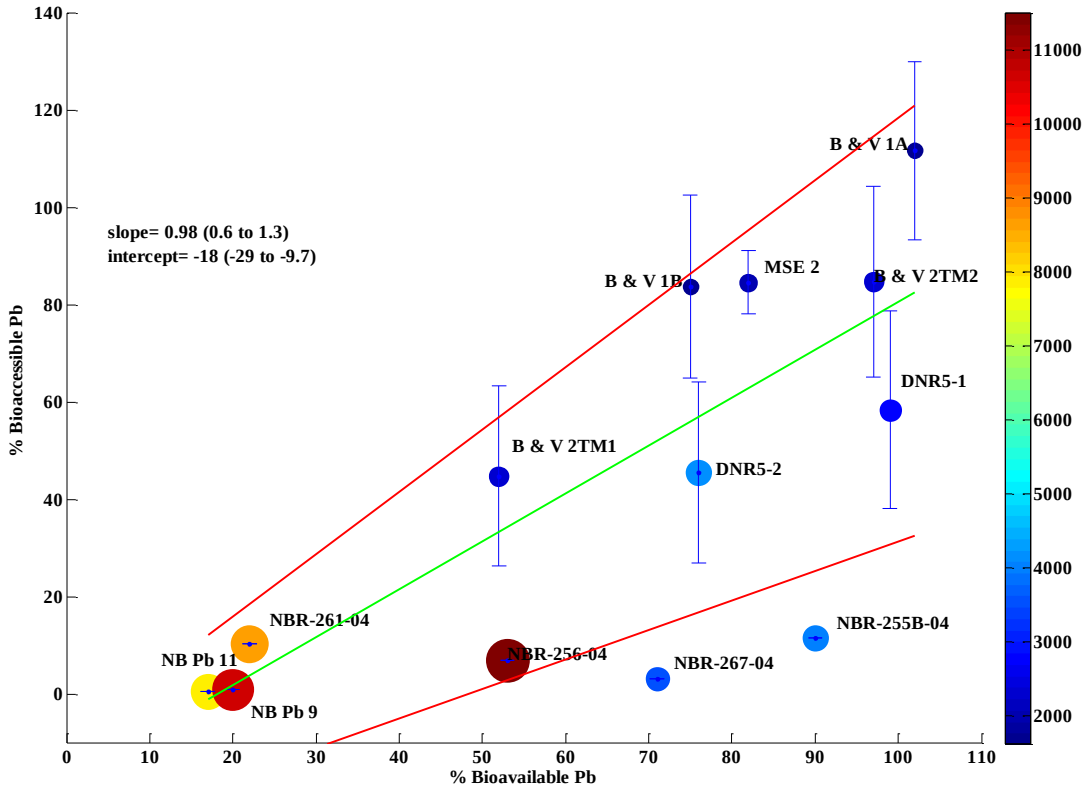


Figure 19 Bioavailability vs Bioaccessibility for Pb in the Pb soils in the 'stomach' phase

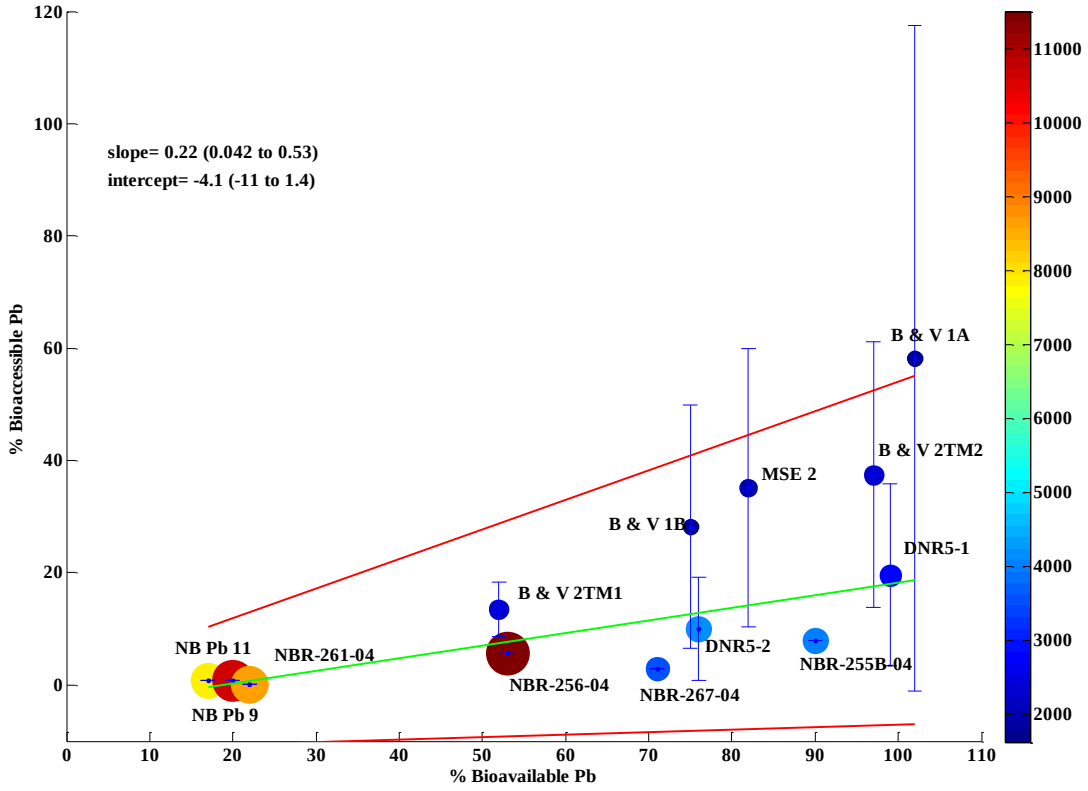


Figure 20 Bioavailability vs Bioaccessibility for Pb in the Pb soils in the 'stomach & intestine' phase

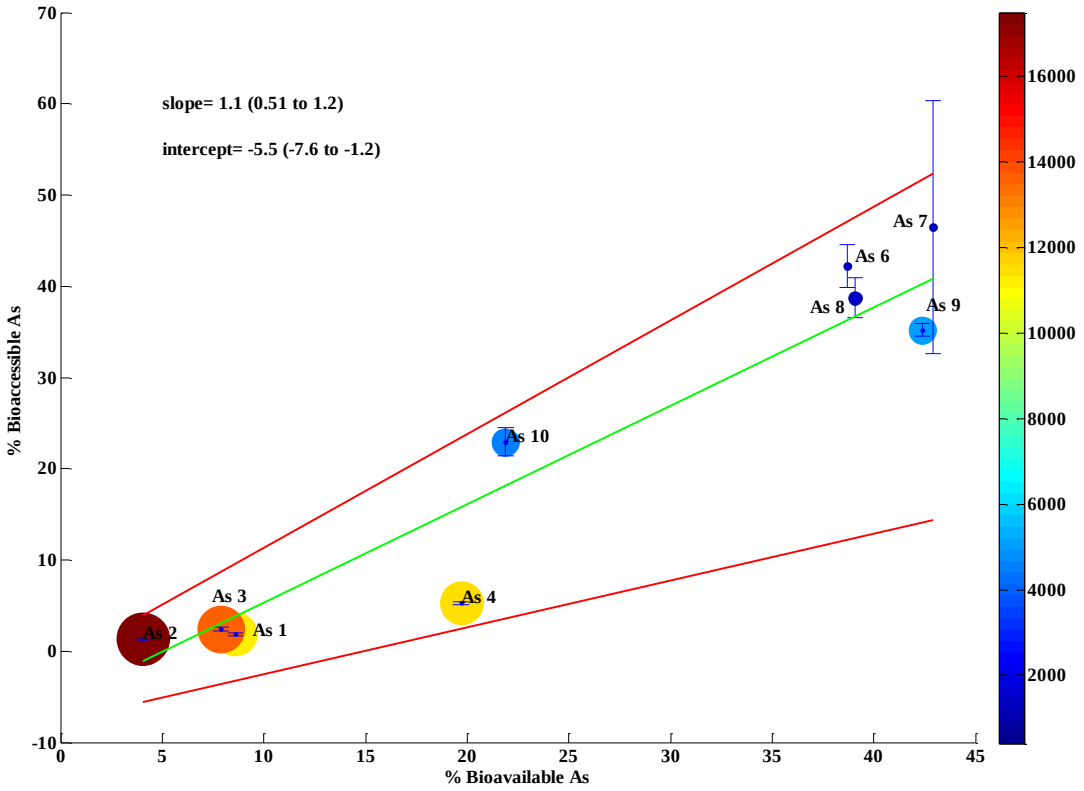


Figure 21 As in the As soils in the 'stomach' phase at 1:1000 solid to liquid ratio

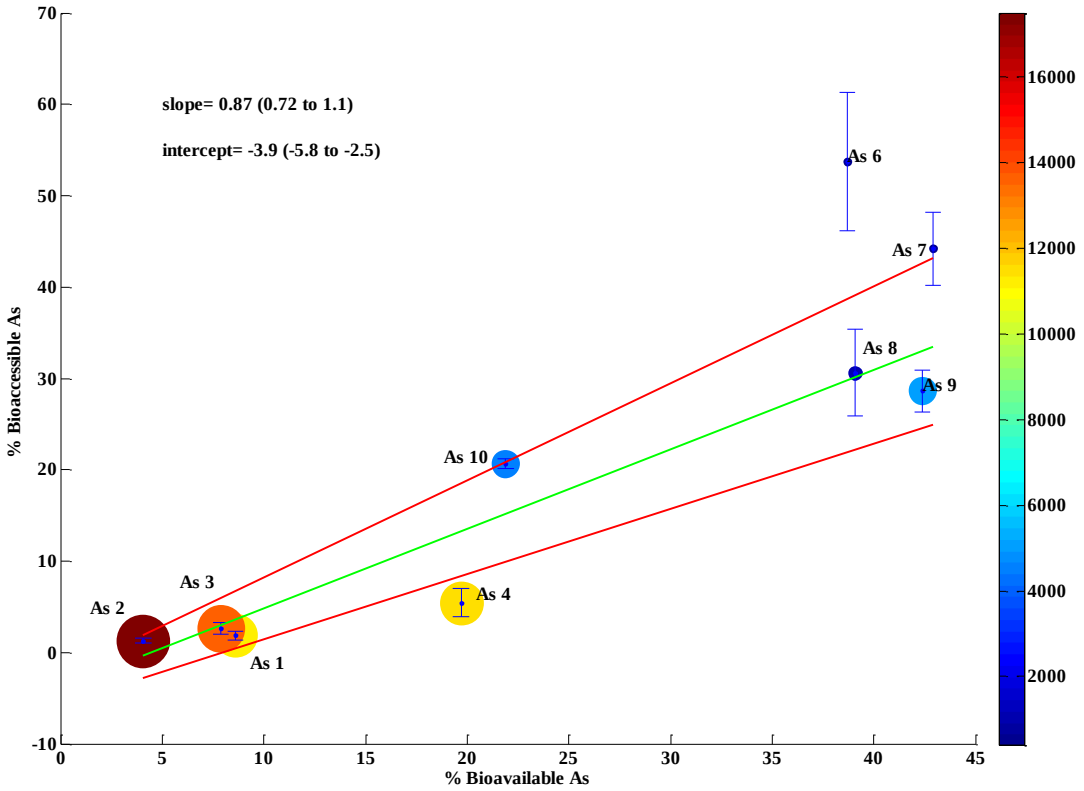


Figure 22 As in the As soils in the 'stomach & intestine' phase at 1:1000 solid to liquid ratio

6.4.1 Basta Soil Total Element Information

A range of major and trace elements present in the test materials supplied by Prof. Basta were determined by the BGS 'in-house' laboratory hot-block mixed acid ($HF/HClO_4^-/HNO_3$) digestion method with associated ICP-AES analysis by the instrument described in section 4.1. The BGS digestion procedure is based on that described by Thompson and Walsh (1983) and has been previously described in full by Wragg (2005; 2007). Each sample was digested in triplicate to get a measure of the repeatability and also to determine whether the BGS laboratories could reproduce the previously reported data from the laboratory of Professor Basta (Rodriguez et al., 1999; Rodriguez et al., 2003; Basta et al., 2007). Table 15 summarises the average of the three replicates determined by the BGS laboratory with the original BGS digestion data presented in Appendix 59. Table 16 summarises that calculated CV's for the three replicate digestions. The calculated % CV are, in general, <10%, however for a number of samples (As 1, As 3, As 4, As 6 and As 8) across a number of elements (Al, Ca, Cu, Fe, Na, Ni, Pb, S and Zn) the % CV has increased to 10-20%.

Table 15 Average total element data for Basta 1-10 soils re-analysed by BGS laboratories (mg kg^{-1})

Element	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10
Al	11754	11633	16570	18004	32001	24766	36229	26761	28419	29830
As	10508	15964	13176	11648	6542	208	301	273	4791	4528
Ca	10231	5457	3270	25225	25738	131054	100247	131059	80217	61926
Cd	41.5	30.3	27.3	31.0	41.6	18.0	25.9	26.9	349	220
Cu	407	366	438	543	794	1471	1241	1598	5979	5909
Fe	289039	303488	259495	217254	140872	226337	119462	217146	196894	174005
K	5545	5387	7418	7230	12059	8518	12455	9084	8984	9176
Mg	891	812	1486	1698	4762	11309	15200	11757	10270	9596
Mn	566	578	566	449	638	2077	1220	2010	1206	1191
Na	863	657	1727	1686	3895	5258	4476	5386	2695	2527
Ni	20.4	19.9	15.3	15.9	17.4	9.58	13.1	10.6	30.4	28.0
P	265	305	428	522	812	1046	1078	1096	1346	1300
Pb	13926	16905	12724	10183	5924	8733	4486	8111	25729	20784
S	41183	31619	38687	48064	33142	11567	6092	11291	15144	15585
Zn	1938	2066	2073	1995	5387	47532	18333	42283	5662	5066

Table 16 % CV's for the three digestion replicates of the Basta As soils

Element	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10
Al	2.33	5.00	10.3	7.25	3.58	2.92	2.57	3.81	3.82	5.94
As	2.09	4.23	9.36	6.45	3.83	9.50	2.53	5.70	4.60	5.26
Ca	3.59	4.48	10.3	6.93	2.22	2.24	2.48	3.01	2.83	6.40
Cd	2.20	4.60	9.46	5.77	4.03	3.71	1.37	2.35	4.42	5.04
Cu	2.43	5.45	11.3	5.91	4.99	6.59	3.38	3.37	2.82	5.63
Fe	20.6	5.09	1.04	15.7	6.67	2.36	4.67	19.0	6.85	8.92
K	2.51	4.85	9.75	6.58	3.72	3.40	3.32	2.84	5.59	5.46
Mg	2.45	4.62	9.29	6.17	3.35	2.64	2.34	2.95	2.76	6.31
Mn	1.82	3.38	8.74	6.21	3.51	3.66	3.22	3.33	2.55	6.82
Na	6.28	2.63	11.1	18.3	5.89	2.77	8.75	3.87	4.24	7.63
Ni	6.61	3.63	8.86	8.46	3.93	10.2	6.42	5.68	4.88	8.11
P	2.55	3.87	9.67	6.56	1.98	2.14	3.37	3.31	1.89	7.48
Pb	17.3	4.70	1.46	14.6	7.14	1.17	1.55	19.0	8.86	9.80
S	2.37	4.76	11.0	7.63	2.48	5.13	2.72	4.16	3.45	6.25
Zn	1.91	4.86	9.10	5.66	3.07	2.38	2.95	18.0	3.29	5.41

In the undertaking of the BGS mixed acid digestion explosive ejections from the digestion tubes were observed for a number of the samples. In addition, the calculated recoveries for the BGS total digestion method in comparison to previously reported data (Table 17) were low (<85%) or high (>115%) for all elements where a comparison could be made, in both the calcine and iron slag materials.

Table 17 Calculated percentage (%) recoveries for BGS mixed acid digestion compared to previously reported data for As 1-10. High and low recoveries highlighted in bold.

Sample	Al	As	Ca	Cu	Fe	Ni	Pb	Zn
As 1	98.8	93.0	85.3	106	97.3	**52.1**	**126**	**121**
As 2	97.8	91.2	**80.3**	115	95.7	**55.3**	**140**	**128**
As 3	**143**	97.6	**79.8**	114	91.1	**48.7**	**116**	**126**
As 4	**153**	101	87.0	104	86.9	**49.3**	**121**	**120**
As 6	102	**51.2**	**458**	**81.3**	108	**39.1**	100	**1235**
As 7	100	**67.0**	**82.8**	**77.1**	102	**53.5**	**66.0**	**1175**
As 8	**83.6**	**23.1**	**153**	**72.3**	**131**	**33.2**	**231**	**1685**
As 9	108	95.4	107	**141**	114	86.0	**204**	**140**
As 10	109	97.4	102	**147**	95.1	**82.2**	**180**	**149**

To assess whether the high and low recoveries were caused by instrumental problems the samples were re-analysed by the same instrument (Varian Vista AX CCD ICP-AES) and also by a second ICP-AES within the BGS laboratories (an ARL 3580). The data returned by both instruments was in agreement, indicating that the instrumental analysis was not at fault for the differences in data compared to that previously reported. In addition, the samples were re-digested by the same methodology, with extra care allowing for the explosive nature of the

samples. The second set of extractions were analysed by the ARL 3580 and returned similar values to the previous digestions of the same samples. No further analysis of the samples by techniques such as XRF was possible because the samples had been consumed by the inter-laboratory trial and the total digestion procedures utilized.

Previous analysis of the As soils was carried out by a variety of methods, INAA, XRF, USEPA 3050B, 3051, 6051A and a variety of oxalate extractions, in the laboratory of Professor Basta. The information provided indicates that only low As recoveries were observed with one method, 3051, that the data from all other methods were in agreement and all subsequent reported data, used to calculate the percentage bioaccessible As, was that returned by the USEPA 3050B methodology.

The range of recoveries obtained by the BGS total digestion method (table 17), compared to the previously reported data, and the information provided by Professor Basta highlight the complicated nature of the primary As samples under investigation by the inter-laboratory trial.

7 Conclusions

- The As soils provided by Professor Basta have a complex physico-chemical composition, which appears to contribute to the reproducibility problems observed in the inter-laboratory trial.

7.1 ARSENIC

- The median relative standard deviation repeatability for As in the stomach phase was 5.7% (range 2.8 – 26.8, n = 15).
- The median relative standard deviation reproducibility for As in the stomach phase was 29.5% (range 6.5 – 74.7, n = 15).
- The median relative standard deviation repeatability for As in the stomach & intestine phase was 6.9% (range 0.63 – 35.7, n = 15).
- The median relative standard deviation reproducibility for As in the stomach & intestine phase was 25.9% (range 6.9 – 81.8, n = 15).
- The slope of the linear regression of percentage bioaccessible As in the stomach phase on the percentage bioavailable As is 0.54 (CI 0.23 – 0.9).

- The slope of the linear regression of percentage bioaccessible As in the stomach & intestine phase on the percentage bioavailable As is 0.48 (CI 0.23 – 0.73).
- The slope of the linear regression of percentage bioaccessible As in the stomach phase (1:1000 soil:solution ratio) on the percentage bioavailable As is 1.1 (CI 0.51 – 1.2).
- The slope of the linear regression of percentage bioaccessible As in the stomach & intestine phase (1:1000 soil:solution ratio) on the percentage bioavailable As is 0.87 (CI 0.72 – 1.1).
- The UBM returns statistically higher bioaccessible As values (paired t-test) in the 'stomach & intestine' phase when an extraction time of 3 hours was employed compared to the standard 4 hour extraction time.

7.2 CADMIUM

- The median relative standard deviation repeatability for Cd in the stomach phase was 3.4% (range 1.3 – 29.9, n = 11).
- The median relative standard deviation reproducibility for Cd in the stomach phase was 7.0% (range 5.2 – 60.3, n = 8).
- The median relative standard deviation repeatability for Cd in the stomach & intestine phase was 6.7% (range 0.8 – 46.5, n = 12).
- The median relative standard deviation reproducibility for Cd in the stomach & intestine phase was 35.3% (range 4.8 – 89.0, n = 8).
- The slope of the linear regression of percentage bioaccessible Cd in the stomach phase on the percentage bioavailable Cd is 0.74 (CI 0.61 – 0.88).
- The slope of the linear regression of percentage bioaccessible Cd in the stomach & intestine phase on the percentage bioavailable Cd is 0.34 (CI -0.0012 – 0.8).

7.3 LEAD

- The median relative standard deviation repeatability for Pb in the stomach phase was 3.6% (range 1.2 – 13.3, n = 10).

- The median relative standard deviation reproducibility for Pb in the stomach phase was 22.8% (range 3.0 – 46.8, n = 10).
- The median relative standard deviation repeatability for Pb in the stomach & intestine phase was 14.6% (range 1.0 – 138.5, n = 10).
- The median relative standard deviation reproducibility for Pb in the stomach & intestine phase was 81.4% (range 35.3 – 140.8, n = 10).
- The slope of the linear regression of percentage bioaccessible Pb in the stomach phase on the percentage bioavailable Pb is 0.98 (CI 0.6 – 1.3).
- The slope of the linear regression of percentage bioaccessible Pb in the stomach & intestine phase on the percentage bioavailable Pb is 0.22 (CI 0.042 – 0.53).

Appendix 1 BARGE UBM Protocol

TITLE: UNIFIED BARGE BIOACCESSIBILITY METHOD

PURPOSE

To define a procedure for the preparation of extracts that are representative of the concentration of potentially harmful elements in a simulated human gastrointestinal tract.

Outline

Soils or other geological materials, in sieved or crushed form, are extracted in an environment that simulates the basic physicochemical conditions of the human gastrointestinal tract.

Sample Preparation

All samples prepared for bioaccessibility testing are dried in a fan assisted oven or air dried at <40°C and sieved to<2mm, <250μm or crushed as required.

Table 1 Equipment

Equipment	Supplier	Comments
Oven/Water Bath	Any suitable	
Rotator	Any suitable	
Centrifuge	Any suitable	Capable of reaching 3000g
pH meter	Any suitable	
Analytical Balance	Any suitable	
Volumetric Flasks	Any suitable	Suitable Grades
Auto pipettes	Any suitable	
Polycarbonate centrifuge tubes with polypropylene screw cap	Nalgene	Catalogue Number 3118-0085 (31 x 101 mm)
2 L HDPE screw top bottle	Nalgene	2125-2000

All glassware and extraction vessels should be cleaned prior to use using a suitable acid rinsing protocol.

Table 2 List of reagents and Suppliers

Reagent	Supplier	Catalogue Number
NaH_2PO_4	Baker	0303
Mucin (pig)	Carl Roth (Germany)	84941
D – Glucuronic acid	Fluka	49335
NaCl	Merck	106404
KSCN	Merck	105125
Anhydrous Na_2SO_4	Merck	106647
KCl	Merck	104936
$CaCl_2.2H_20$	Merck	102382
NH_4Cl	Merck	101145
$NaHCO_3$	Merck	106329
KH_2PO4	Baker	0240
$MgCl_2.6H_20$	Merck	105833
NaOH	Merck	6498
HCl	Merck	100317
Urea	Merck	108487
Uric Acid	Merck-Prolabo	20745134
Anhydrous D + Glucose	Merck	8337
D-glucosaminehydrochloride	Merck	104113
Pepsin (pig)	Merck	107185
Bovine serum albumin (BSA)	Merck	112018
Pancreatin (pig)	Merck	107130
α-amylase (bacillus species)	Sigma	A-6814
Lipase (pig)	Sigma	L-3126
Bile salts (bovine)	Sigma	B-3883
69% HNO_3	Merck	1.01518.1000

Preparation of the Simulated Fluids

All simulated gastric and intestinal fluids are prepared one day prior to carrying out bioaccessibility extractions to ensure that all of the reagents are thoroughly dissolved. All simulated gastrointestinal fluids are combinations of separately prepared 500ml inorganic and organic phases with the additional solid phase chemicals.

Simulated Saliva Fluid (1000 ml): The chemical composition of the inorganic, organic solutions and the solid chemical reagents required to prepare 1000 ml of simulated saliva fluid are given in Tables 3, 4 and 5 respectively. Add the solid chemical reagents to a suitable container (preferably a 2 L HDPE screw top bottle) according to the weights given in Table 5. Simultaneously pour the separately prepared 500 ml volumes of the inorganic and organic saliva

phase reagents into the bottle containing the solid reagents and mix thoroughly. Measure the pH of the simulated saliva fluid, which should be at 6.5 ± 0.5. If required, adjust the pH of the saliva fluid to the correct pH with either 1.0 M NaOH or 37% HCl.

Table 3 Inorganic Saliva Phase Reagent (500 ml)

Reagent	Final Concentration in 500ml mg l^{-1}	Volume/Weight made up to 500 ml
KCl	1792	896 mg
NaH_2PO_4	1776	888 mg
KSCN	400	200 mg
Na_2SO_4	1140	570 mg
NaCl	596	298 mg
NaOH	144	1.80 ml of 1.0 M

Table 4 Organic Saliva Phase Reagent (500 ml)

Reagent	Final Concentration in 500ml mg l^{-1}	Volume/Weight made up to 500 ml
Urea	400	200 mg

Table 5 Additional Constituents for Saliva Phase Reagent

Reagent	Final Concentration in 1000ml mg l^{-1}	Volume/Weight made up to 1000 ml
Amylase	145	145 mg
Mucin	50.0	50.0 mg
Uric Acid	15.0	15.0 mg

Simulated Gastric Fluid (1000 ml): The chemical composition of the inorganic, organic solutions and the solid chemical reagents required to prepare 1000 ml of simulated gastric fluid are given in Tables 6, 7 and 8 respectively. Add the solid chemical reagents to a suitable container (preferably a 2 L HDPE screw top bottle) according to the weights given in table 8.

Simultaneously pour the separately prepared 500 ml volumes of the inorganic and organic gastric phase reagents into the bottle containing the solid reagents and mix thoroughly. Measure the pH of the simulated gastric fluid, which should be at 0.9 – 1.0. If required, adjust the pH of the gastric fluid to the correct pH with either 1.0 M NaOH or 37% HCl.

Check that the final pH of the mixed saliva and gastric phase (1ml of saliva and 1.5 ml of gastric) is 1.2-1.4. If the mixture is not within specification adjust the pH of the gastric fluid to with either 1.0 M NaOH or 37% HCl and recheck an aliquot of the mixed saliva-gastric phase.

Table 6 Inorganic Gastric Phase Reagent (500 ml)

Reagent	Final Concentration in 500ml mg l^{-1}	Volume/Weight made up to 500 ml
NaCl	5504	2752 mg
NaH_2PO_4	533	266 mg
KCl	1649	824 mg
$CaCl_2$	799	400 mg
NH_4Cl	612	306 mg
HCl	0.31%	8.3 ml of 37% HCl

Table 7 Organic Gastric Phase Reagent (500 ml)

Reagent	Final Concentration in 500ml mg l^{-1}	Volume/Weight made up to 500 ml
Glucose	1300	650 mg
Glucuronic acid	40.0	20.0 mg
Urea	170	85.0 mg
Glucosaminehydrochloride	660	330 mg

Table 8 Additional Constituents for Gastric Phase Reagent

Reagent	Final Concentration in 1000 ml mg l^{-1}	Volume/Weight made up to 1000 ml
Bovine Serum Albumin	1000	1000 mg
Mucin	3000	3000 mg
Pepsin	1000	1000 mg

Simulated Duodenal Fluid (1000 ml): The chemical composition of the inorganic, organic solutions and the solid chemical reagents required to prepare 1000 ml of simulated duodenal fluid are given in Tables 9, 10 and 11 respectively. Add the solid chemical reagents to a suitable container (preferably a 2 L HDPE screw top bottle) according to the weights given in Table 11. Simultaneously pour the separately prepared 500 ml volumes of the inorganic and organic duodenal phase reagents into the bottle containing the solid reagents and mix thoroughly. Measure the pH of the simulated duodenal fluid, which should be at 7.4 ± 0.2. If required, adjust the pH of the duodenal fluid to the correct pH with either 1.0 M NaOH or 37% HCl.

Table 9 Inorganic Duodenal Phase Reagent (500 ml)

Reagent	Final Concentration in 500ml mg l^{-1}	Volume/Weight made up to 500 ml
NaCl	14024	7012 mg
$NaHCO_3$	11214	5607 mg
KH_2PO_4	160	80 mg
KCl	1129	564 mg
$MgCl_2$	100	50.0 mg
HCl	0.01%	180µl of 37% HCl

Table 10 Organic Duodenal Phase Reagent (500 ml)

Reagent	Final Concentration in 500ml mg l^{-1}	Volume/Weight made up to 500 ml
Urea	200	100 mg

Table 11 Additional Constituents for Duodenal Phase Reagent

Reagent	Final Concentration in 1000ml mg l^{-1}	Volume/Weight made up to 1000 ml
$CaCl_2$	200	200 mg
Bovine Serum Albumin	1000	1000 mg
Pancreatin	3000	3000 mg
Lipase	500	500 mg

Simulated Bile Fluid (1000 ml): The chemical composition of the inorganic, organic solutions and the solid chemical reagents required to prepare 1000 ml of simulated bile fluid are given in tables 12, 13 and 14 respectively. Add the solid chemical reagents to a suitable container (preferably a 2 L HDPE screw top bottle) according to the weights given in table 14. Simultaneously pour the separately prepared 500 ml volumes of the inorganic and organic bile phase reagents into the bottle containing the solid reagents and mix thoroughly. Leave the solution for c.1 hour at room temperature to allow for the complete dissolution of all reagents and measure the pH of the simulated bile fluid. The simulated bile fluid should be at 8.0 ± 0.2. If required, adjust the pH of the duodenal fluid to the correct pH with either 1.0 M NaOH or 37% HCl.

Check that the final pH of the mixed gastro-intestinal fluid (1.0 ml of saliva, 1.5 ml of gastric, 3.0 ml of duodenal and 1.0 ml of bile) is 6.3 ± 0.5. If the mixture is not within specification adjust the pH of the duodenal fluid to with either 1.0 M NaOH or 37% HCl and recheck an aliquot of the mixed gastro-intestinal phase.

Table 12 Inorganic Bile Phase Reagent

Reagent	Final Concentration in 500ml mg l^{-1}	Volume/Weight made up to 500 ml
NaCl	10518	5259 mg
$NaHCO_3$	11570	5785 mg
KCl	753	376 mg
HCl	0.01%	180µl of 37% HCl

Table 13 Organic Bile Phase Reagent (500 ml)

Reagent	Final Concentration in 500ml mg kg^{-1}	Volume/Weight made up to 500 ml
Urea	500	250 mg

Table 14 Additional Constituents for Bile Phase Reagent

Reagent	Reagent Concentration in 1000ml mg l^{-1}	Volume/Weight made up to 1000 ml
$CaCl_2$	222	222 mg
Bovine Serum Albumin	1800	1800 mg
Bile	6000	6000 mg

The simulated gastrointestinal fluids are stored at room temperature over night prior to completion of bioaccessibility extractions. The fluids are heated to 37 ± 2°C at least two hours prior to carrying out the extraction methodology on the day following preparation of the fluids.

Table 15 pH adjustment reagent concentrations

Reagent	Volume/weight in 1000ml
0.1 M HNO_3	6.30ml
1.0M NaOH	40.0g
10M NaOH	400g

Preparation of the Samples

Weigh two 0.6 g sub-samples of each test soil (dry weight) accurately into uniquely labelled suitable extraction vessels capable of undergoing centrifugation. Label one sub-sample of the soil for the stomach phase of the extraction and the other sub-sample stomach + intestine. Store the weighed test samples at room temperature prior to completion of the extraction methodology. For every ten unknown soil samples extract a blank sample, a sample duplicate and a reference material. The reference material subjected to bioaccessibility testing as an internal control is NIST Montana soil 2710 or 2711.

Bioaccessibility extraction protocol

1. Switch on the extractor at least 2 hours prior to beginning the bioaccessibility extraction and set the temperature to 37 ± 2°C.

2. Warm the simulated gastrointestinal fluids (prepared on the previous day) to 37 ± 2°C prior to their use in the bioaccessibility extraction method.

3. Check that the final pH of the mixed saliva and gastric phase (1ml of saliva and 1.5 ml of gastric) is 1.2-1.4 and the mixed gastro-intestinal fluid (1.0 ml of saliva, 1.5 ml of gastric, 3.0 ml of duodenal and 1.0 ml of bile) is 6.3 ± 0.5. If the saliva-gastric mixture is not within specification, adjust the pH of the gastric fluid to with either 1.0 M NaOH or 37% HCl and recheck an aliquot of the mixed saliva-gastric phase. If the gastro-intestinal mixture is not within specification, adjust the pH of the duodenal fluid to with either 1.0 M NaOH or 37% HCl and recheck an aliquot of the mixed gastro-intestinal phase.

4. Once the extractor set-up and simulated gastrointestinal fluids have reached an operating temperature of 37 ± 2°C, carry out the following extraction protocol (a flow diagram is shown in figure 1).

5. Accurately add, to each extraction vessel, via pipette 9.0 ml of simulated saliva fluid.

6. Cap each extraction vessel and manually shake to thoroughly mix the soil and simulated fluids.

7. Accurately add to each extraction vessel, via pipette 13.5 ml of simulated gastric fluid, 5-15 minutes after the addition of the simulated saliva fluid.

8. Place the extraction vessels in the extractor and incubate the samples using end-over-end rotation, at 37 ± 2°C for 1 hour.

9. Switch off the extractor and remove both the 'stomach' and 'stomach + intestine' soil suspensions.

10. Measure the pH of each of the soil suspensions ('stomach' and 'stomach + small intestine'); the pH should be 1.2 – 1.7. *If the pH of an individual suspension is not within this tolerance, the extraction for both sub-samples must be discarded and the sub-samples re-extracted. For the repeat extraction of sub-samples add an additional aliquot of 37% HCl, up to a maximum of 1.0 ml.*

11. If the pH of the suspension is within the required tolerance (see section 10), collect the stomach phase solution of the bioaccessibility extraction protocol by centrifuging the soil suspensions at 3000g for 5 minutes. Remove the supernatant and preserve by pipetting 1.0 ml into a uniquely labelled container and adding 9.0 ml of 0.1 M HNO_3. Store the sample at <8°C prior to determination of the bioaccessible contaminant content.

12. To prepare the 'stomach + intestine' phase solution of the protocol add, via a pipette, 27.0 ml of simulated duodenal fluid and 9.0 ml of simulated bile fluid to the sub-sample labelled 'stomach + intestine' after checking its pH on completion of the stomach phase in section 10.

13. Cap each extraction vessel and manually shake to thoroughly mix the soil and simulated fluids.

14. Adjust the pH each of the resulting suspensions to 6.3 ± 0.5, with the dropwise addition of 37% HCl, 1M or 10 M NaOH (as required).

15. Replace the extraction vessels and incubate in the end-over–end rotator for a further 4

hours at 37 ± 2°C.

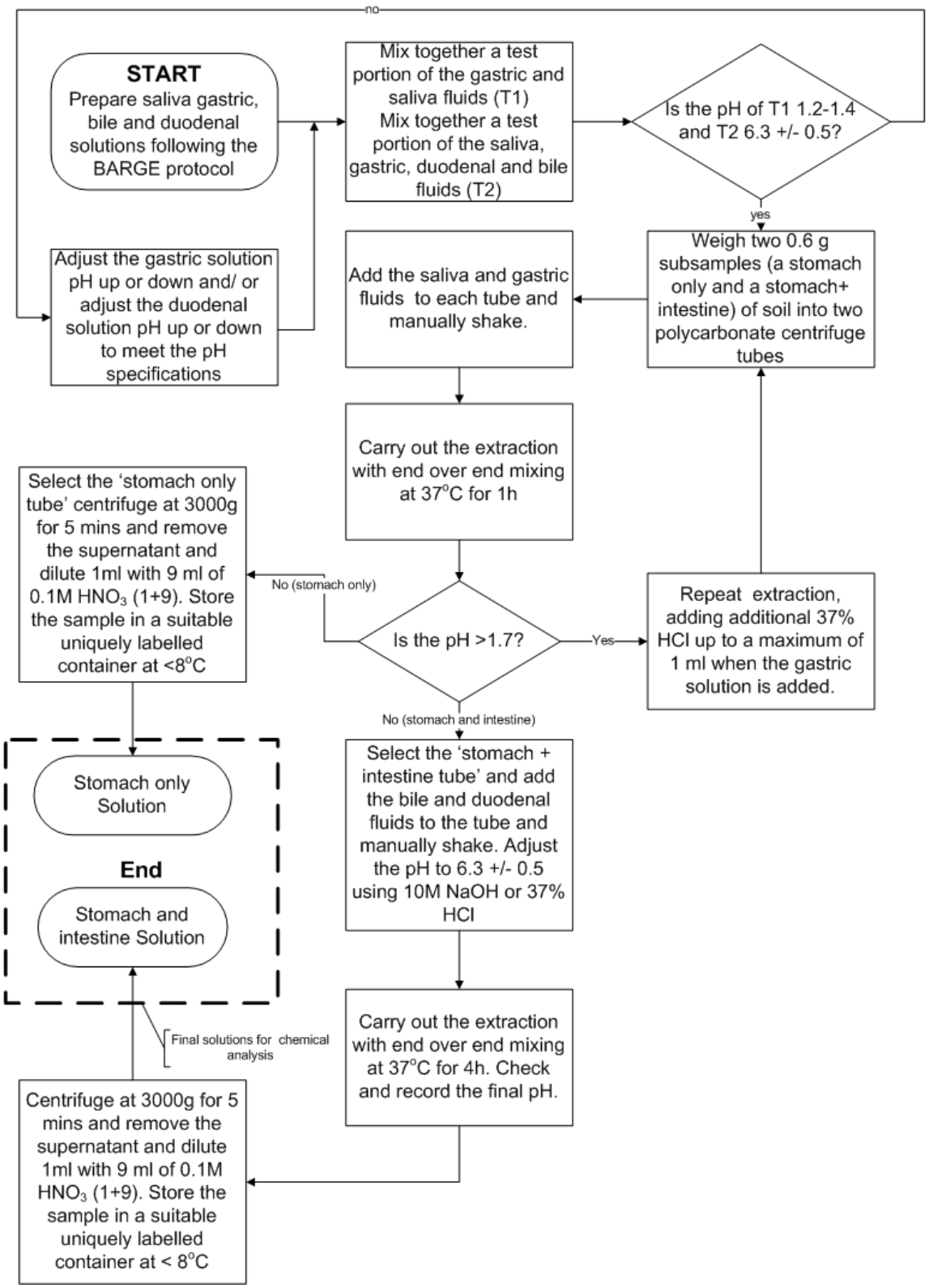
16. Switch off the extractor and remove the 'stomach + intestine' soil suspensions.

17. Measure and record the pH of each of the soil suspensions; the pH should be 6.3 ± 0.5.

18. Collect the intestine phase solution of the bioaccessibility extraction protocol by centrifuging the soil suspensions at 3000g for 5 minutes. Remove the supernatant and preserve by pipetting 1.0 ml into a uniquely labelled container and adding 9.0 ml of 0.1 M HNO_3. Store the sample at <8°C prior to determination of the bioaccessible contaminant content.

Quality Control

Incorporate at least one certified reference material, a duplicate suspension and a procedural blank with every extraction run of 10 unknown samples.


START
Prepare saliva gastric, bile and duodenal solutions following the BARGE protocol
Mix together a test portion of the gastric and saliva fluids (T1)
Mix together a test portion of the saliva, gastric, duodenal and bile fluids (T2)
Is the pH of T1 1.2-1.4 and T2 6.3 +/- 0.5?
no
yes
Adjust the gastric solution pH up or down and/ or adjust the duodenal solution pH up or down to meet the pH specifications
Add the saliva and gastric fluids to each tube and manually shake.
Weigh two 0.6 g subsamples (a stomach only and a stomach+ intestine) of soil into two polycarbonate centrifuge tubes
Carry out the extraction with end over end mixing at 37°C for 1h
Select the 'stomach only tube' centrifuge at 3000g for 5 mins and remove the supernatant and dilute 1ml with 9 ml of 0.1M HNO3 (1+9). Store the sample in a suitable uniquely labelled container at <8°C
No (stomach only)
Is the pH >1.7?
Yes
Repeat extraction, adding additional 37% HCl up to a maximum of 1 ml when the gastric solution is added.
No (stomach and intestine)
Stomach only Solution
End
Stomach and intestine Solution
Select the 'stomach + intestine tube' and add the bile and duodenal fluids to the tube and manually shake. Adjust the pH to 6.3 +/- 0.5 using 10M NaOH or 37% HCl
Carry out the extraction with end over end mixing at 37°C for 4h. Check and record the final pH.
Final solutions for chemical analysis
Centrifuge at 3000g for 5 mins and remove the supernatant and dilute 1ml with 9 ml of 0.1M HNO3 (1+9). Store the sample in a suitable uniquely labelled container at < 8°C

Appendix 2 ISO Form A: As Soils As 'stomach' phase data (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	166	186	275	499	342	74.1	91.0	17.4	585	431	63.4	84.3	258	53.3	5.26
	153	188	266	498	367	74.2	85.6	66.8	597	470	68.2	113	261	54.3	5.13
DHI	227	269	463	783	494	127	78.5	605	1992	885	70.9	55.8	348	56.7	3.34
	221	255	466	684	493	94.1	79.4	647	2065	1043	72.2	53.6	347	57.4	3.34
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4.92
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4.82
GHENT	159	172	246	371	295	30.3	40.7	140	311	461	N/S	N/S	N/S	N/S	7.11
	N/S	N/S	N/S	400	N/S	N/S	38.9	N/S	314	N/S	N/S	N/S	N/S	N/S	3.36
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	3.35
INERIS	226	261	410	784	486	86.4	143	125	924	870	N/S	N/S	N/S	59.3	3.39
	227	258	356	771	509	84.4	141	124	977	874	N/S	N/S	N/S	64.2	8.23
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	61.1	6.75
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	56.9	5.10
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	5.29
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	3.36
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	7.97
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4.51
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4.50
OHIO	183	191	331	534	419	129	215	509	2077	1130	N/S	N/S	339	N/S	5.06
	189	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	329	N/S	5.50
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	331	N/S	5.50
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	354	N/S	N/S
RIVM	167	209	342	538	356	88	119	219	901	673	N/S	N/S	323	N/S	2.87
	164	193	320	593	373	78	103	219	822	662	N/S	N/S	372	N/S	3.42
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	5.35
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4.14
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	10.2
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	10.3
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	10.2
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	10.4
RMC	111	130	194	313	285	134	90	486	1278	989	N/S	N/S	N/S	47.3	3.47
	112	121	179	311	291	145	104	482	1473	854	N/S	N/S	N/S	48.0	3.69

Appendix 3 ISO Form B: Average As 'stomach' phase data for As soils (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	160	187	271	498	355	74.1	88.3	42.1	591	450	65.8	98.5	259*	53.8	5.20
DHI	224	262	465	733	494	110	78.9	626	2029	964	71.6	54.7	348	57.1	4.10
GHENT	159	172	246	386	295	30.3	39.8	140	313	461	N/S	N/S	N/S	N/S	4.60
INERIS	227	259	383	778	498	85.4	142	124	951	872	N/S	N/S	N/S	61.7	5.66
OHIO	186	191	331	534	419	130	215	509	2077	1130	N/S	N/S	338	N/S	5.35
RIVM	165	201	331	566	364	83.1	111	219	861	667	N/S	N/S	347	N/S	3.15
RMC	111	125	186	312	288	140	96.8	484	1375	922	N/S	N/S	N/S	47.6	3.58

N/S Sample not analysed by the laboratory, * Statistical straggler

Appendix 4 ISO Form C: As 'stomach standard deviation data for As soils (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	13.6	2.49	9.18	0.87	25.2	0.10	5.40	49.3	12.5	38.8	4.84	28.3	2.85	0.98	0.12
DHI	6.03	14.3	2.62	100	0.89	32.8	0.96	42.1	72.1	158	1.31	2.27	1.30	0.71	0.88
GHENT	-	-	-	29.0	-	-	1.82	-	3.20	-	N/S	N/S	N/S	N/S	2.17
INERIS	1.19	2.31	54.5	13.1	23.1	2.07	1.93	1.52	53.1	4.67	N/S	N/S	N/S	4.84*	1.73
OHIO	7.57	-	-	-	-	32.7	-	-	-	-	N/S	N/S	11.1	N/S	0.25
RIVM	3.38	16.0	21.9	55.1	17.5	9.83	15.8	0.55	79.4	10.8	N/S	N/S	49.2	N/S	3.44
RMC	1.29	9.53	15.0	1.15	6.31	10.6	13.7	3.91	196**	135	N/S	N/S	N/S	0.61	0.22

N/S Sample not analysed by the laboratory, - Only one replicate extracted by the laboratory, * Statistical straggler , ** Statistical outlier

Appendix 5 ISO Form A: As Soils As 'stomach & intestine' phase data (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	148	148	242	417	300	53.0	56.7	50.7	220	190	37.9	67.7	243	47.2	8.79
	138	156	239	409	303	48.2	36.6	56.1	180	172	43.9	71.7	245	38.2	8.74
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4.07
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	3.46
DHI	229	269	426	652	388	38.6	<17.6	95.3	510	465	40.5	40.7	278	54.9	8.75
	229	252	454	686	391	41.1	<17.6	139	622	437	45.2	40.5	280	58.0	8.66
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4.18
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	3.88
GHENT	159	175	246	405	266	31.8	48.8	92.9	314	517	N/S	N/S	N/S	N/S	8.72
	N/S	N/S	N/S	417	N/S	N/S	44.6	N/S	322	N/S	N/S	N/S	N/S	N/S	8.61
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	8.73
INERIS	227	266	430	780	454	55.4	30.1	36.8	488	N/S	N/S	N/S	N/S	N/S	8.83
	233	302	374	763	439	N/S	28.2	30.0	490	106	N/S	N/S	N/S	N/S	8.78
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	8.77
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4.35
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	14.6
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4.75
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	8.75
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	8.61
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4.13
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4.00
OHIO	188	206	298	581	341	<1.98	9.41	24.9	254	128	N/S	N/S	269	N/S	4.42
	196	N/S	N/S	N/S	N/S	<1.98	N/S	N/S	N/S	N/S	N/S	N/S	270	N/S	4.41
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	273	N/S	3.86
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	267	N/S	N/S
RIVM	186	221	319	553	255	26.6	62.9	69.6	589	488	N/S	N/S	263	N/S	3.52
	184	208	299	516	283	31.1	58.7	81.4	681	527	N/S	N/S	342	N/S	3.37
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	3.34
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	3.48
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	10.2
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	9.56
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	12.1
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	10.1
RMC	156	168	300	423	305	<18.0	<17.6	<17.7	21.5	<17.7	N/S	N/S	N/S	38.5	2.82
	194	178	278	421	289	<17.7	<17.9	<17.5	<17.7	<17.6	N/S	N/S	N/S	37.1	3.30

Appendix 6 ISO Form B: Average As 'stomach & intestine' phase data for As Soils (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	143	152	240	413	302	50.6	46.6	53.4	200	181	40.9	69.7	244	42.7	6.26
DHI	229	260	440	669	390	39.8	9.00	117	566	451	42.9	40.6	279	56.4	6.35
GHENT	159	175	246	411	266	31.8	46.7	92.9	318	517	N/S	N/S	N/S	N/S	8.70
INERIS	230	284	402	771	447	55.4	29.1	33.4	489	106	N/S	N/S	N/S	N/S	5.58
OHIO	192	206	298	581	341	0.99	9.41	24.9	254	128	N/S	N/S	270	N/S	4.23
RIVM	185	214	309	535	269	28.8	60.8	75.5	635	507	N/S	N/S	302	N/S	3.45
RMC	175	173	289	422	297	9.00	9.00	8.87	15.3	9.00	N/S	N/S	N/S	37.8	3.06

N/S Sample not analysed by laboratory

Appendix 7 ISO Form C: As 'stomach & intestine' standard deviation data for As soils (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	10.4	8.30	3.50	8.05	3.09	4.80	20.1**	5.44	40.3	18.1	6.04	3.97	2.24	8.92	2.90
DHI	0.06	17.2	28.1	33.7	2.63	2.47	0.00	44.1*	112	28.9	4.72	0.17	2.01	3.15	2.68
GHENT	-	-	-	12.3	-	-	4.15	-	7.27	-	N/S	N/S	N/S	N/S	3.01
INERIS	5.76	35.6	56.0	16.6	15.5	-	1.91	6.80	1.93	-	N/S	N/S	N/S	N/S	3.49
OHIO	10.3	-	-	-	-	2.33	-	-	-	-	N/S	N/S	2.78	N/S	0.32
RIVM	1.95	12.7	20.5	37.2	27.3	4.46	4.22	11.8	92.8	38.6	N/S	N/S	79.6**	N/S	3.8
RMC	38.1*	9.88	21.9	1.66	16.0	0.00	0.00	0.26	12.5	0.00	N/S	N/S	N/S	1.33	0.47

N/S Sample not analysed by the laboratory, - Only one replicate extracted by the laboratory, * Statistical straggler , ** Statistical outlier

Appendix 8 ISO Form A: As Soils Cd 'stomach' phase data (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	<0.89	<0.89	<0.90	1.53	17.1	3.40	9.56	<0.89	174	115	13.7	13.7	14.7	34.2	0.21
	<0.90	<0.89	<0.89	1.55	17.3	3.80	9.03	3.45	178	118	14.1	14.9	14.7	35.0	0.21
DHI	<0.89	<0.89	<0.90	1.86	18.4	12.6	2.37	88.4	280	122	14.8	7.52	15.7	35.0	0.45
	<0.90	<0.89	<0.90	1.79	18.5	10.2	2.46	94.8	296	143	14.9	6.69	15.6	35.4	0.45
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.21
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.20
GHENT	<0.89	<0.89	<0.89	1.37	16.6	1.64	4.63	51.6	115	108	N/S	N/S	N/S	N/S	0.45
	N/S	N/S	N/S	1.39	N/S	N/S	4.53	N/S	115	N/S	N/S	N/S	N/S	N/S	0.45
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.45
INERIS	<0.91	<0.89	<0.89	1.81	17.3	3.44	13.1	5.02	245	164	N/S	N/S	N/S	35.7	0.45
	<0.91	<0.89	<0.91	1.77	17.9	2.68	12.7	5.09	255	160	N/S	N/S	N/S	33.1	0.45
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	39.4	0.45
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	36.0	0.29
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	36.2	0.28
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.45
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	1.20
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.50
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.27
OHIO	<0.02	<0.02	<0.02	<0.02	19.6	13.0	22.7	83.2	282	156	N/S	N/S	15.6	N/S	0.01
	<0.02	N/S	N/S	N/S	N/S	14.7	N/S	N/S	N/S	N/S	N/S	N/S	17.2	N/S	0.01
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	13.7	N/S	0.01
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	14.9	N/S	N/S
RIVM	<0.90	<0.88	<0.91	1.33	15.4	3.63	6.51	64.1	196	127	N/S	N/S	14.6	N/S	0.21
	<0.87	<0.89	<0.88	1.40	16.2	3.40	6.09	62.7	195	126	N/S	N/S	12.6	N/S	0.27
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.30
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.24
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.28
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.28
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.26
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.32
RMC	<0.90	<0.89	<0.91	1.25	17.8	10.9	11.6	82.0	266	146	N/S	N/S	N/S	29.1	0.25
	<0.89	<0.89	<0.89	1.11	17.7	11.7	13.0	77.7	275	152	N/S	N/S	N/S	29.6	0.26

Appendix 9 ISO Form A: As Soils Cd 'stomach & intestine' phase data (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR1	AR2	NIST 2710	NIST 2711	BGS 102
BGS	<2.34	<2.34	<2.33	<2.32	7.06	8.04	9.55	2.78	202	140	3.74	4.00	13.8	N/S	1.17
	<2.34	<2.34	<2.33	<2.34	6.95	4.95	9.54	3.30	204	140	3.60	4.09	9.59	N/S	1.17
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	6.87	N/S	0.12
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	6.94	N/S	0.12
DHI	<2.33	<2.33	<2.34	<2.34	6.49	7.65	<2.35	85.5	239	113	2.78	2.74	6.83	18.5	1.17
	<2.33	<2.33	<2.33	<2.33	4.97	6.40	<2.35	84.1	251	123	3.17	2.66	6.50	19.3	1.16
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	5.98	N/S	0.12
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	3.82	N/S	0.12
GHENT	<2.34	<2.32	<2.33	<2.34	9.45	<2.31	<2.32	24.2	66.7	84.2	N/S	N/S	N/S	N/S	1.17
	N/S	N/S	N/S	<2.31	N/S	N/S	<2.33	N/S	65.1	N/S	N/S	N/S	N/S	N/S	1.15
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	1.17
INERIS	<2.36	<2.32	<2.30	<2.35	10.5	<2.32	4.87	<2.30	182	N/S	N/S	N/S	N/S	18.6	1.18
	<2.37	<2.32	<2.36	<2.34	10.8	N/S	5.02	<2.35	180	141	N/S	N/S	N/S	21.1	1.17
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	20.1	1.17
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	19.2	0.12
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	14.9	0.77
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.12
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	1.17
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	1.15
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.12
OHIO	<0.05	<0.05	<0.05	<0.05	7.30	<0.05	9.82	35.2	112	83.7	N/S	N/S	7.59	N/S	0.03
	<0.05	N/S	N/S	N/S	N/S	3.87	N/S	N/S	N/S	N/S	N/S	N/S	6.48	N/S	0.03
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	6.48	N/S	0.03
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	6.48	N/S	N/S
RIVM	<2.33	<2.35	<2.38	<2.35	3.94	<2.34	<2.33	29.7	135	87.9	N/S	N/S	6.24	N/S	0.07
	<2.33	<2.45	<2.31	<2.34	4.27	<2.45	<2.33	32.6	137	93.5	N/S	N/S	<22.3	N/S	0.08
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.12
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.11
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.27
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.66
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.54
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.29
RMC	<2.33	<2.36	<2.33	<2.32	9.95	7.84	9.20	74.6	174	83.7	N/S	N/S	N/S	11.5	0.11
	<3.06	<2.34	<2.35	<2.36	9.48	8.23	10.5	72.2	161	78.3	N/S	N/S	N/S	10.1	0.14

Appendix 10 ISO Form A: As Soils Pb 'stomach' phase data (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	8.98	7.39	6.36	8.95	92.8	712	524	180	4479	4375	234	198	3135	926	17.6
	7.93	18.9	6.62	8.56	94.2	853	429	626	4494	4731	231	221	3138	947	16.2
DHI	5.16	7.04	7.47	15.2	129	3107	113	3574	13355	6739	245	134	4091	955	11.9
	4.58	5.02	9.90	14.3	129	1191	105	3851	13647	8221	248	125	4103	964	12.7
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	10.5
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	10.6
GHENT	<2.22	<2.23	<2.23	8.39	94.1	19.7	18.2	292	1278	4816	N/S	N/S	N/S	N/S	8.44
	N/S	N/S	N/S	6.63	N/S	N/S	21.4	N/S	1352	N/S	N/S	N/S	N/S	N/S	1.12
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	1.12
INERIS	4.11	2.47	11.7	8.51	127	286	609	248	8839	8804	N/S	N/S	N/S	984	17.1
	4.12	3.10	8.65	10.5	136	246	635	215	9527	8764	N/S	N/S	N/S	910	18.9
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	1072	18.6
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	980	15.0
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	947	16.1
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	11.4
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	13.7
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	10.5
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	8.68
OHIO	12.2	8.18	8.80	8.48	124	3717	2583	3193	11687	7982	N/S	N/S	4142	N/S	19.9
	8.37	N/S	N/S	N/S	N/S	3747	N/S	N/S	N/S	N/S	N/S	N/S	4088	N/S	20.7
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	3879	N/S	20.7
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4083	N/S	N/S
RIVM	<2.25	3.04	5.78	<2.23	93.2	900	593	1856	8102	6720	N/S	N/S	3568	N/S	10.0
	<2.18	<2.22	2.92	6.71	98.2	877	511	1833	9459	6595	N/S	N/S	4150	N/S	12.3
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	38.3
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	26.8
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	42.8
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	41.9
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	43.9
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	48.8
RMC	<5.83	<5.90	<5.83	<5.80	36.5	99.7	83.3	40.9	202	127	N/S	N/S	N/S	805	9.81
	<7.66	<5.84	<5.88	<5.89	37.2	84.6	67.3	25.4	148	153	N/S	N/S	N/S	854	10.4

Appendix 11 ISO Form A: As Soils Pb 'stomach & intestine' phase data (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	<5.85	<5.84	<5.82	<5.81	48.1	104	241	108	1427	1283	92.6	140	1629	70.4	2.93
	<5.85	<5.84	<5.82	<5.85	47.4	53.3	151	105	1111	1150	90.0	150	1658	18.3	2.91
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	1.34
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.29
DHI	<5.82	<5.82	<5.86	<5.85	<5.84	90.2	6.76	362	3022	3356	54.7	65.7	177	20.0	2.92
	<5.81	<5.83	<5.82	<5.82	<5.84	120	6.43	356	3579	3013	58.3	63.3	126	27.0	2.89
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	207	N/S	0.29
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	98.3	N/S	0.29
GHENT	<5.85	<5.79	<5.82	<5.84	63.5	84.1	48.1	305	1481	4578	N/S	N/S	N/S	N/S	2.91
	N/S	N/S	N/S	<5.76	N/S	N/S	49.6	N/S	1540	N/S	N/S	N/S	N/S	N/S	2.87
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	2.91
INERIS	12.1	11.4	<5.76	7.78	79.3	173	85.3	53.6	3701	N/S	N/S	N/S	N/S	322	2.94
	10.2	17.3	<5.90	8.53	78.3	N/S	95.8	39.8	3837	811	N/S	N/S	N/S	319	2.92
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	77.2	1.71
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	197	42.6
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	210	1.46
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	2.92
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	2.87
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.88
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.83
OHIO	4.91	<0.99	<0.99	<0.99	31.26	5.20	28.7	89.1	1403	803	N/S	N/S	1530	N/S	0.50
	3.96	N/S	N/S	N/S	N/S	5.29	N/S	N/S	N/S	N/S	N/S	N/S	507	N/S	0.50
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	494	N/S	0.50
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	510	N/S	N/S
RIVM	<5.83	<5.88	<5.94	<5.88	8.95	186	250	442	4088	4056	N/S	N/S	1334	N/S	0.35
	<5.83	<6.13	<5.79	<5.85	<5.84	206	235	545	4686	4352	N/S	N/S	2659	N/S	0.20
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.50
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	0.31
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	11.5
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	11.6
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	20.8
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	17.4
RMC	<5.83	<5.90	<5.83	<5.80	36.5	99.7	83.3	40.9	202	127	N/S	N/S	N/S	123	0.52
	<7.66	<5.84	<5.88	<5.89	37.2	84.6	67.3	25.4	148	153	N/S	N/S	N/S	102	0.43

Appendix 12 ISO Form A: Cd Soils As 'stomach' phase data (mg kg^{-1})

Laboratory	ETM 1	ETM 2	ETM 3	ETM 4	NB Cd 1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04
BGS	41.8	247	29.5	<6.80	N/S	N/S	N/S	N/S	N/S
	42.9	252	31.4	<6.83	N/S	N/S	N/S	N/S	N/S
DHI	78.9	281	30.0	<6.86	52.0	<6.73	77.5	27.5	83.8
	85.4	271	28.0	<6.84	53.4	<6.74	71.5	26.8	82.2
GHENT	35.8	664	76.4	<19.8	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	45.6	299	33.4	<6.90	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	71.6	286	31.8	<0.90	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	21.6	243	24.5	<7.29	32.1	N/S	N/S	N/S	N/S
	21.1	228	23.8	<7.06	33.5	N/S	N/S	N/S	N/S
RMC	44.7	274	29.3	<6.81	N/S	N/S	N/S	N/S	N/S
	40.6	256	29.6	<7.00	N/S	N/S	N/S	N/S	N/S

Appendix 13 ISO Form A: Cd Soils As 'stomach & intestine' phase data (mg kg^{-1})

Laboratory	ETM 1	ETM 2	ETM 3	ETM 4	NB Cd 1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04
BGS	18.9	243	24.0	<17.6	N/S	N/S	N/S	N/S	N/S
	24.8	244	22.1	<17.6	N/S	N/S	N/S	N/S	N/S
DHI	23.3	303	19.4	<17.6	39.1	<17.5	41.9	27.6	35.3
	29.9	266	<17.5	<17.6	38.7	<17.6	40.3	29.1	31.7
GHENT	80.5	1190	<51.6	<50.6	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	35.0	297	28.9	<17.8	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	26.7	248	17.1	<2.34	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	<18.7	242	<19.0	<19.1	21.3	N/S	N/S	N/S	N/S
	<18.1	233	<18.0	<17.2	23.4	N/S	N/S	N/S	N/S
RMC	33.3	251	25.3	<17.3	N/S	N/S	N/S	N/S	N/S
	35.9	266	<17.5	<17.3	N/S	N/S	N/S	N/S	N/S

Appendix 14 ISO Form A: Cd Soils Cd 'stomach' phase data (mg kg^{-1})

Laboratory	ETM 1	ETM 2	ETM 3	ETM 4	NB Cd 1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04
BGS	3255	391	80.4	16.2	N/S	N/S	N/S	N/S	N/S
	3284	386	82.0	16.1	N/S	N/S	N/S	N/S	N/S
DHI	3424	382	88.6	17.6	309	91.6	13.7	10.1	16.5
	3644	366	79.4	17.1	321	86.8	13.2	10.3	16.5
GHENT	6864	1080	230	17.4	312	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	3681	402	88.0	17.9	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	3458	383	81.3	16.3	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	3364	351	78.8	13.6	284	N/S	N/S	N/S	N/S
	3294	320	74.6	13.9	317	N/S	N/S	N/S	N/S
RMC	3491	395	84.9	16.5	N/S	N/S	N/S	N/S	N/S
	3730	390	86.7	16.6	N/S	N/S	N/S	N/S	N/S

Appendix 15 ISO Form B: Average Cd 'stomach' phase data for Cd Soils (mg kg^{-1})

Laboratory	ETM 1	ETM 2	ETM 3	ETM 4	NB Cd 1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	NIST 2710	NIST 2711	BGS 102
BGS	3269	389	81.2	16.1	N/S	N/S	N/S	N/S	N/S	14.7	34.6	0.21
DHI	3534	374	84.0	17.3	315	89.2	13.4	10.2	16.5	15.6	35.2	0.33
GHENT	6864**	1080**	230**	17.4	312	N/S	N/S	N/S	N/S	N/S	N/S	0.45
INERIS	3681	402	88.0	17.9	N/S	N/S	N/S	N/S	N/S	N/S	36.1	0.48
OHIO	3458	383	81.3	16.3	N/S	N/S	N/S	N/S	N/S	15.4	N/S	0.01
RIVM	3329	335*	76.7	13.7*	301	N/S	N/S	N/S	N/S	13.6	N/S	0.24
RMC	3610	392	85.8	16.6	N/S	N/S	N/S	N/S	N/S	N/S	29.3*	0.25

N/S Sample not analysed by the laboratory, * Statistical straggler , ** Statistical outlier

Appendix 16 ISO Form C: Cd 'stomach' standard deviation data for Cd soils (mg kg^{-1})

Laboratory	ETM1	ETM2	ETM3	ETM4	NBCd1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	NIST 2710	NIST 2711	BGS 102
BGS	108	254	517**	15.1	N/S	N/S	N/S	N/S	N/S	9.31	N/S	0.64
DHI	1079	146	50.5	10.0	229	68.6	10.1	4.99	9.04	6.67	18.9	1.28
GHENT	674	1134**	138*	13.5	219	N/S	N/S	N/S	N/S	N/S	N/S	1.16
INERIS	2147	321	83.3	12.0	N/S	N/S	N/S	N/S	N/S	N/S	18.8	0.65
OHIO	1037	214	49.7	9.9	N/S	N/S	N/S	N/S	N/S	6.76	N/S	0.03
RIVM	772	203	46.5	5.66	215	N/S	N/S	N/S	N/S	8.70	N/S	0.27
RMC	2289	255	76.1	5.35	N/S	N/S	N/S	N/S	N/S	N/S	10.8*	0.12

N/S Sample not analysed by the laboratory, * Statistical straggler , ** Statistical outlier

Appendix 17 ISO Form A: Cd Soils Cd 'stomach & intestine' phase data (mg kg^{-1})

Laboratory	ETM1	ETM2	ETM3	ETM4	NBCd1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04
BGS	1304	254	63.3	19.0	N/S	N/S	N/S	N/S	N/S
	1171	253	61.9	11.3	N/S	N/S	N/S	N/S	N/S
DHI	938	137	49.6	8.65	230	69.3	10.0	5.37	8.99
	1220	155	51.4	11.4	229	67.8	10.3	4.62	9.09
GHENT	674	1134	138	13.5	219	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	2147	321	83.3	12.0	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	1037	214	49.7	9.85	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	771	201	43.9	5.85	226	N/S	N/S	N/S	N/S
	773	205	49.1	5.47	205	N/S	N/S	N/S	N/S
RMC	2307	237	85.8	5.46	N/S	N/S	N/S	N/S	N/S
	2270	274	66.4	5.23	N/S	N/S	N/S	N/S	N/S

Appendix 18 ISO Form B: Average Cd 'stomach & intestine' phase data for Cd Soils (mg kg^{-1})

Laboratory	ETM1	ETM2	ETM3	ETM4	NBCd1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	NIST 2710	NIST 2711	BGS 102
BGS	29.6	4.99	1.61	0.09	N/S	N/S	N/S	N/S	N/S	0.09	0.77	0.0
DHI	220	15.4	9.14	0.50	11.8	4.89	0.58	0.28	0.00	0.07	0.43	0.14
GHENT	-	-	-	-	-	N/S	N/S	N/S	N/S	N/S	N/S	0.00
INERIS	-	-	-	-	N/S	N/S	N/S	N/S	N/S	N/S	2.26	0.28*
OHIO	-	-	-	-	N/S	N/S	N/S	N/S	N/S	1.47	N/S	0.00
RIVM	69.7	31.3	4.21	0.34	32.3	N/S	N/S	N/S	N/S	1.95	N/S	0.034
RMC	239	5.37	1.80	0.12	N/S	N/S	N/S	N/S	N/S	N/S	0.42	0.01

N/S Sample not analysed by the laboratory, - Only one replicate extracted by the laboratory, * Statistical straggler

Appendix 19 ISO Form C: Cd 'stomach' standard deviation data for Cd soils (mg kg^{-1})

Laboratory	ETM1	ETM2	ETM3	ETM4	NBCd1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	NIST 2710	NIST 2711	BGS 102
BGS	133	0.99	31.4	7.70	N/S	N/S	N/S	N/S	N/S	3.27	N/S	0.61
DHI	282**	17.8	1.77	2.74	0.45	1.51	0.28	0.74	0.10	0.33	0.84	0.60
GHENT	-	-	-	-	-	N/S	N/S	N/S	N/S	N/S	N/S	0.01
INERIS	-	-	-	-	N/S	N/S	N/S	N/S	N/S	N/S	2.35	0.53
OHIO	-	-	-	-	N/S	N/S	N/S	N/S	N/S	0.55	N/S	0.00
RIVM	2.10	4.28	5.24	0.38	20.9	N/S	N/S	N/S	N/S	3.48	N/S	0.22
RMC	37.7	36.6	19.4*	0.22	N/S	N/S	N/S	N/S	N/S	N/S	1.38	0.03

N/S Sample not analysed by the laboratory, - Only one replicate extracted by the laboratory, * Statistical straggler , ** Statistical outlier

Appendix 20 ISO Form A: Cd Soils Pb 'stomach' phase data (mg kg^{-1})

Laboratory	ETM 1	ETM 2	ETM 3	ETM 4	NB Cd 1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04
BGS	615	354	689	38.3	N/S	N/S	N/S	N/S	N/S
	629	362	696	45.3	N/S	N/S	N/S	N/S	N/S
DHI	884	449	664	44.2	921	460	804	896	114
	896	459	627	44.4	938	474	772	867	109
GHENT	14.8	1006	95.5	<6.59	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	730	484	788	45.1	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	862	469	735	38.8	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	294	397	517	13.1	567	N/S	N/S	N/S	N/S
	301	381	471	12.0	640	N/S	N/S	N/S	N/S
RMC	770	396	739	47.3	N/S	N/S	N/S	N/S	N/S
	717	401	751	38.6	N/S	N/S	N/S	N/S	N/S

Appendix 21 ISO Form A: Cd Soils Pb 'stomach & intestine' phase data (mg kg^{-1})

Laboratory	ETM 1	ETM 2	ETM 3	ETM 4	NB Cd 1	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04
BGS	119	254	515	19.0	N/S	N/S	N/S	N/S	N/S
	96.5	253	518	11.3	N/S	N/S	N/S	N/S	N/S
DHI	89.8	<5.85	318	<5.86	478	353	666	7.18	107
	128	8.04	285	<5.86	443	298	646	<5.79	103
GHENT	1054	1252	290	<16.9	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	400	308	615	11.2	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	162	63.0	388	9.74	N/S	N/S	N/S	N/S	N/S
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	7.67	159	147	<6.36	365	N/S	N/S	N/S	N/S
	9.50	173	167	<5.73	331	N/S	N/S	N/S	N/S
RMC	361	190	820	<5.77	N/S	N/S	N/S	N/S	N/S
	349	179	358	<5.78	N/S	N/S	N/S	N/S	N/S

Appendix 22 ISO Form A: Pb Soils As 'stomach' phase data (mg kg^{-1})

Laboratory	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2 TM1	B & V 2 TM2	DNR5 1	DNR5 2	MSE 2
BGS	N/S	N/S	N/S	N/S	N/S	N/S	10.1	<6.66	6.83	<6.75	<6.70	<6.73	<6.74
	N/S	N/S	N/S	N/S	N/S	N/S	10.5	<6.71	<6.79	<6.73	<6.66	<6.70	<6.67
DHI	51.7	108	<6.73	77.5	27.5	83.8	9.63	<6.75	<6.74	<6.71	<6.73	<6.74	<6.73
	62.6	88.3	<6.74	71.5	26.8	82.2	9.40	<6.71	<6.65	<6.74	<6.75	<6.63	<6.73
GHENT	N/S	N/S	N/S	N/S	N/S	N/S	<20.2	<20.2	<19.5	<19.5	<19.2	<20.3	<20.1
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	N/S	N/S	N/S	N/S	N/S	N/S	38.5	9.01	<6.97	<6.67	<6.70	<6.81	<6.70
	N/S	N/S	N/S	N/S	N/S	N/S	38.1	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	N/S	N/S	N/S	N/S	N/S	N/S	<0.90	<0.90	<0.90	<0.90	<0.90	<0.90	<0.90
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	N/S	N/S	N/S	N/S	N/S	N/S	7.4	<6.82	<6.92	<7.34	<7.15	<7.27	<7.36
	N/S	N/S	N/S	N/S	N/S	N/S	8.5	<6.76	<6.58	<6.98	<7.36	<7.29	<7.12
RMC	N/S	N/S	N/S	N/S	N/S	N/S	<6.78	<6.85	<6.68	<7.02	<6.74	<6.67	<6.95
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	<6.77	<6.85	<6.71	<6.75	<6.85	<6.80

Appendix 23 ISO Form A: Pb Soils As 'stomach & intestine' phase data (mg kg^{-1})

Laboratory	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2 TM1	B & V 2 TM2	DNR5 1	DNR5 2	MSE 2
BGS	N/S	N/S	N/S	N/S	N/S	N/S	<47.8	<17.5	<17.5	<17.4	<17.5	<17.5	<17.4
	N/S	N/S	N/S	N/S	N/S	N/S	<48.8	<17.5	<17.5	<17.5	<17.4	<17.5	<17.5
DHI	31.2	19.2	<17.5	41.9	27.6	35.3	<17.5	<17.5	<17.6	<17.4	<17.5	<17.4	<17.6
	33.4	24.8	<17.6	40.3	29.1	31.7	<17.6	<17.5	<17.5	<17.5	<17.6	<17.2	<17.2
GHENT	N/S	N/S	N/S	N/S	N/S	N/S	<52.1	<51.4	<50.7	<50.9	<51.9	<52.5	<52.3
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	N/S	N/S	N/S	N/S	N/S	N/S	<71.8	<17.7	<18.0	<17.4	<17.4	<17.7	<17.4
	N/S	N/S	N/S	N/S	N/S	N/S	<64.8	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	N/S	N/S	N/S	N/S	N/S	N/S	7.94	4.36	<2.34	<2.34	<2.34	<2.34	<2.34
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	N/S	N/S	N/S	N/S	N/S	N/S	<17.9	<16.7	<17.4	<18.8	<19.0	<19.1	<18.4
	N/S	N/S	N/S	N/S	N/S	N/S	<17.1	<16.7	<17.0	<18.8	<18.4	<19.1	<17.7
RMC	N/S	N/S	N/S	N/S	N/S	N/S	<17.3	<17.2	<17.9	<17.8	<17.7	<17.7	<17.3
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	<17.7	<17.3	<17.5	<17.6	<17.1	<17.3

Appendix 24 ISO Form A: Pb Soils Cd 'stomach' phase data (mg kg^{-1})

Laboratory	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2 TM1	B & V 2 TM2	DNR5 1	DNR5 2	MSE 2
BGS	N/S	N/S	N/S	N/S	N/S	N/S	1.51	2.33	19.9	34.4	11.4	14.0	<0.90
	N/S	N/S	N/S	N/S	N/S	N/S	1.86	2.28	18.6	24.9	11.4	13.5	<0.89
DHI	<0.89	<0.89	91.6	13.7	10.1	16.5	1.72	2.45	19.0	23.7	12.3	14.2	<0.90
	<0.90	<0.89	86.8	13.2	10.3	16.5	1.73	2.39	18.8	24.4	12.3	14.1	<0.90
GHENT	N/S	N/S	N/S	N/S	N/S	N/S	3.81	5.84	16.2	57.8	31.1	34.9	<2.67
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	N/S	N/S	N/S	N/S	N/S	N/S	8.31	3.01	19.3	25.3	13.5	14.3	<0.89
	N/S	N/S	N/S	N/S	N/S	N/S	7.54	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	N/S	N/S	N/S	N/S	N/S	N/S	<0.02	<0.02	15.1	23.9	12.2	14.1	<0.02
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	N/S	N/S	N/S	N/S	N/S	N/S	1.49	1.97	14.9	22.1	11.1	12.8	<0.98
	N/S	N/S	N/S	N/S	N/S	N/S	1.41	1.95	15.5	21.5	10.7	12.9	<0.95
RMC	N/S	N/S	N/S	N/S	N/S	N/S	1.39	2.18	18.4	24.0	12.7	14.3	<0.93
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	2.02	19.5	23.2	12.3	14.6	<0.91

Appendix 25 ISO Form A: Pb Soils Cd 'stomach &intestine' phase data (mg kg^{-1})

Laboratory	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2 TM1	B & V 2 TM2	DNR5 1	DNR5 2	MSE 2
BGS	N/S	N/S	N/S	N/S	N/S	N/S	<6.37	<2.34	10.8	10.4	11.0	3.89	<2.32
	N/S	N/S	N/S	N/S	N/S	N/S	<6.51	<2.33	12.2	3.35	3.56	3.88	<2.34
DHI	<2.33	<2.31	69.3	9.98	5.37	8.99	<2.34	<2.33	10.7	9.71	<2.33	<2.33	<2.35
	<2.32	<2.32	67.8	10.3	4.62	9.09	<2.34	<2.33	10.5	10.1	2.35	<2.30	<2.29
GHENT	N/S	N/S	N/S	N/S	N/S	N/S	<6.94	<6.86	13.9	22.6	<6.92	<7.01	<6.98
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	N/S	N/S	N/S	N/S	N/S	N/S	<9.6	<2.36	8.15	16.1	6.75	4.79	<2.32
	N/S	N/S	N/S	N/S	N/S	N/S	<8.63	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	N/S	N/S	N/S	N/S	N/S	N/S	<0.05	<0.05	8.78	11.2	4.26	4.94	<0.05
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	N/S	N/S	N/S	N/S	N/S	N/S	<2.39	<2.23	6.95	8.48	<2.53	<2.54	<2.46
	N/S	N/S	N/S	N/S	N/S	N/S	<2.28	<2.22	6.92	9.24	<2.46	<2.55	<2.36
RMC	N/S	N/S	N/S	N/S	N/S	N/S	<2.30	<2.29	11.3	10.3	2.69	4.94	<2.30
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	<2.36	10.5	11.0	3.85	5.66	<2.31

Appendix 26 ISO Form A: Pb Soils Pb 'stomach' phase data (mg kg^{-1})

Laboratory	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2 TM1	B & V 2 TM2	DNR5 1	DNR5 2	MSE 2
BGS	N/S	N/S	N/S	N/S	N/S	N/S	1479	975	1415	1514	916	1552	1564
	N/S	N/S	N/S	N/S	N/S	N/S	2013	999	1329	1543	986	1604	1615
DHI	42.8	101	460	804	896	114	1962	1402	762	1991	2053	2465	1798
	50.0	92.6	474	772	867	109	1848	1432	709	1970	2097	2447	1739
GHENT	N/S	N/S	N/S	N/S	N/S	N/S	2316	1887	253	2868	1296	855	4858
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	N/S	N/S	N/S	N/S	N/S	N/S	6614	1571	1396	2037	2318	1817	1854
	N/S	N/S	N/S	N/S	N/S	N/S	5804	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	N/S	N/S	N/S	N/S	N/S	N/S	1832	1396	1084	1970	2329	3360	1787
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	N/S	N/S	N/S	N/S	N/S	N/S	1594	1242	995	1747	1565	1633	1554
	N/S	N/S	N/S	N/S	N/S	N/S	1653	1157	944	1693	1462	1644	1539
RMC	N/S	N/S	N/S	N/S	N/S	N/S	1607	1166	1334	1592	1170	1745	1622
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	1100	1397	1602	1125	1797	1810

Appendix 27 ISO Form B: Average Pb 'stomach' data for Pb soils (mg kg^{-1})

Laboratory	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2 TM1	B & V 2 TM2	DNR 5-1	DNR 5-2	MSE 2	NIST 2710	NIST 2711	BGS 102
BGS	N/S	N/S	N/S	N/S	N/S	N/S	1746	987	1372	1529	951	1578	1589	3136	936	16.9
DHI	46.4	96.8	467	788	881	112	1905	1417	735	1980	2075	2456	1768	4097	960	11.4
GHENT	N/S	N/S	N/S	N/S	N/S	N/S	2316*	1887	253	2868*	1296	855	4858**	N/S	N/S	3.56
INERIS	N/S	N/S	N/S	N/S	N/S	N/S	6209**	1571	1396	2037	2318	1817	1854	N/S	979	14.4
OHIO	N/S	N/S	N/S	N/S	N/S	N/S	1832	1396	1084	1970	2329	3360	1787	4048	N/S	20.4
RIVM	N/S	N/S	N/S	N/S	N/S	N/S	1653	1157	944	1693	1462	1644	1539	3859	N/S	33.1
RMC	N/S	N/S	N/S	N/S	N/S	N/S	1607	1133	1366	1597	1148	1771	1716	N/S	829.3	10.1

N/S Sample not analysed by the laboratory, * Statistical straggler, ** Statistical outlier

Appendix 28 ISO Form B: Average Pb 'stomach & intestine' data for Pb soils (mg kg^{-1})

Laboratory	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2 TM1	B & V 2 TM2	DNR 5-1	DNR 5-2	MSE 2	NIST 2710	NIST 2711	BGS 102
BGS	N/S	N/S	N/S	N/S	N/S	N/S	1091	719	341	860	911	841	847	1644	44	1.87
DHI	61.0	83.1	325	656	5.04	105	16.3	106	446	320	66.1	27.8	38.9	152	23	1.60
GHENT	N/S	N/S	N/S	N/S	N/S	N/S	1921	839	261	1916*	915	521	1397	N/S	N/S	2.90
INERIS	N/S	N/S	N/S	N/S	N/S	N/S	2561	839	221	1029	1197	996	963	N/S	225	6.57
OHIO	N/S	N/S	N/S	N/S	N/S	N/S	247	182	169	328	245	418	222	760	N/S	0.50
RIVM	N/S	N/S	N/S	N/S	N/S	N/S	46.8	34.4	215	795	171	39.9	792	1997	N/S	7.83
RMC	N/S	N/S	N/S	N/S	N/S	N/S	839	499	407	791	379	115	703	N/S	112.2	0.48

N/S Sample not analysed by the laboratory, * Statistical straggler

Appendix 29 ISO Form A: Pb Soils Pb 'stomach & intestine' phase data (mg kg^{-1})

Laboratory	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2 TM1	B & V 2 TM2	DNR5 1	DNR5 2	MSE 2
BGS	N/S	N/S	N/S	N/S	N/S	N/S	1073	775	338	1048	1114	829	819
	N/S	N/S	N/S	N/S	N/S	N/S	1108	662	343	673	708	852	874
DHI	58.0	77.8	353	666	7.18	107	13.9	64.1	447	274	43.9	21.2	45.4
	64.0	88.5	298	646	<5.79	103	18.7	149	445	366	88.3	34.5	32.3
GHENT	N/S	N/S	N/S	N/S	N/S	N/S	1921	839	261	1916	915	521	1397
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
INERIS	N/S	N/S	N/S	N/S	N/S	N/S	2610	839	221	1029	1197	996	963
	N/S	N/S	N/S	N/S	N/S	N/S	2512	N/S	N/S	N/S	N/S	N/S	N/S
OHIO	N/S	N/S	N/S	N/S	N/S	N/S	247	182	169	328	245	418	222
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S
RIVM	N/S	N/S	N/S	N/S	N/S	N/S	66.0	42.2	186	736	209	44.8	792
	N/S	N/S	N/S	N/S	N/S	N/S	27.6	26.7	244	855	134	35.1	793
RMC	N/S	N/S	N/S	N/S	N/S	N/S	839	459	409	770	490	149	499
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	540	404	812	268	82.2	906

Appendix 30 Pb 'stomach' standard deviation data for Pb soils (mg kg^{-1})

Laboratory	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2TM1	B & V 2 TM2	DNR 5-1	DNR 5-2	MSE 2	NIST 2710	NIST 2711	BGS 102
BGS	N/S	N/S	N/S	N/S	N/S	N/S	534	23.8	86.3	28.5	69.4	51.7	50.7	2.73	21.0	1.37
DHI	7.13	8.48	14.3	32.1	28.8	4.37	114	29.9	53.0	20.5	43.7	18.3	59.2	12.1	9.03	1.06
GHENT	N/S	N/S	N/S	N/S	N/S	N/S	-	-	-	-	-	-	-	N/S	N/S	4.23
INERIS	N/S	N/S	N/S	N/S	N/S	N/S	810	-	-	-	-	-	-	N/S	59.9	3.64
OHIO	N/S	N/S	N/S	N/S	N/S	N/S	-	-	-	-	-	-	-	116	N/S	0.50
RIVM	N/S	N/S	N/S	N/S	N/S	N/S	58.9	85.8	50.5	54.4	102	11.0	15.2	582*	N/S	15.0**
RMC	N/S	N/S	N/S	N/S	N/S	N/S	-	65.2	63.6	10.4	45.6	51.9	188	N/S	49.5	0.63

N/S Sample not analysed by the laboratory, - Only one replicate extracted by the laboratory, * Statistical straggler , ** Statistical outlier

Appendix 31 ISO Form C: Pb 'stomach & intestine' standard deviation data for Pb soils (mg kg^{-1})

Laboratory	NB Pb 11	NB Pb 9	NBR-255B-04	NBR-256-04	NBR-261-04	NBR-267-04	B & V 1A	B & V 1B	B & V 2 TM1	B & V 2 TM2	DNR 5-1	DNR 5-2	MSE 2	NIST 2710	NIST 2711	BGS 102
BGS	N/S	N/S	N/S	N/S	N/S	N/S	35.9	113	5.03	376	407	22.3	54.7	29.5	52.0	1.29
DHI	6.07	10.8	54.7	20.0	4.29	3.72	4.76	84.7	1.57	91.6	44.4	13.3	13.1	48.8	7.00	1.51
GHENT	N/S	N/S	N/S	N/S	N/S	N/S	-	-	-	-	-	-	-	N/S	N/S	0.02
INERIS	N/S	N/S	N/S	N/S	N/S	N/S	97.3	-	-	-	-	-	-	N/S	102	13.5
OHIO	N/S	N/S	N/S	N/S	N/S	N/S	-	-	-	-	-	-	-	513	N/S	0.00
RIVM	N/S	N/S	N/S	N/S	N/S	N/S	38.4	15.5	57.4**	119	75.7	9.71	1.09	1325	N/S	8.55
RMC	N/S	N/S	N/S	N/S	N/S	N/S	-	80.6	5.39	41.7	222	66.4	407**	N/S	21.3	0.09

N/S Sample not analysed by the laboratory, - Only one replicate extracted by the laboratory, ** Statistical outlier

Appendix 32 ISO Form A: As Soils As 'stomach' phase data 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	189	233	308	618	520	157	259	438	1809	1136	287
	206	226	311	616	548	172	247	441	1767	1092	280
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	374
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	372
RMC	213	269	359	589	N/S	174	167	488	1771	991	338
	226	230	331	599	N/S	178	163	458	1723	1047	343

Appendix 33 ISO Form B: As Average 'stomach' data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	198	230	310	617	534	165	253	440	1788	1114	328
RMC	220	249	345	594	N/S	176	165	473	1747	1019	341

Appendix 34 ISO Form C: As 'stomach' standard deviation data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	17.6	7.20	3.40	2.30	27.3	15.4	12.0	3.20	42.2	43.7	51.9
RMC	12.7	39.5	27.7	10.0	N/S	4.30	3.60	30.8	48.0	56.0	5.10

Appendix 35 ISO Form A: As Soils As 'stomach & intestine' phase data 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	247	270	468	753	605	253	218	323	1482	992	246
	247	234	305	746	617	223	187	329	1546	960	254
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	380
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	407
RMC	175	192	322	501	N/S	199	196	431	1326	930	310
	170	195	321	503	N/S	195	195	362	1393	961	311

Appendix 36 ISO Form B: As Average 'stomach & intestine' data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	247	252	387	749	611	238	202	326	1514	976	322
RMC	172	193	322	502	N/S	197	195	397	1359	946	310

Appendix 37 ISO Form C: As 'stomach & intestine' standard deviation data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	**0.28**	**35.8**	**163**	**6.70**	**11.7**	**29.7**	**30.4**	**6.60**	**63.9**	**32.3**	**84.0**
RMC	**5.10**	**2.82**	**0.90**	**2.10**	N/S	**4.00**	**0.82**	**70.0**	**67.5**	**32.0**	**1.20**

Appendix 38 ISO Form A: As Soils Cd 'stomach' phase data 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	<9.12	<9.23	<9.36	9.12	33.6	24.5	37.8	90.7	260	165	14.2
	<9.76	<8.36	<8.44	9.63	33.3	27.2	37.1	87.0	256	151	13.9
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	14.8
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	14.7

Appendix 39 ISO Form B: Cd Average 'stomach' data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	4.70	4.40	4.40	9.37	33.5	25.9	37.5	88.9	258	158	14.4

Appendix 40 ISO Form C: Cd 'stomach' standard deviation data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	0.32	0.44	0.46	0.50	0.28	2.60	0.73	3.76	3.35	13.4	0.45

Appendix 41 ISO Form A: As Soils Cd 'stomach & intestine' phase data 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	<21.8	<21.7	<23.6	<24.0	59.7	57.5	49.3	60.4	131	96.8	6.49
	<23.5	<21.7	<22.8	29.0	61.5	52.2	49.9	61.7	124	99.0	6.97
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	<24.2
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	<22.1

Appendix 42 ISO Form B: Cd Average 'stomach & intestine' data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	11.3	10.9	11.6	20.5	60.6	54.8	49.6	61.0	128	97.9	6.73

Appendix 43 ISO Form C: Cd 'stomach & intestine' standard deviation data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	0.84	0.00	0.42	17.0	1.80	5.30	0.65	1.26	7.24	2.21	2.80

Appendix 44 ISO Form A: As Soils Pb 'stomach' phase data 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	<22.80	<23.1	<23.4	<21.4	129	2544	2820	2856	10786	8005	3240
	<24.41	<20.9	<21.1	<23.1	144	2908	2742	2825	10493	7601	3077
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4335
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	4254

Appendix 45 ISO Form B: Pb Average 'stomach' data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	11.8	11.0	11.1	11.1	137	2726	2781	2841	10639	7803	3726

Appendix 46 ISO Form C: Pb 'stomach' standard deviation data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	0.80	1.09	1.15	0.86	15.6	364	78.5	30.9	292	403	660

Appendix 47 ISO Form A: As Soils Pb 'stomach & intestine' phase data 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	<54.6	<54.3	94.3	<60.1	82.0	2392	270	296	1315	1058	1346
	<58.8	<54.3	<56.9	<57.2	73.7	2239	292	306	860	1246	1422
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	2848
	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	N/S	2855

Appendix 48 ISO Form B: Pb Average 'stomach & intestine' data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	28.4	27.2	61.4	29.3	77.8	2315	281	301	1088	1152	2118

Appendix 49 ISO Form C: Pb 'stomach & intestine' standard deviation data for As soils 1:1000 ratio (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	NIST 2710
RIVM	2.10	0.00	66.0	1.50	8.30	152	21.2	10.0	455	189	848

Appendix 50 ISO Form A: As Soils As 'stomach & intestine' phase 3 hr data (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	179	161	257	392	280	63.8	115	106	390	335	240	56.3	194	51.0	3.84
	166	172	259	429	318	97.4	106	75.3	251	347	257	58.3	175	45.0	4.09

Appendix 51 ISO Form B: As Average 3 hr 'stomach & intestine' data for As soils (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	173	166	258	410	299	80.6	111	90.7	321	341	249	57.3	184	48.0	3.96

Appendix 52 ISO Form C: As 3 hr 'stomach & intestine' standard deviation data for As soils (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	13.0	10.8	2.38	37.2	38.3	33.6	8.50	30.8	139	11.2	17.4	1.98	19.7	6.04	0.25

Appendix 53 ISO Form A: As Soils Cd 'stomach & intestine' phase 3 hr data (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	<2.33	<2.31	<2.32	<2.32	7.43	5.66	17.8	4.25	217	67.1	2.47	3.34	6.19	15.9	<0.23
	<2.34	<2.32	<2.31	<2.32	8.42	7.14	16.1	3.90	217	71.6	<2.33	3.06	5.90	14.6	<0.23

Appendix 54 ISO Form B: Cd Average 3 hr 'stomach & intestine' data for As soils (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	1.17	1.16	1.16	1.16	7.93	6.40	17.0	4.07	217	69.0	1.82	3.20	6.05	15.0	0.12

Appendix 55 ISO Form C: Cd 3 hr 'stomach & intestine' standard deviation data for As soils (mg kg^{-1})

Laboratory	As1	As2	As3	As4	As5	As6	As7	As8	As9	As10	AR1	AR2	NIST 2710	NIST 2711	BGS 102
BGS	0.02	0.01	0.01	0.00	0.98	1.49	1.70	0.36	0.38	4.40	1.30	0.28	0.21	0.91	0.00

Appendix 56 ISO Form A: As Soils Pb 'stomach & intestine' phase 3 hr data (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	7.92	9.75	13.6	<5.81	59.0	101	1266	131	2501	2987	59.5	91.7	1429	285	1.67
	22.0	8.21	<5.79	<5.80	63.4	191	1175	140	1578	3208	73.5	97.1	1333	268	1.01

Appendix 57 ISO Form B: Pb Average 3 hr 'stomach & intestine' data for As soils (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	15.0	8.98	8.24	2.90	61.2	146	1220	136	2040	3097	66.5	94.0	1381	277	1.34

Appendix 58 ISO Form C: Pb 3 hr 'stomach & intestine' standard deviation data for As soils (mg kg^{-1})

Laboratory	As 1	As 2	As 3	As 4	As 5	As 6	As 7	As 8	As 9	As 10	AR 1	AR 2	NIST 2710	NIST 2711	BGS 102
BGS	14.0	1.50	11.0	0.00	4.50	90.2	90.6	9.47	923	221	14.0	5.36	95.5	16.9	0.66

Appendix 59 Total digestion data for Basta As soils 1-10 (mg kg^{-1})

Sample Code	Al	As	Ca	Cd	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	S	Zn
As 1	11842	10565	9954	41.6	411	357669	5536	902	573	805	21.1	270	16672	41034	1941
As 1	11447	10265	10091	40.5	395	257539	5410	866	554	872	18.8	257	12909	40292	1900
As 1	11972	10692	10648	42.3	414	251909	5688	904	571	912	21.2	267	12197	42222	1974
As 2	11093	15339	5175	29.4	348	320832	5159	778	564	644	20.1	296	17724	30071	1970
As 2	12248	16681	5600	31.9	387	298359	5672	852	600	651	20.4	318	16853	33077	2170
As 2	11559	15872	5597	29.6	363	291271	5330	806	568	677	19.0	299	16137	31710	2058
As 3	15368	12305	3042	25.7	408	262435	6939	1383	531	1635	15.2	395	12550	35524	1921
As 3	18522	14587	3657	30.3	495	258924	8250	1643	623	1946	16.7	474	12920	43548	2284
As 3	15819	12637	3112	26.0	411	257124	7065	1432	545	1599	14.0	413	12703	36990	2014
As 4	17041	11126	24115	29.6	530	254765	6900	1627	427	1430	15.5	495	11804	45559	1902
As 4	17482	11309	24320	30.4	520	208982	7015	1649	439	2029	14.8	509	9870	46361	1963
As 4	19488	12509	27240	33.0	580	188015	7775	1818	480	1600	17.4	560	8873	52272	2121
As 5	33321	6829	26378	43.5	839	151483	12574	4946	664	4141	18.1	827	6406	34063	5573
As 5	31262	6365	25275	40.5	764	137552	11843	4683	627	3857	16.8	795	5757	32489	5256
As 5	31418	6433	25561	40.7	779	133581	11760	4658	624	3687	17.2	814	5610	32874	5333
As 6	25536	230	134439	18.7	1583	220184	8834	11654	2165	5414	10.7	1068	8626	12248	46246
As 6	24104	194	129495	17.4	1411	229787	8264	11149	2029	5232	9.17	1024	8830	11298	48374
As 6	24658	199	129228	17.8	1419	229040	8457	11124	2038	5126	8.87	1046	8743	11156	47977
As 7	36332	300	100368	26.0	1232	121234	12487	15263	1221	4903	13.9	1094	4477	6163	18336
As 7	37105	309	102674	26.2	1287	123941	12851	15521	1259	4391	13.1	1104	4559	6211	18872
As 7	35251	294	97701	25.5	1205	113209	12025	14817	1180	4134	12.2	1037	4422	5903	17791
As 8	27597	290	133391	27.7	1649	264610	9237	12036	2057	5550	11.2	1131	9893	11548	51031
As 8	27059	268	133284	26.5	1605	191406	9229	11867	2041	5458	10.6	1100	7144	11576	37272
As 8	25626	260	126503	26.7	1541	195422	8787	11368	1934	5151	9.97	1058	7297	10749	38545
As 9	29158	4914	81738	358	6130	208748	9176	10413	1232	2793	31.2	1348	27685	15532	5801
As 9	27172	4536	77612	331	5797	182215	8414	9943	1172	2570	28.7	1319	23225	14550	5451
As 9	28927	4922	81301	357	6010	199719	9360	10454	1214	2723	31.2	1370	26276	15349	5734
As 10	28960	4421	59040	215	5719	185670	8891	9238	1123	2428	26.7	1224	22365	15270	4945
As 10	31870	4801	66442	233	6293	156400	9755	10295	1281	2749	30.6	1409	18485	16678	5380
As 10	28660	4362	60298	212	5715	179944	8883	9255	1167	2404	26.6	1266	21503	14807	4874

Glossary

Bioaccessibility- The fraction that is soluble in the gastrointestinal environment and is available for absorption (Paustenbach, 2000).

Bioavailability- The fraction of an administered dose that reaches the central (blood) compartment from the gastrointestinal tract (Paustenbach, 2000).

In vitro- Performed in a laboratory dish or test tube; an artificial environment (Latin for *in glass*)

In vivo- Performed in a living organism.

Repeatability conditions- Conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time. [ISO 3534: 3.15].

Repeatability- Precision estimated under repeatability conditions. [ISO 3534: 3.15].

Reproducibility conditions- Conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment. [ISO 3534: 3.20].

Reproducibility- Precision under reproducibility conditions. [ISO 3534: 3.20].

References

BASTA, N T, FOSTER, J N, DAYTON, E A, RODRIGUEZ, R R, and CASTEEL, S W. 2007. The effect of dosing vehicle on arsenic bioaccessibility in smelter-contaminated soils. *Journal of Environmental Science and Health, Part A*, Vol. 42, 1275 - 1281.

CAVE, M R, and WRAGG, J. 2002. Determination of Major and Trace Cations in Aqueous Samples by Inductively Coupled Plasma Atomic Emission Spectrometry (Varian/Vista). *British Geological Survey UKAS Technical Procedure*, AGN 2.3.5.

CAVE, M R, WRAGG, J, PALUMBO, B, and KLINCK, B A. 2003. Measurement of the Bioaccessibility of Arsenic in UK soils. *Environment Agency*, P5-062/TR1.

ELLICKSON, K M, MEEKER, R J, GALLO, M A, BUCKLEY, B T, and LIOY, P J. 2001. Oral bioavailability of lead and arsenic from a NIST standard reference soil material. *Archives of Environmental Contamination and Toxicology*, Vol. 40, 128-135.

GLAISTER, P. 2005. Robust linear regression using Theil's method. *Journal of Chemical Education*, Vol. 82, 1472-1473.

GRUBBS, F E. 1950. Sample Criteria for testing outlying observations. *Ann. Math. Stat.*, Vol. 21, 27-58.

ISO. 5725-2:1994,Accuracy (Trueness and Precision) of measurement methods and results. Part2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

OOMEN, A G. 2000. Determination of oral bioavailability of soil-borne contaminants. PhD thesis, University of Utrecht.

OOMEN, A G, HACK, A, MINEKUS, M, ZEIJDNER, E, CORNELIS, C, SCHOETERS, G, VERSTRAETE, W, VAN DE WIELE, T, WRAGG, J, ROMPELBERG, C J M, SIPS, A, and VAN WIJNEN, J H. 2002. Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. *Environmental Science & Technology*, Vol. 36, 3326-3334.

PAUSTENBACH, D J. 2000. The practice of exposure assessment: A state-of-the-art review (Reprinted from Principles and Methods of Toxicology, 4th edition, 2001). *Journal of Toxicology and Environmental Health-Part B-Critical Reviews*, Vol. 3, 179-291.

R DEVELOPMENT CORE TEAM. 2007. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*.

RODRIGUEZ, R R, BASTA, N T, CASTEEL, S, and PACE, L. 1999. An in vitro gastrointestinal method to estimate bioavailable arsenic in contaminated soils and solid media. *Environmental Science & Technology*, Vol. 33, 642-649.

RODRIGUEZ, R R, BASTA, N T, CASTEEL, S W, ARMSTRONG, F P, and WARD, D C. 2003. Chemical extraction methods to assess bioavailable arsenic in soil and solid media. *Journal of Environmental Quality*, Vol. 32, 876-884.

SCHRODER, J L, BASTA, N T, CASTEEL, S W, EVANS, T J, PAYTON, M E, and SI, J. 2004. Validation of the in vitro gastrointestinal (IVG) method to estimate relative bioavailable lead in contaminated soils. *Journal of Environmental Quality*, Vol. 33, 513-521.

SCHRODER, J L, BASTA, N T, SI, J T, CASTEEL, S W, EVANS, T, and PAYTON, M. 2003. In vitro gastrointestinal method to estimate relative bioavailable cadmium in contaminated soil. *Environmental Science & Technology*, Vol. 37, 1365-1370.

SNEDECOR, G W, and COCHRAN, W G. 1980. *Statistical Methods.* (Iowa State University Press.)

THOMPSON, M, and WALSH, J M. 1983. Chapter 5 Multielement applications of ICP in applied geochemistry, 5.4.4. *A Handbook of Inductively Coupled Plasma Spectrometry*, (Ist Edition edition). 144-145. (Glasgow and London: Blackie.)

VAN DE WIELE, T R, OOMEN, A G, WRAGG, J, CAVE, M, MINEKUS, M, HACK, A, CORNELIS, C, ROMPELBERG, C J M, DE ZWART, L L, KLINCK, B, VAN WIJNEN, J, VERSTRAETE, W, and SIPS, A E N J A M. 2007. Comparison of five in vitro digestion models to in vivo experimental results: Lead bioaccessibility in the human gastrointestinal tract. *Journal of Environmental Science and Health, Part A*, Vol. 42, 1203 - 1211.

WRAGG, J. 2005. A Study of the Relationship between Arsenic Bioaccessibility and its Solid Phase Distribution in Wellingborough Soils. Nottingham.

WRAGG, J, and KLINCK, B. 2007. The bioaccessibility of lead from Welsh mine waste using a respiratory uptake test. *Journal of Environmental Science and Health, Part A*, Vol. 42, 1223 - 1231.