Lead Bioaccessibility Testing

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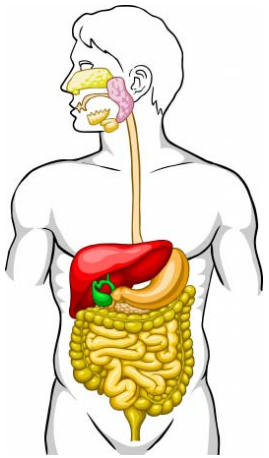
Bioaccessibility & Bioavailability



- Bioavailability
  - In-vivo availability
- Bioaccessibility
  - In-vitro availability
- Laboratory testing is in-vitro
- Simulated human digestive system
- Methodologies recognised to produce results that correlate with bioavailability
- % Bioaccessible Fraction

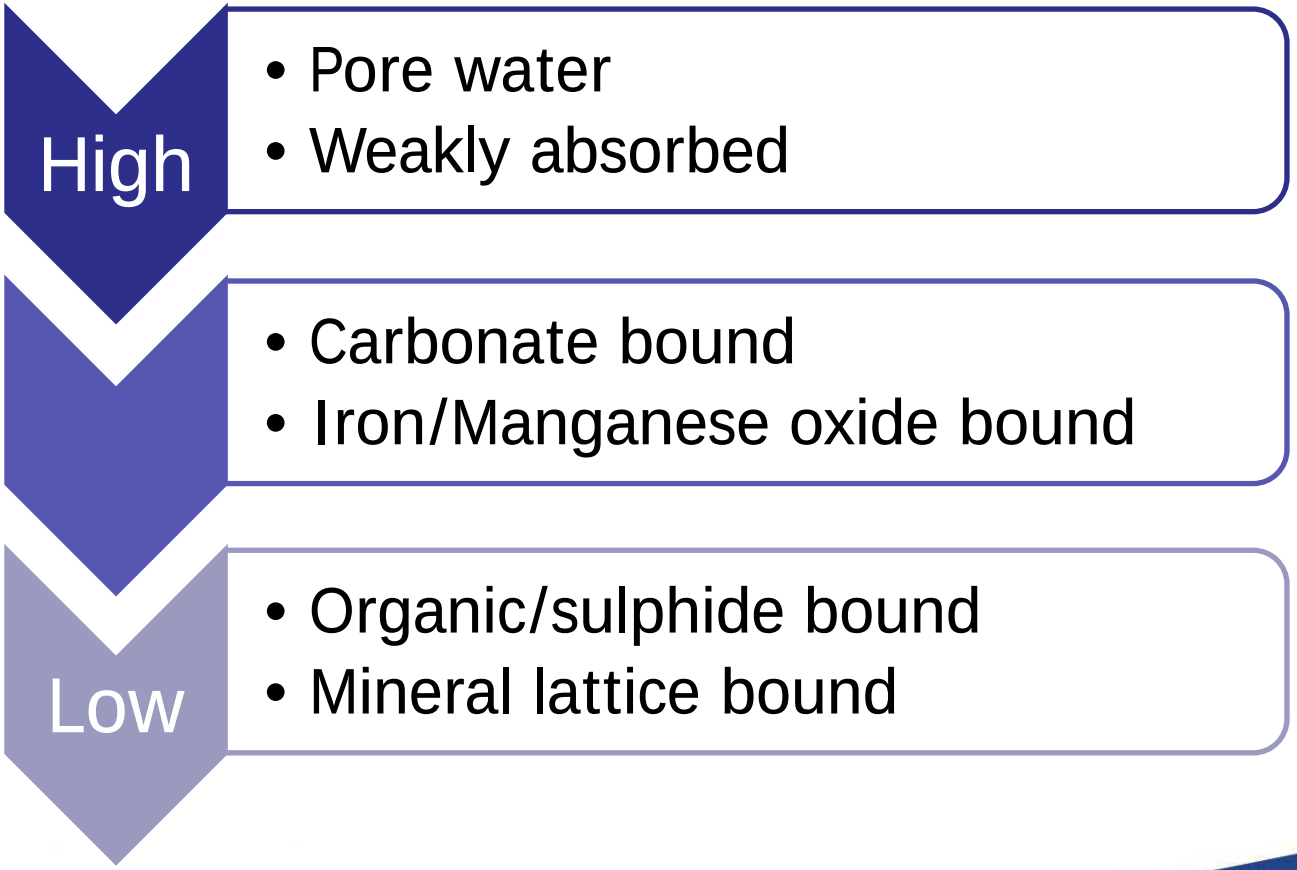
  = [Bioaccessible] / [total] x 100
- Maximum concentration [Bioaccessible] used for calculation

Method Validation

- Data for bioaccessibility (*in-vitro*) assessed against bioavailability (*in-vitro*)
- Bioavailability assessed using animals as a surrogate
  - Juvenile swine model
- Empirical relationship between concentrations from the *in-vitro* method and *in-vivo* studies established
- Validated using a variety of soil types, metal concentrations, either naturally occurring or anthropogenic.

Bioaccessibility

- Total > Acid Extractable > Bioaccessible > Bioavailable



Lead Mineralogy - Bioavailability

High
- Cerrusite
- Mn(M) Oxide

- Lead Phosphate
- Lead Oxide

Low
- Fe(M) Sulphate
- Anglesite
- Galena
- Pb(M) Oxide
- Fe(M) Oxide

Bioavailability

(USEPA OSWER 9285.7-77, 2007)

Selecting a Method

- Guideline documents:
  - ISO/TS 17924 (2007) Technical Specification
    - Guidelines on application and selection
  - Environment Agency Technical Report (2002)
    - Critical review
    - Recommends validated methods
    - Holistic approach with geochemistry
    - Analysis of reference materials
    - Clear reporting

Lead Specific- Recommendations

- Pb solubility pH dependent, Pb soluble at stomach pH
- Intestine conditions, Pb precipitation / insolubility as chlorophosphates and other compounds, excreted as solid
- Bioaccessibility testing simulates worst case scenario, stomach testing conditions most suitable for Pb
  - ISO/TS 17924 (2007) Technical Specification

Selected Laboratory Methods

- PBET (Ruby, 1996) Physiologically Based Extraction Test
  - Modified for ease of performing test
  - Metals including arsenic
- SBET (Drexler, 1999) Simplified Bioaccessibility Extraction Test
  - USEPA 9200.1-86, Nov 2009 (IVBA)
  - Validated for lead
- BARGE UBM (BGS, 2009)
  - Inorganic and organic contaminants (fed or fasted state), validated with bioavailability data, inter-laboratory and inter-method comparisons
- DIN 19738 (2004)
  - Organic and inorganic contaminants, fasted model
- FOREhST (2010)
  - Fed Organic Estimation human Simulation Test, organics

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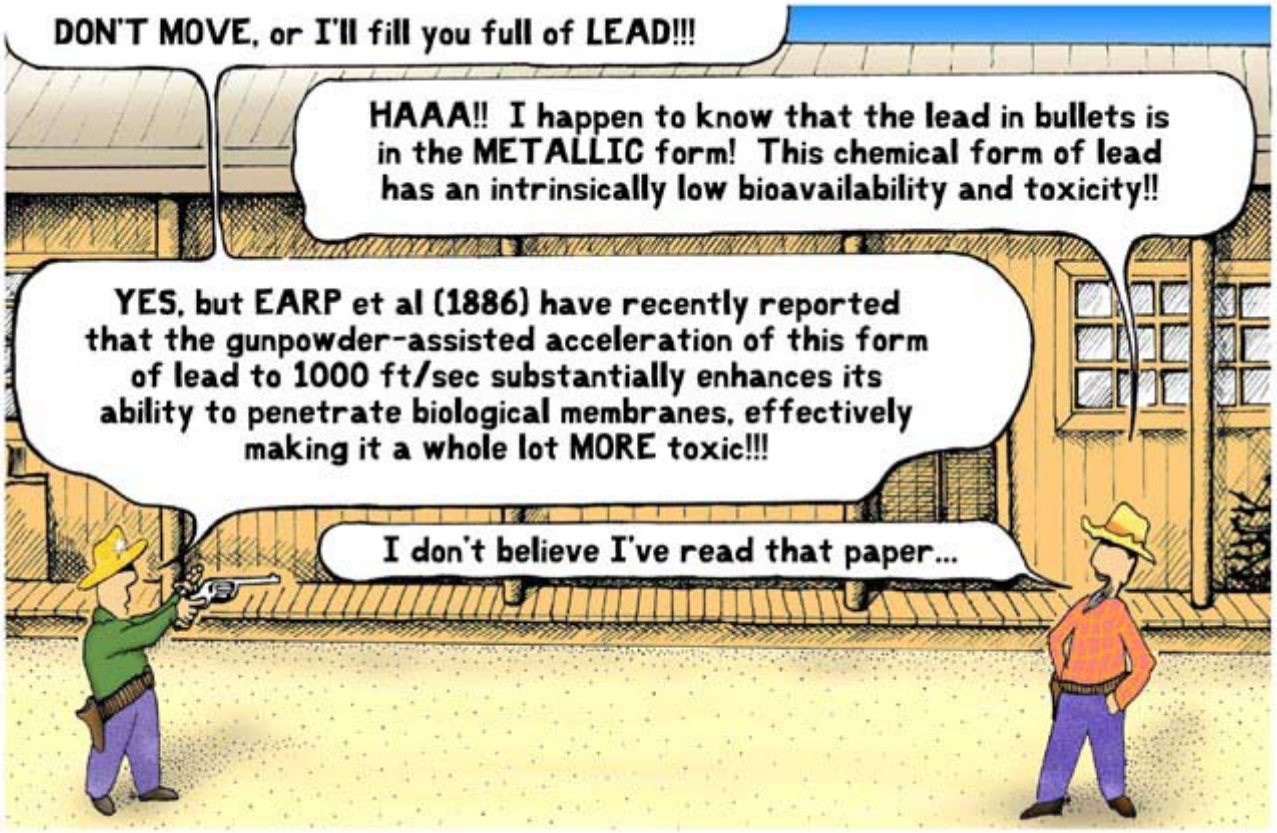
Sample Preparation

- Samples received in small amber jars (PTFE seal)
- Samples air dried to constant weight at < 30 °C (MCERTS)
- Samples sieved and crushed to pass 250 µm sieve
- 250 µm particle size optimum for adherence to children's hands

Analysis of Total Pb

- MCERTS validated and accredited method
- Aqua-regia extraction:
  - 1 g dry sample
  - 2.5 ml HNO_3
  - 7.5 ml HCl
  - Heat under reflux in digestion block for 1 hr 15 mins, at 115 °C
  - Allow to cool, dilute extract to 50 ml with deionised water
  - Filter extract and analyse by ICP-OES

Methods of Analysis



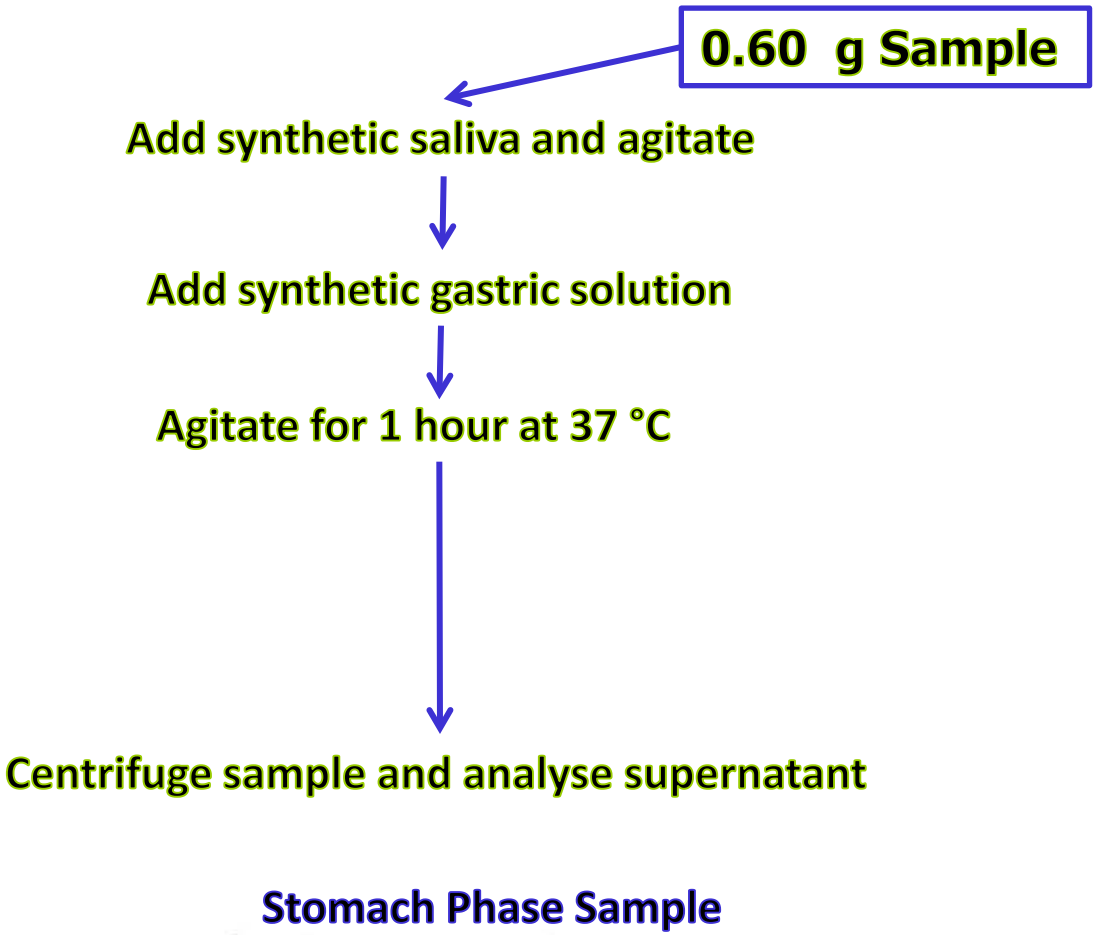
Environmental Scientists in the Wild West

Cartoon by Nick D Kim, Strangematter.net

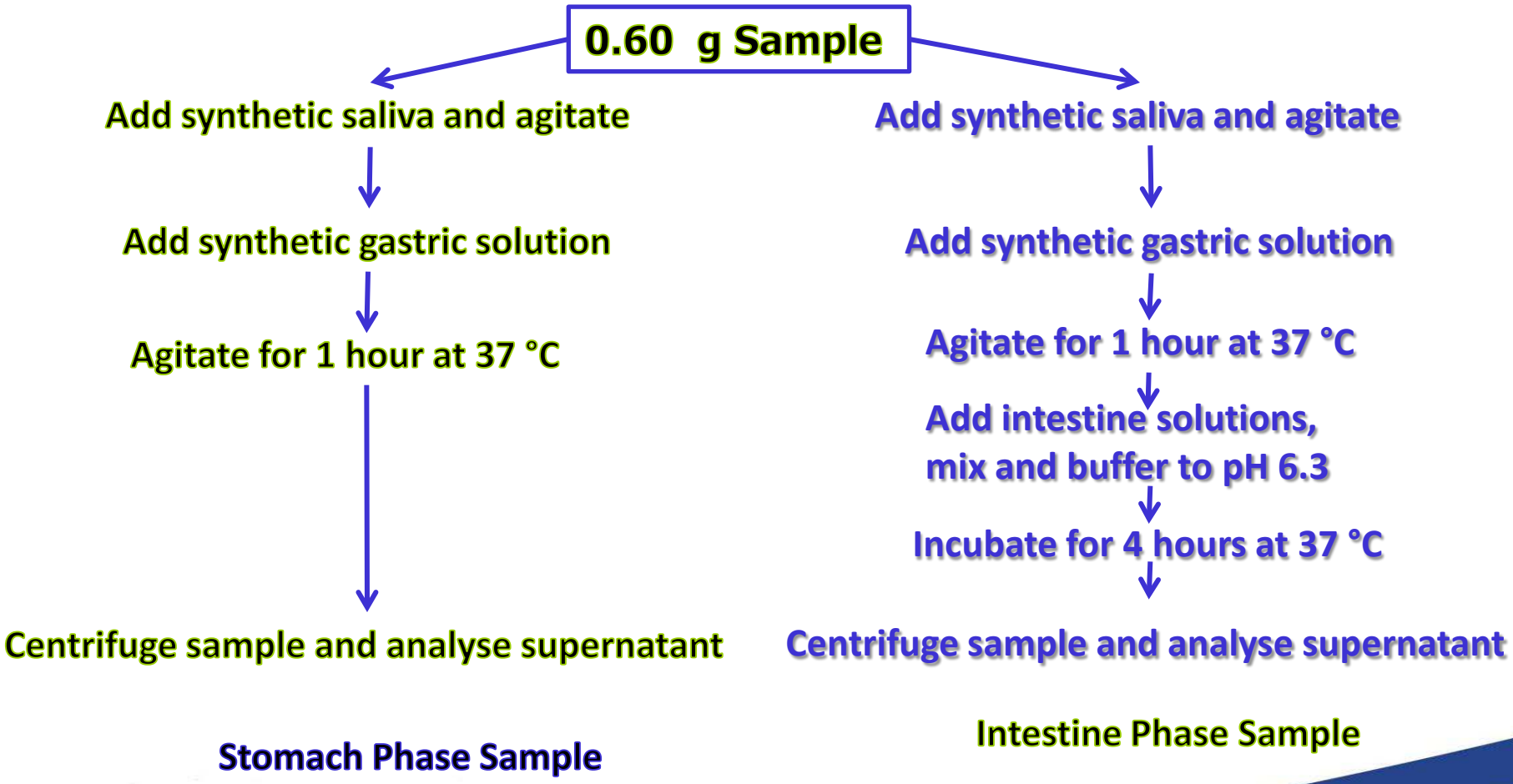
Methodology - UBM

- Developed by BARGE, BGS preferred methodology
- Standard procedure
- Physiologically based, validated against the juvenile swine model for various metals including Pb
- i2 recommended this method where a synergistic approach is required (various metals, more cost effective)
- Gastric (stomach) and intestinal bioaccessibility assessed
- Linear regression indicates good correlation for stomach phase, poorer for stomach and intestine phase
- Analysis of extract solutions by ICP-OES

Testing Procedure - Summary



Testing Procedure - Summary



UBM Methodology Data

Total Pb mg/kg	Stomach		Stomach + Intestine	
	Pb mg/kg	% Bioaccessible	Pb mg/kg	% Bioaccessible
40.674	17.63	43.34	0.00	0.00
53.601	17.88	33.35	0.00	0.00
315	191.75	60.87	0.88	0.28
540.06	321.75	59.58	3.81	0.70
582.09	36.18	6.22	2.49	0.43
604.7	141.74	23.44	53.38	8.83
786.11	70.87	9.02	26.69	3.39
1080.12	643.51	59.58	7.61	0.70

Methodology – EPA 9200 / SBET

- Simplified Bioaccessibility Extraction Test established technique for Lead
- EPA 9200 Method : *in-vitro* bioaccessibility (IVBA)
- Standard method: defines conditions, equipment, permissible deviations from standard procedure, reporting and quality control
- Validated using juvenile swine model:
  - Relative Bioavailability (RBA) correlated to IVBA
- Gastric solution 0.4 M Glycine, acidified to pH 1.5 using HCl
- 1 g soil extracted with 100 ml simulated gastric solution @ 37 °C for 1 hour
- pH checked and/or adjusted during extraction
- Sample allowed to settle (up to 4 hours) and supernatant filtered at 0.45 µm, acidified to 0.1 % HNO_3, analysis by ICP-OES

Experimental Data

Total Pb mg/kg	Extract Pb mg/kg	IVBA -	IVBA %	RBA -
40.674	17.98	0.44	44	0.36
53.601	30.75	0.57	57	0.48
315	209.3	0.66	66	0.56
582.09	248.68	0.43	43	0.35
604.7	149.6	0.25	25	0.19
1080.12	806.5	0.75	75	0.63

- RBA = 0.878*IVBA – 0.028
- Samples prepared from various contaminated land sites, mixed matrices of loam, sand and clay

Method Quality Control

- Samples:
  - Homogenous, sample size sufficient for small scale heterogeneity to be mitigated
- Confidence in analytical data:
  - Standard methods of analysis for both *in-vitro* test and analysis of extracts
- Quality Control Samples (minimum once per batch)
  - Duplicates of test samples
  - Recovery tests on fraction matrices
  - System and extract solution blanks
  - Routine AQC (for both test itself and analytical technique used to measure metal)
  - Certified or In House Reference materials

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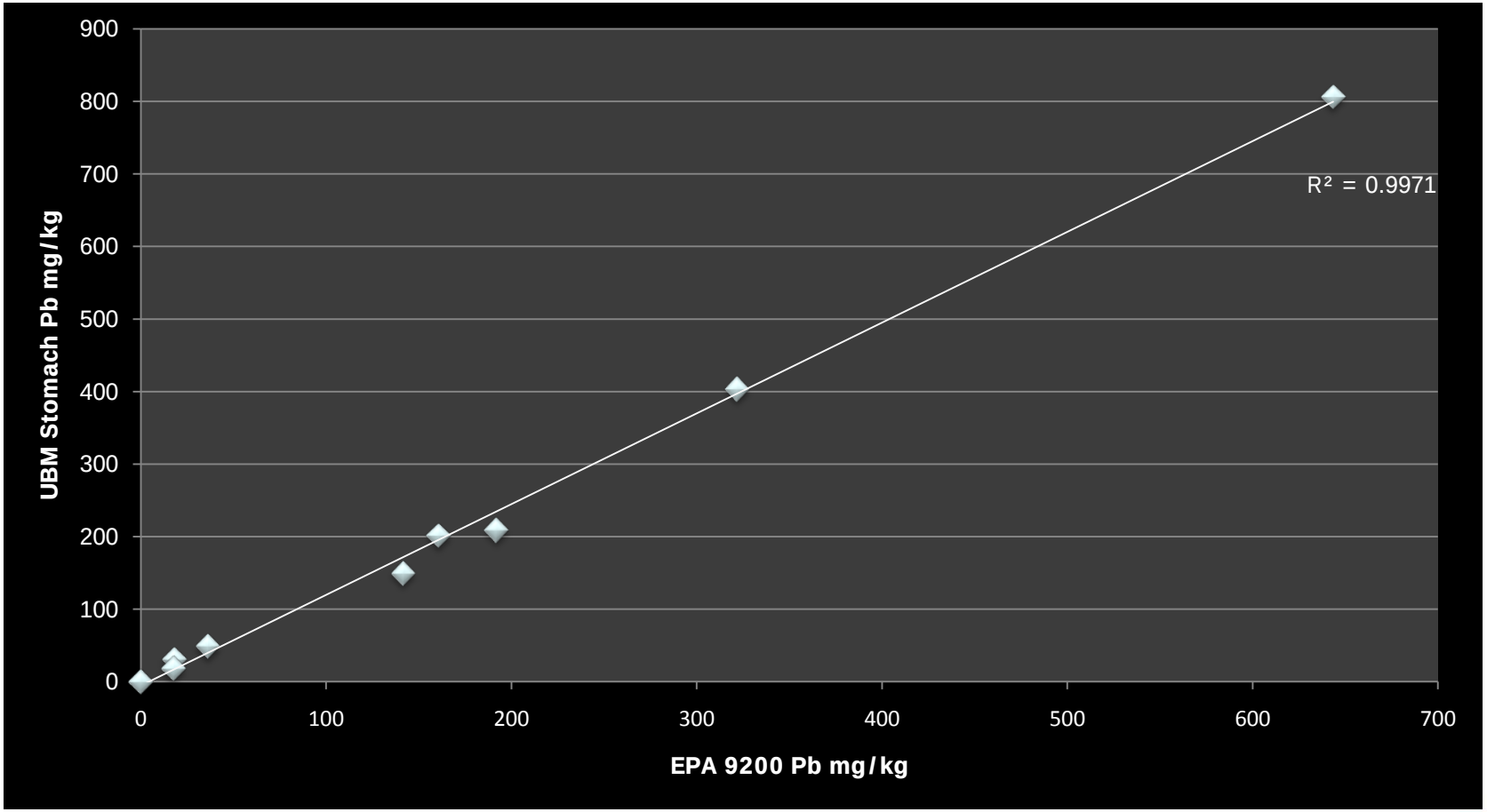
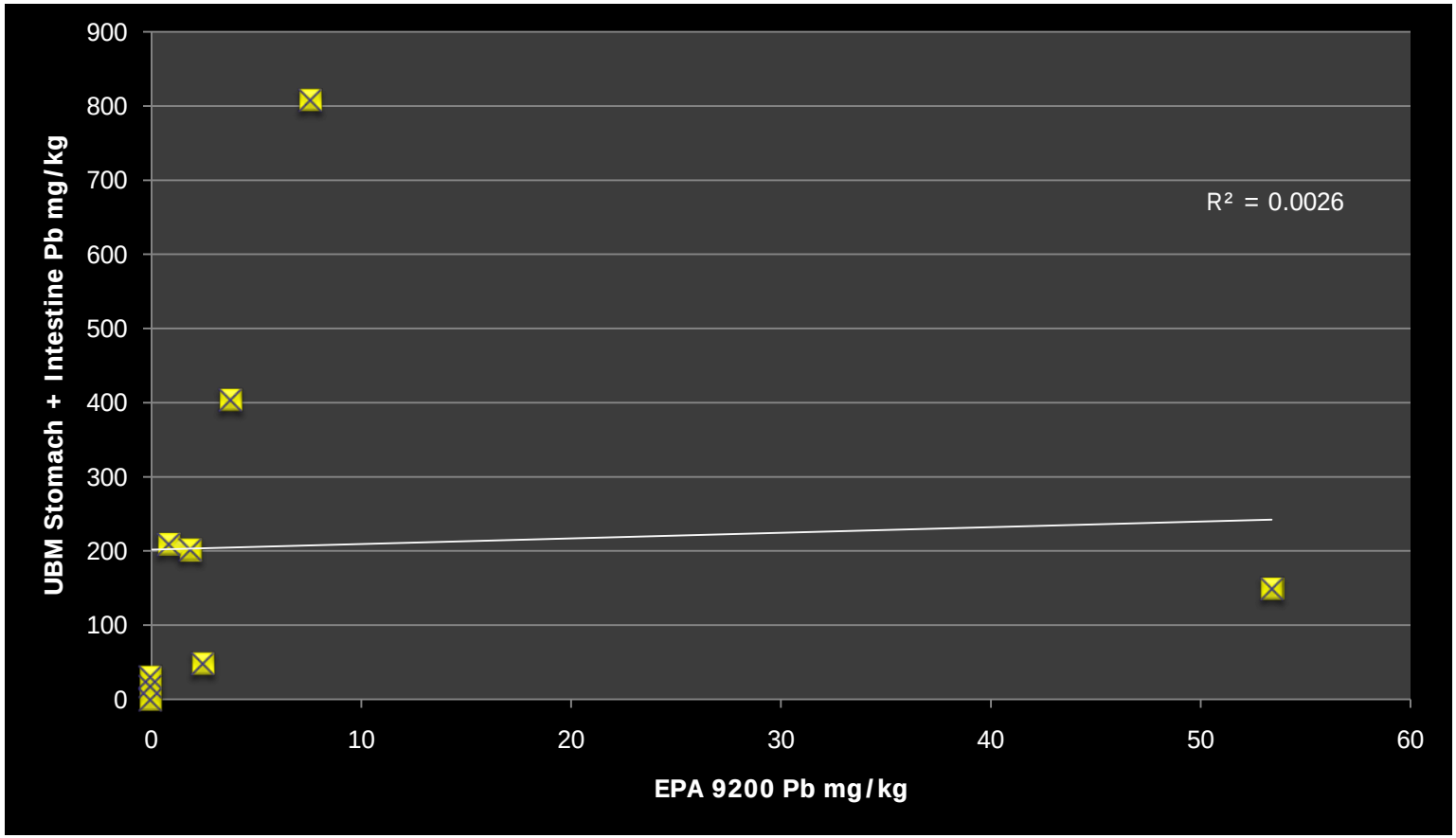
- Samples:
  - Homogenous, sample size sufficient for small scale heterogeneity to be mitigated
- Confidence in analytical data:
  - Standard methods of analysis for both *in-vitro* test and analysis of extracts
- Quality Control Samples (minimum once per batch), must meet defined criteria for batch to pass and be reported
  - Duplicates of test samples
  - Recovery tests on fraction matrices
  - System and extract solution blanks
  - Routine AQC (for both test itself and analytical technique used to measure metal)
  - Certified or In House Reference materials

Comparison of Methods



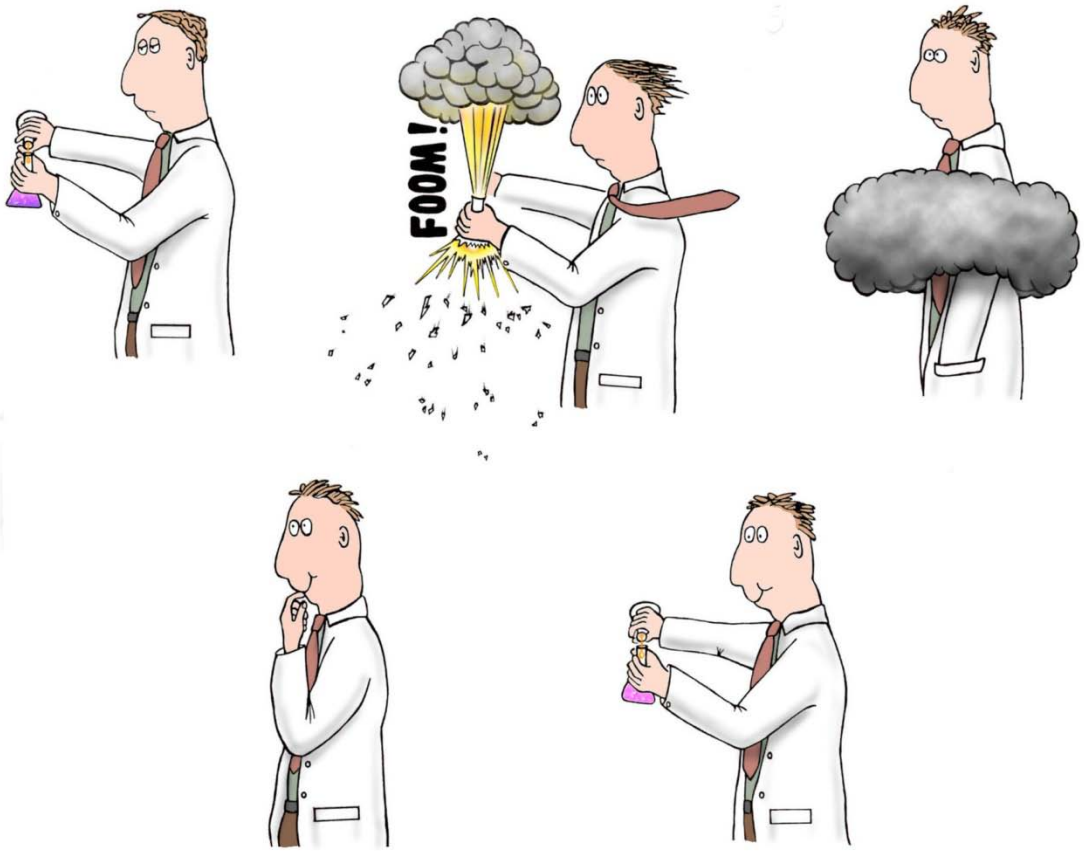
Comparison of Methods



Pb Bioaccessibility

- Bioaccessibility – in-vitro test
  - Laboratory testing
- Selecting methods
  - Suitable for Pb, worst case scenario
- UBM Methodology
- EPA 9200 Methodology
- Correlation between methods, real contaminated land samples

Thank you



Cartoon by Nick D Kim, Strangematter.net

Use of Bioaccessibility Data in Lead Risk Assessment

Society of Brownfield Risk Assessment
2011 Workshop

Lead Risk Assessment for Contaminated Land: The Key Issues

The Mechanics Institute, Manchester
21 June 2011

MIKE QUINT AND ED STUTT

Contents

- Introduction
- US Approaches
- Dutch Approaches
- Practical Experience
- Conclusions

Introduction

- *"The assumption in the CLEA software is that the relative bioavailability is one (that is, the absolute bioavailability of the chemical in the soil sample is the same as the absolute bioavailability in the media used in the relevant toxicological studies on which the HCV is based)."*

CLEA Software (Version 1.05) Handbook. Science Report SC050021/SR4. Environment Agency, 2009.

US Approaches

- Absolute bioavailability (ABA) is the ratio of the amount absorbed over the amount ingested
- ABA of 'soluble lead' (eg, lead in drinking water or food) assumed to be ~50% (as a long-term average)
- $ABA_{soil} = ABA_{soluble} \times RBA_{soil}$
- Based on a literature review, the USEPA estimate the relative bioavailability (RBA) of lead in soil to be approximately 60%
- ABA of lead in soil is therefore approximately 30% (this is used as a default in the US EPA's IEUBK model)
- Can vary on a site-specific basis

US Approaches (cont)

- USEPA *in vitro* bioaccessibility assay (IVBA) methodology:
  - utilises a test system of 0.4 M glycine adjusted to pH 1.5 to resemble fasting conditions within the human stomach. This is rotated for 1 hr in a water bath at 37°C
  - relatively cheap at £50-60 per sample BUT it only seems to work for lead and not other metals
- Based on the relationship between results for *in vitro* bioaccessibility and measurements of relative bioavailability (RBA) determined by the juvenile swine test, the USEPA (2007) have derived the following relationship between *in vitro* bioaccessibility (IVBA) and RBA:

$$RBA = 0.878*IVBA - 0.028 \ (r^2 = 0.924)$$

(Validation Assessment of *In Vitro* Lead Bioaccessibility Assay for Predicting Relative Bioavailability of Lead in Soils and Soil-like Materials at Superfund Sites . US EPA 2009. OSWER 9200.3-51)

Dutch Approaches

- Considerable amount of work done on the differences in the bioavailability of lead under fasted and fed conditions (based on the observation that lead is better absorbed in fasted than in fed conditions).
- Dutch Soil Intervention Value (DIV) for lead has a "generic intervention correction factor" of 0.74 (based on the 80th percentile of measured / assumed RBA factors).
- Previously, a provisional 'relative absorption factor' value of 0.6 was used as a default value in human health risk assessment.
- Variation in lead RBA has also been linked with variation in organic matter content.

Dutch approaches (cont)

- RIVM *in vitro* bioaccessibility test:
  - More complex than the USEPA's IVBA
  - Physiologically based, with optimisation of certain parameters including soil loading, temperature, pH and retention time.
  - Comprises mixing with a saliva analogue and two sequential extraction stages to simulate both stomach and intestinal compartments.
- Can use the results in site-specific risk assessment:
  - "...the calculated lead exposure due to introduction of a relative bioavailability is decreased when the bioaccessibility of lead from soil is less than 50%. When bioaccessibility of lead from soil is higher than 50%, a relative bioavailability greater than 1 is obtained."

Oomen et al, 2006. How can information on oral bioavailability improve human health risk assessment for lead-contaminated soils? Implementation and scientific basis. RIVM report 711701042/2006

Practical Experience – Site 1

- 15 shallow soil samples analysed for lead bioaccessibility by Unified BARGE Method (UBM)
- Results ranged from 41-95%.
- Higher than expected and would be higher than generic values used in other jurisdictions.
- Material with highest measurements of lead bioaccessibility (90%+) was ash-type fill and not 'typical soil'.

Practical Experience – Site 2

- More like 'typical soil' but still some relatively high measurements of bioaccessibility
- Utilised UBM and IVBA approaches
  - Bioaccessibility measured by UBM: 27-52%
  - Bioaccessibility measured by IVBA: 27-75%
- Some indication of a consistent relationship in measured values with IVBA giving higher bioaccessibility than UBM
- IVBA result of 74.5% can be converted to a RBA of 62.6% (see above)

	% Bioaccessibility	
	IVBA	UBM
Sample 1	74.5	52.3
Sample 2	32.7	27.2
Sample 3	63.4	44.0

Conclusions

- Site-specific bioaccessibility measurements of lead can be used (with care!) in risk assessment
- As with all laboratory testing, the methodology used can influence the results
- Validation of *in vitro* methods with *in vivo* data is important
- The USA and the Netherlands have both developed testing protocols and guidance on the use of *in vitro* bioaccessibility measurements in risk assessment
- Basis of the dose-response criteria (eg, HCV) is important

Thanks for listening!

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