Lead Bioaccessibility Testing

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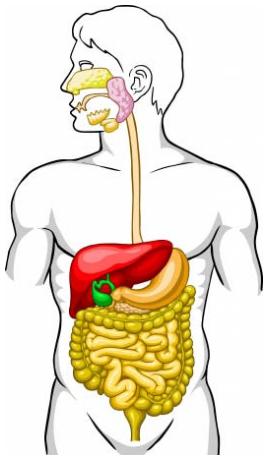
Bioaccessibility & Bioavailability



- 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

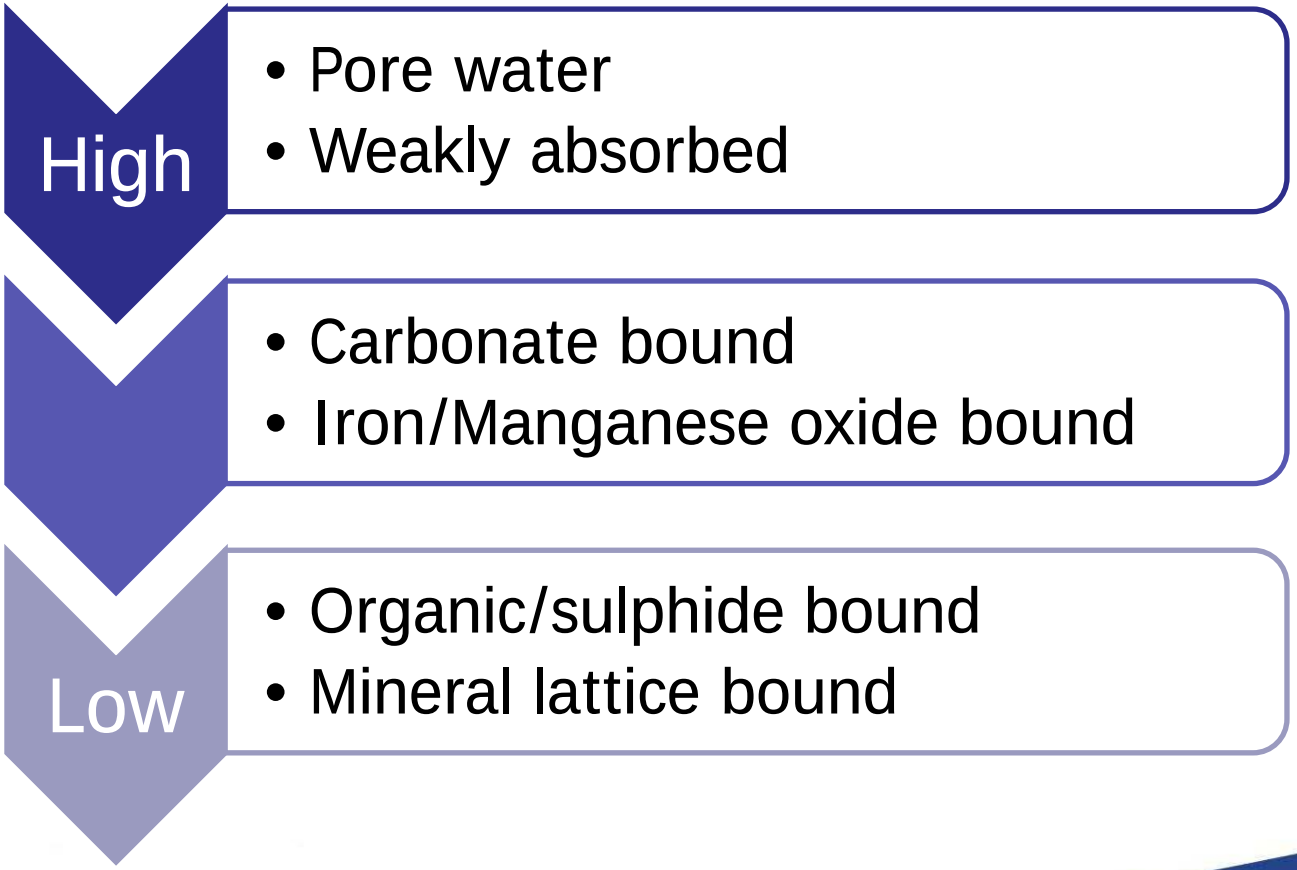
  = [Bioaccessible] / [total] x 100
- Maximum concentration [Bioaccessible] used for calculation

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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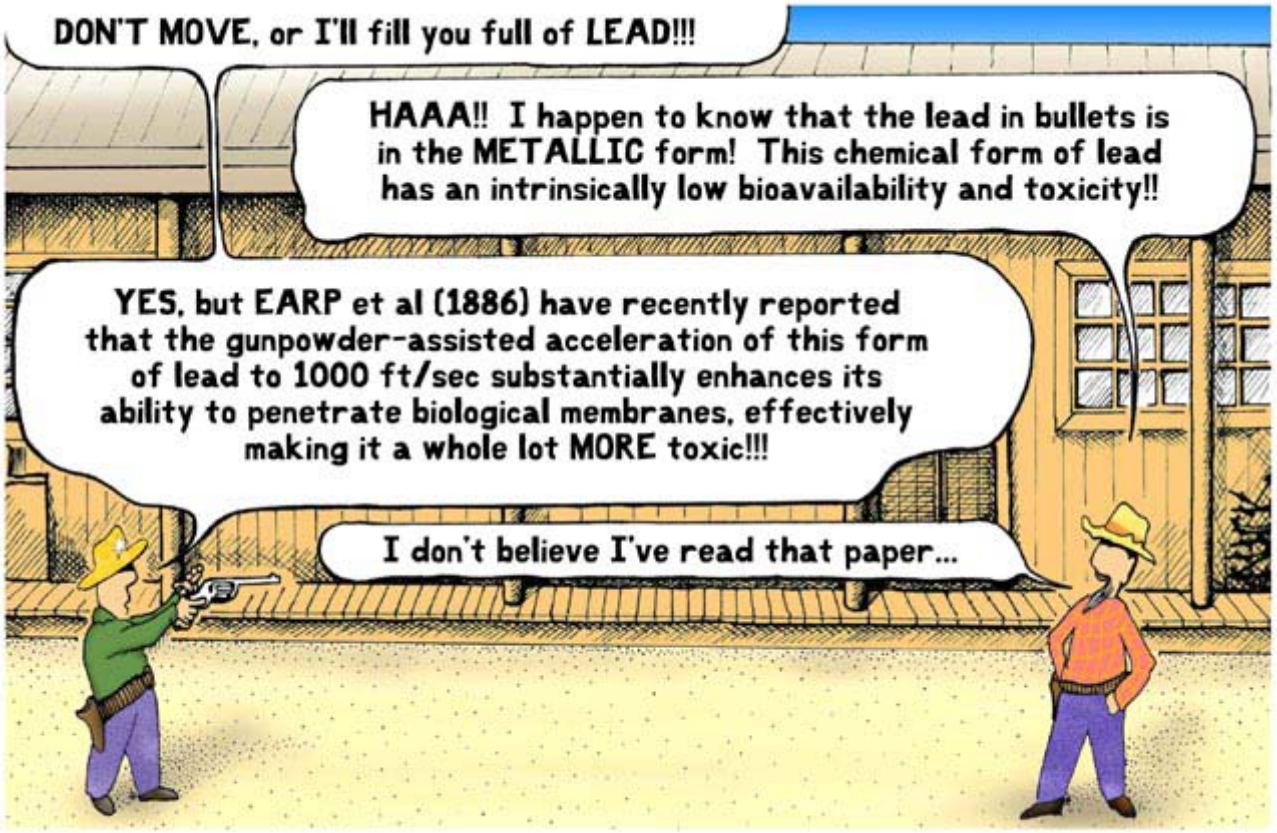
Sample Preparation

- 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

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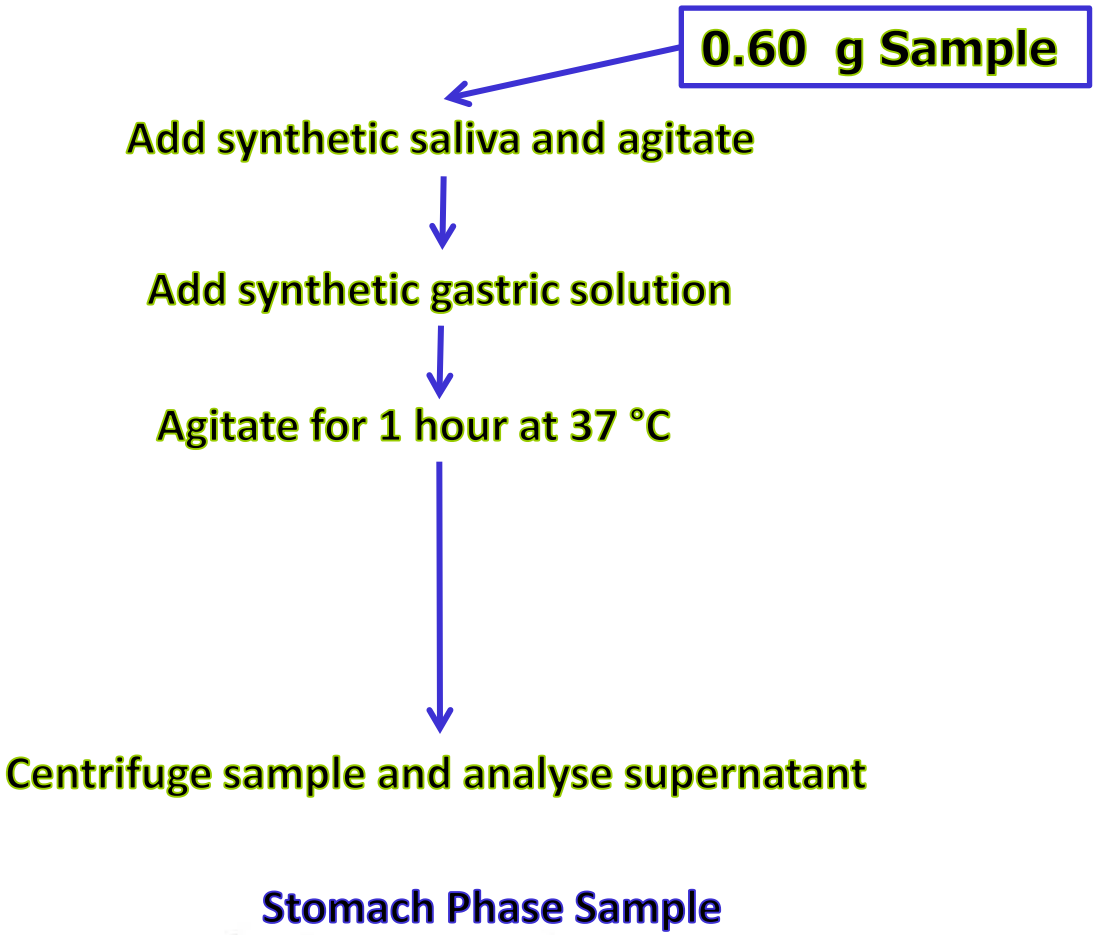
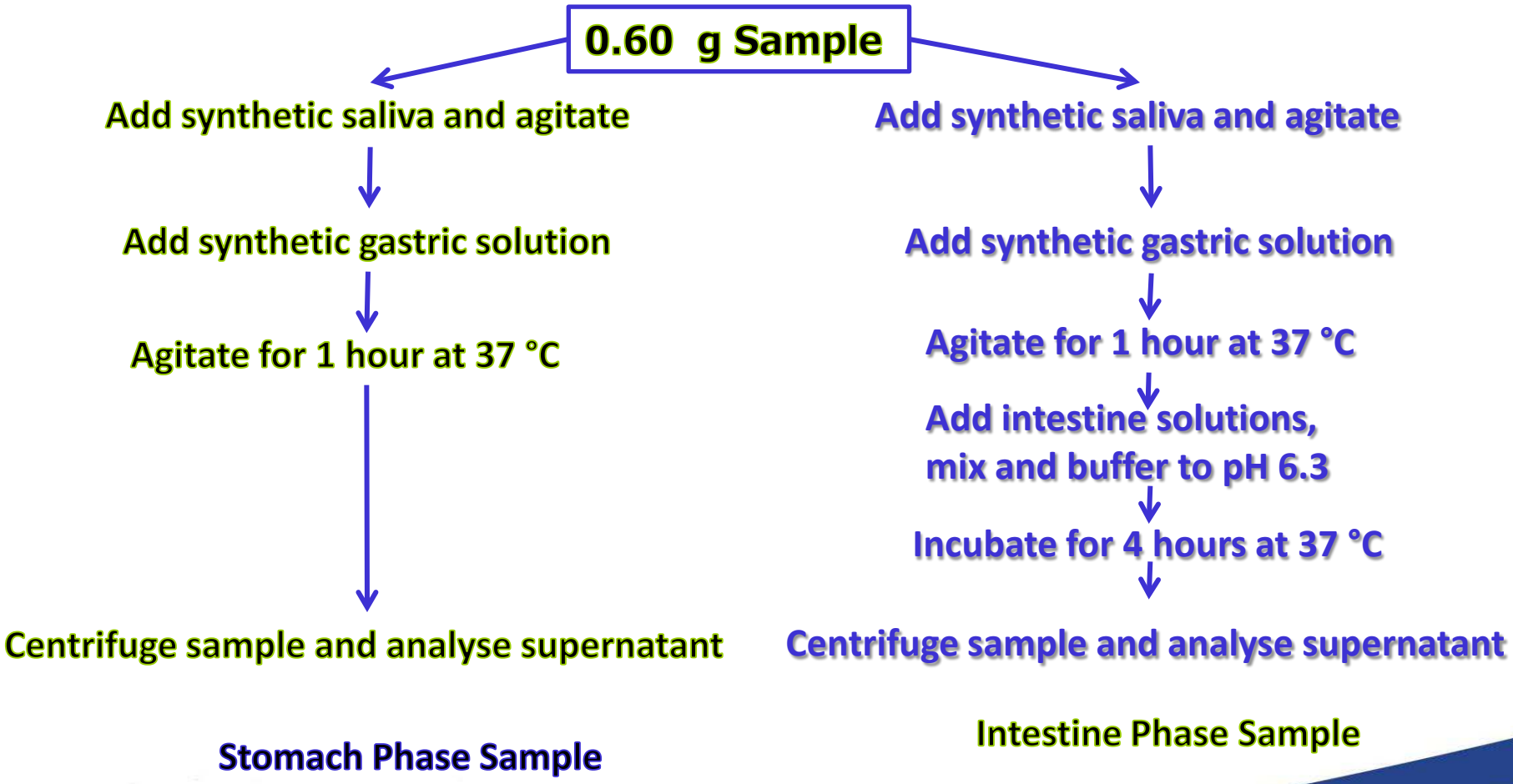
Methodology - UBM

- 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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  - 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
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  - System and extract solution blanks
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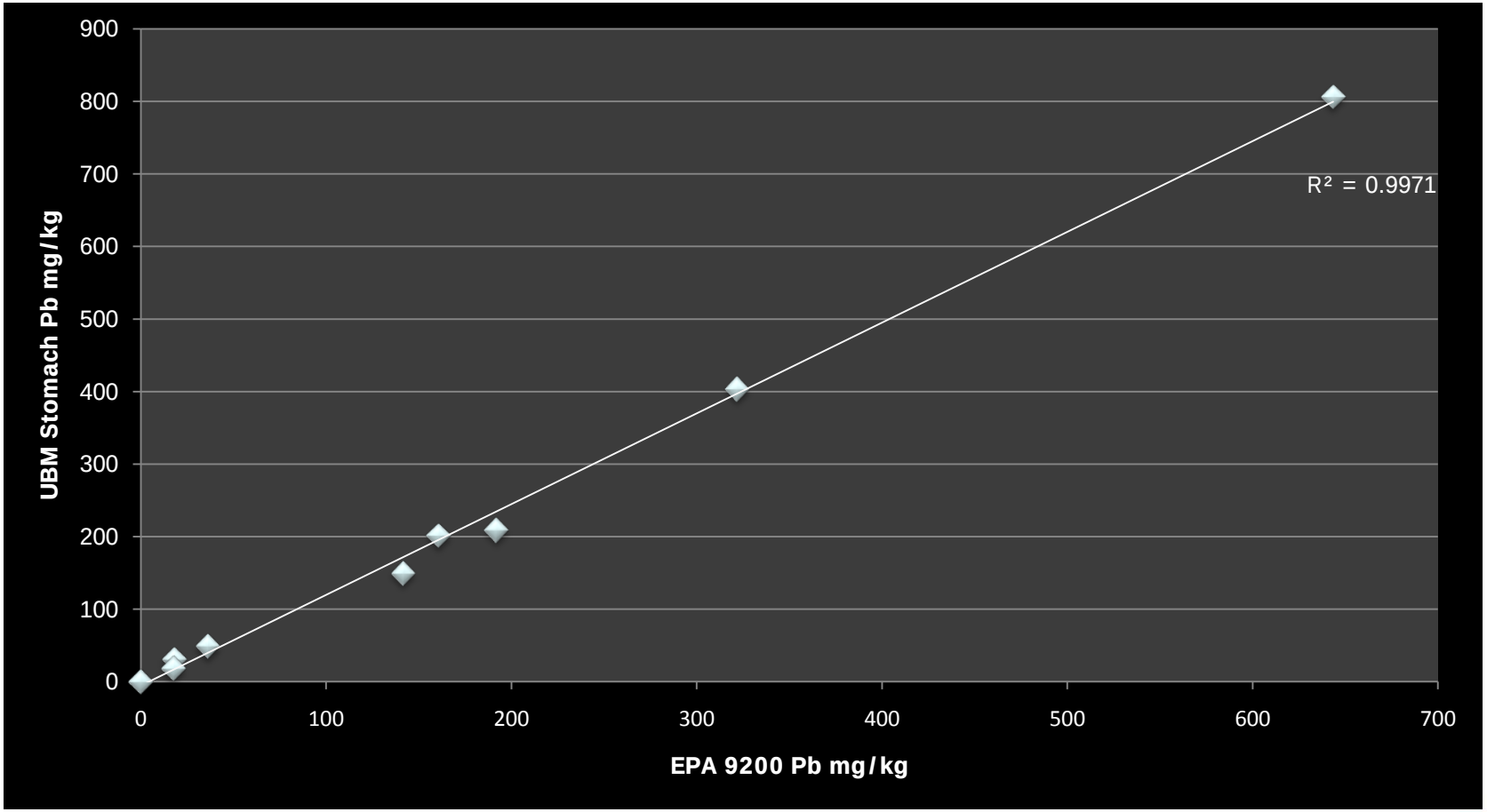
Comparison of Methods



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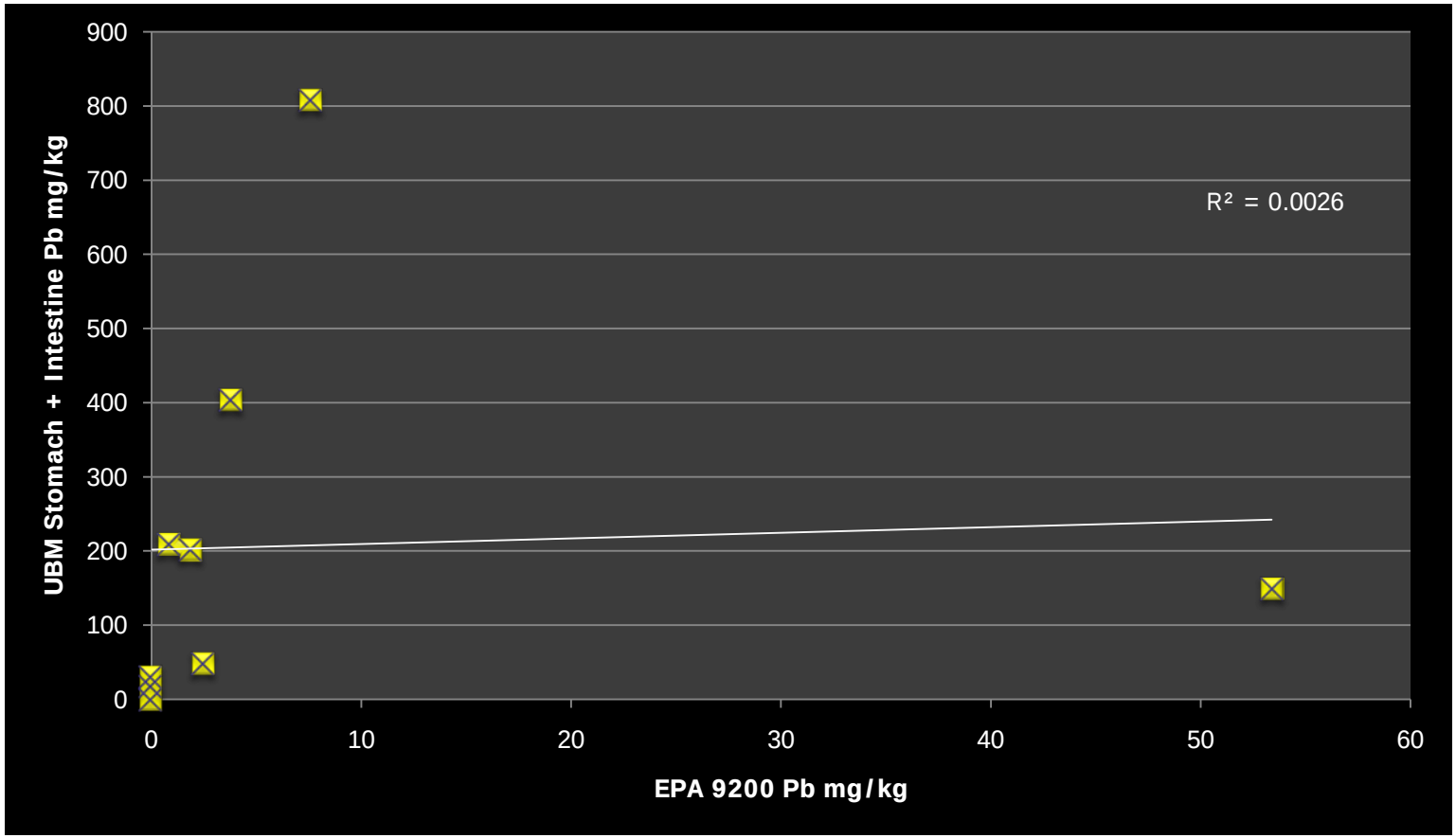
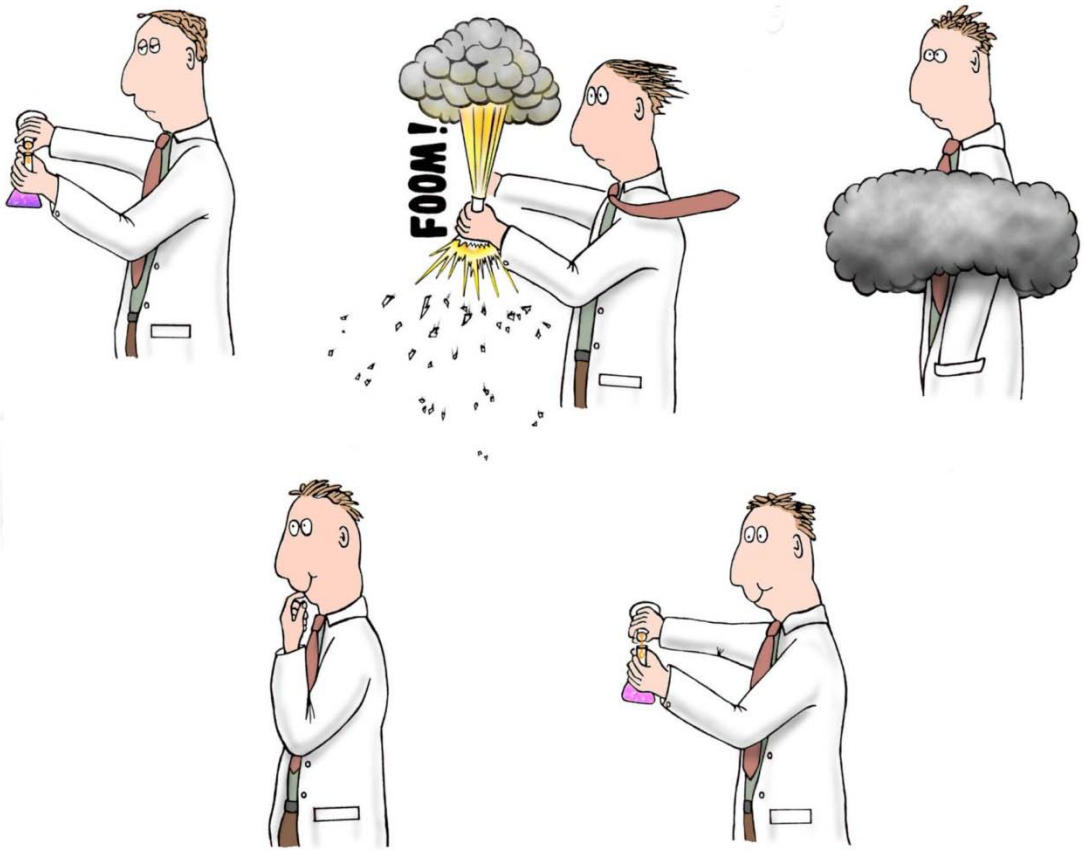


Pb Bioaccessibility

- 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



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