Alternative Methods for the Evaluation of Bioavailability of Arsenic in Mining Soils

Valerie Mitchell, PhD
Staff Toxicologist
California Environmental Protection Agency
Department of Toxic Substances Control
The Mission of DTSC is to protect California’s people and environment from harmful effects of toxic substances by restoring contaminated properties, identifying and promoting safer ingredients in consumer products, and ensuring stewardship through enforcement, regulation and pollution prevention.

Cleanup at Abandoned Mine Lands typically regulated by the Brownfields and Environmental Restoration Program of DTSC
Arsenic RBA Study

47,000 Abandoned Mines in California as estimated by Department of Conservation
Arsenic RBA Study

U.S. EPA Grant to Conduct Study

- DTSC applied for a Brownfields Training, Research and Technical Assistance Grant in 2008.
- DTSC awarded a grant to quantify the risk of arsenic found in mine-scarred lands, comparing in vivo and in vitro toxicity testing for different geological forms of arsenic by U.S. EPA.
- Cooperative Agreement between DTSC and U.S. EPA signed in March 2009. The grant is for a total of $900,000 over five years (2008-2013).
Objective of the research

Provide better tools to assess health risks at mine-scarred lands (MSL) that allow use of bioavailability (RBA) in risk assessment and risk management decisions by:

- Developing cost effective methods to determine arsenic bioavailability:
  - Improve correlation between in vitro and in vivo methods through improvement of the in vitro simulated gastro-intestinal assay.
  - Identify geochemical and mineralogical parameters which control RBA of soil-bound As, and inexpensive bench procedures for estimation of RBA acceptable in a regulatory setting.
Objective cont.

- Identifying wet chemical, spectroscopic, and physical measurements to use in characterizing MSL.
- Developing a database of mine wastes and corresponding in vitro and mineralogy data.
- Establishing a methodology for implementation at sites throughout California.
- Developing guidance for the use of bioavailability at MSL.
Arsenic RBA Study

Partners

- USGS
- Ohio State University
- University of Missouri
- Chapman University
Arsenic RBA Study

Major Tasks for Study

1. Sample and Analysis Plan with Field Work (done).
2. Develop Database to Assist in Predicting Bioavailability.
5. In Vivo Bioavailability Testing.
7. Public Outreach.
Arsenic RBA Study

- Tens of thousands of abandoned / inactive gold mines in California

- Main contaminants of concern at hardrock (lode) gold mines: arsenic, lead, and mercury

- Numerous areas in Sierra Nevada where development is encroaching on historical mine sites

- Challenge for risk assessment – How bioavailable is arsenic in hardrock mine waste?
Arsenic RBA Study

- Standard Toxicity Criteria is based on readily soluble forms of arsenic such as Sodium Arsenate in drinking water.
- Mining Soils are rich in minerals that are known to bind arsenic and potentially reduce its bioavailability and therefore its toxicity.
Common Arsenic-rich Minerals associated with Low-Sulfide Gold-Quartz Veins

**Primary (Hydrothermal)**

- **Pyrite**
  - FeS₂
  - “Fool’s Gold”
  - 0-5 wt % As

- **Arsenopyrite**
  - FeAsS
  - 46 wt % As

**Secondary (Weathering)**

- **Hydrous ferric oxides (HFO)**
  - (“rust”) containing arsenic
  - (up to 20 wt % As)
  - Goethite FeO(OH)
  - Ferrihydrite 5Fe₂O₃·9H₂O

- **Jarosite**
  - KFe₃(SO₄)₂(OH)₆
  - Up to 1 wt % As
Relatively soluble secondary arsenic minerals

Yukonite
\[ \text{Ca}_7\text{Fe}_{12}(\text{AsO}_4)_{10}(\text{OH})_{20} \cdot 15\text{H}_2\text{O} \]

Arseniosiderite
\[ \text{Ca}_2\text{Fe}_3(\text{AsO}_4)_{3}\text{O}_2 \cdot 3\text{H}_2\text{O} \]

Pharmacosiderite
\[ \text{KFe}_4(\text{AsO}_4)_{3}(\text{OH})_4 \cdot 6-7(\text{H}_2\text{O}) \]

Amorphous ferric arsenate
\[ \text{FeAsO}_4 \cdot n\text{H}_2\text{O} \]

Hydrous ferric arsenate (HFA)
\[ [\text{Fe(OH)}_3]_x[\text{FeAsO}_4]_y \cdot n\text{H}_2\text{O} \]

Higher solubility
Higher bioaccessibility & Higher bioavailability

Soluble phases may represent a small amount of the total arsenic in a sample (~1%)

But, may represent a large amount of the bioavailable arsenic (~50%)

De Sisto et al. (2011) Appl. Geochem
Why Measure Bioavailability?

- Improve Accuracy of Exposure and Risk Calculations
- Minimize Unnecessary Site Cleanups
- “Gold Standard”
- US EPA Approved Methodology
Why NOT Measure Bioavailability?

- Expensive
- Time Consuming
- Ethical Issues
Using Soil Data to Estimate Arsenic Bioavailability and Adjust/Predict Risk

Both Approaches are evaluated in our study

Contaminated Soil

Use In Vitro Gastro(intestinal) Methods (Correlated with Arsenic Bioavailability) to Predict Arsenic Bioavailability
OSU IVG, SBRC

Use Soil Sequential Extraction Procedure (SEP) to Estimate Arsenic Bioavailability
Conceptual Model for Arsenic Toxicokinetics

- Intravenous dose ($D_{iv}$)
- Ingested dose ($D_{oral}$)
- Blood
- Urine
- Feces
- Bile
- Tissue
- Absorbed ($AF_0$)
- Non-Absorbed ($1-AF_0$)

$K_b$, $K_u$, $K_t$
Arsenic Biokinetics Model

- Absorbed As primarily excreted in urine

- Bioavailability is estimated by Urine Excretion Factor (UEF)

\[
UEF = \frac{\text{Excreted As}}{\text{Dosed As}}
\]
Sample Collection

- 25 Soil Samples Collected from Empire Mine State Historic Park
- 15 to 12,095 mg/kg As; median 2,980 mg/kg As
Soil Selection for In Vivo Study

- Total As Concentration
- In Vitro Bioaccessibility
- Mineralogy
Materials and Methods

*In vivo* RBA Study

- Groups of 5 pigs dosed daily
- Absorbed As estimated by As excreted in urine over 48 hrs
- Urinary As excretion--a linear function of dose and independent of time after day 5
RBA Study Design

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Sample</th>
<th>Arsenic (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EM01</td>
<td>302</td>
</tr>
<tr>
<td>2</td>
<td>EM03</td>
<td>2541</td>
</tr>
<tr>
<td>3</td>
<td>EM08</td>
<td>633</td>
</tr>
<tr>
<td>4</td>
<td>EM18</td>
<td>10482</td>
</tr>
<tr>
<td>5</td>
<td>EM19</td>
<td>370</td>
</tr>
<tr>
<td>6</td>
<td>EM21</td>
<td>12041</td>
</tr>
<tr>
<td>7</td>
<td>Sodium Arsenate</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>Negative Control</td>
<td>0</td>
</tr>
</tbody>
</table>

Administered Daily Dose: 60 mg/kg
RBA Data Evaluation

- As excreted in urine = C X V (L/48 hrs)
- Plot As urine vs As dosed
  - UEF is slope of this line
- $RBA_{(x)} = \frac{UEF_{(x)}}{UEF_{(Sodium arsenate)}}$
- Note: Each RBA is a ratio of slopes
Example Dose Response

EM08
Day 6/7

\[ y = 0.1449x + 49.8 \]

Excreted As (µg/L) vs. Arsenic Dose per 48 Hours
## Estimated RBA for EMSHP Samples

<table>
<thead>
<tr>
<th>Test Material</th>
<th>90% Confidence Interval</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBA Day 6/7</td>
<td>RBA Day 9/10</td>
</tr>
<tr>
<td>EM01</td>
<td>26.8 (20.3-33.4)</td>
<td>29.2 (24.1-34.3)</td>
</tr>
<tr>
<td>EM03</td>
<td>17.0 (13.4-20.6)</td>
<td>15.9 (13.0-18.8)</td>
</tr>
<tr>
<td>EM08</td>
<td>20.3 (18.4-22.2)</td>
<td>19.5 (14.2-24.8)</td>
</tr>
<tr>
<td>EM18</td>
<td>6.8 (5.8-7.7)</td>
<td>4.4 (2.2-6.5)</td>
</tr>
<tr>
<td>EM19</td>
<td>13.8 (11.1-16.4)</td>
<td>11.7 (9.5-13.9)</td>
</tr>
<tr>
<td>EM21</td>
<td>23.5 (19.1-28.0)</td>
<td>26.0 (22.3-29.8)</td>
</tr>
</tbody>
</table>
### Effects of RBA on Risk

<table>
<thead>
<tr>
<th></th>
<th>As (mg/kg)</th>
<th>Default Risk</th>
<th>RBA</th>
<th>Adjusted Risk</th>
<th>RBC (1x10⁻⁶)</th>
<th>RBC (1x10⁻⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM01</td>
<td>302</td>
<td>7.E⁻⁰⁴</td>
<td>0.237</td>
<td>2.E⁻⁰⁴</td>
<td>1.8</td>
<td>179</td>
</tr>
<tr>
<td>EM03</td>
<td>2541</td>
<td>6.E⁻⁰³</td>
<td>0.153</td>
<td>9.E⁻⁰⁴</td>
<td>2.8</td>
<td>278</td>
</tr>
<tr>
<td>EM08</td>
<td>633</td>
<td>1.E⁻⁰³</td>
<td>0.192</td>
<td>3.E⁻⁰⁴</td>
<td>2.2</td>
<td>221</td>
</tr>
<tr>
<td>EM18</td>
<td>10482</td>
<td>2.E⁻⁰²</td>
<td>0.04</td>
<td>1.E⁻⁰³</td>
<td>10.6</td>
<td>1062</td>
</tr>
<tr>
<td>EM19</td>
<td>370</td>
<td>9.E⁻⁰⁴</td>
<td>0.117</td>
<td>1.E⁻⁰⁴</td>
<td>3.6</td>
<td>363</td>
</tr>
<tr>
<td>EM21</td>
<td>12041</td>
<td>3.E⁻⁰²</td>
<td>0.23</td>
<td>7.E⁻⁰³</td>
<td>1.8</td>
<td>185</td>
</tr>
</tbody>
</table>

Residential Scenario (Ingestion only)
Estimated Bioaccessibility

Most As (>85%) is not extracted by GI methods

Bioaccessible As < 10%

IVG GE ≈ IVG IE

Amount extracted by IVG or SBRC is soil dependent
in vivo vs. in vitro

IVBA vs RBA

R² = 0.8237
Soil Chemical Pools Control Arsenic Fractionation and Bioaccessibility

Relationship between sequential extraction fractions and bioaccessibility

- **F1**: Non-specifically sorbed
  - Solution: 0.05M (NH₄)SO₄

- **F2**: Specifically sorbed
  - Solution: 0.05M (NH₄)H₂PO₄

- **F3**: Amorphous and poorly-crystalline oxides of Fe and Al
  - Solution: 0.2M oxalate (ox), pH 3

- **F4**: Well-crystallized oxides of Fe and Al
  - Solution: 0.1 M ascorbic + 0.2M ox

Results Soil As Fractions and RBA As (6 soils)

\[ \sum F1-F3 > RBA As > \sum F1-F2 \]
Results Oxalate Extractions
Step “2.5”

200mM oxalate is ≥ % RBA
50mM oxalate ≥ % RBA As
with the exception of EM19;
and the % As extracted by
the 5mM
Summary and Conclusions

- *In vivo* RBA test results (n=6) correlate with *in vitro* IVGA results, but relation not predictive

- Sequential extractions bracket *in vivo* RBA: F1+F2 too low, F1+F2+F3 too high

- Sample with most arsenopyrite has lowest RBA and IVGA

- Samples with Ca-Fe-arsenates have the highest RBA and IVGA, reflecting solubility
Ongoing Work

- Six additional samples selected for *in vivo* testing completed September 2012 (total n=12)
- Further Investigation of “F2.5” -- lower oxalate concentration, between F2 and F3 of sequential extraction
- Speciation (As-EXAFS) of fine-grained fractions
- Differential XRD using leached material from “F2.5” and IVG (*in vitro*) tests
- Additional characterization of Ca-Fe-arsenate minerals (electron probe & μ-XRD)
Acknowledgements

Funded by USEPA Brownfields Training, Research and Technical Assistance Grant: TR-83415101

DTSC:
Perry Myers, Valerie Mitchell, Rick Fears, John Christopher (retired)

USGS:
Charles Alpers, Andrea Foster, Alex Blum

University of Missouri:
Stan Casteel, Laura Naught

Ohio State University:
Nicholas Basta, Shane Whitacre

Chapman University:
Christopher Kim

Sacramento State University
Tamsen Burlak, Lisa Hammersley

University of Utah
Erich Petersen
Arsenic RBA Study

Information can be found on DTSC’s website

http://www.dtsc.ca.gov/InformationResources/Arsenic_Relative_Study.cfm