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In vitro oral bioavailability testing in human health risk assessment of CCA-contaminated soils: short review & case study

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Outline

- Background
  - Oral bioavailability in HHRA
  - Definitions: bioaccessibility vs bioavailability
  - In vitro or in vivo?
- Bioaccessibility
  - What we know
  - What we do not know
- Case study
  - Soils near CCA-treated utility poles (As, Cr, Cu)
- Conclusions

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Remediation of contaminated sites

Using bioavailability

- Generic criteria for site remediation
  - Maximal concentrations allowed according to soil use
  - General approach
- Human Health Risk Assessment
  - Remediation goals specific to the site
  - Consideration of different exposure pathways
  - Inclusion of contaminant bioavailability in exposure assessment
- Determination of oral bioavailability
  - In vivo
  - In vitro

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Why do we need bioavailability ?
Risk assessment

- Non carcinogenic risk
  - Calculated with the Hazard Index:
  - HI = CDI / RfD
  - CDI = Chemical Daily Intake (mg/kg/d) ; RfD = Reference Dose (mg/kg/d)
- Carcinogenic risk
  - Probabilistic approach
  - Risk = CDI x CSF
  - CSF = Cancer Slope Factor (mg/kg/d)
- Adjusted dose
  - $CDI_{adjusted}$ = CDI x RBA

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Risk assessment: Exposure
Daily intake

Chemical daily intake (CDI) absorbed by incidental ingestion of soil (µg/d) *(Hemond and Solo-Gabriele, 2004)*:

Exposure Point Concentration mg/kg

% Bioavailability ?

$$CDI_{adjusted} = EPC \times SIR \times EF \times CF \times RBA$$

- SIR: soil ingestion rate: 100 mg/d *(US EPA, 1997)*
- EF: exposure frequency: 130-260 d/y (*Dubé et al., Hemond and Solo-Gabriele, Ursitti et al., 2004)*
- CF: unit conversion factor: 10^{-3}

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Definitions

Bioavailability

In vivo bioavailability: the fraction of a contaminant that reaches the central compartment (blood) from the GI tract *(Ruby et al., 1999)*

- Absolute: ABA = $\frac{\text{Absorbed dose}}{\text{Ingested dose}}$

- Relative: RBA = $\frac{\text{ABA study soil}}{\text{ABA ref. material}} = \frac{\text{UEF study soil}}{\text{UEF réf. material}}$

$Na_2AsO_4 \cdot 7H_2O$

- UEF = Urinary Excretion Fraction

Rodriguez et al., 1999

Assessing in vivo bioavailability

- Principle: measurement of contaminant concentration in tissues or excreta at various time points after feeding
- Choice of study design based on element behaviour in the body
  - As: well absorbed, rapidly excreted in the urine
  - Pb: accumulated in bones
  - Cd and Hg: accumulated in kidney and liver

Kelley et al., 2002; NRC, 2003

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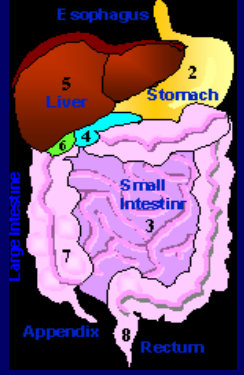


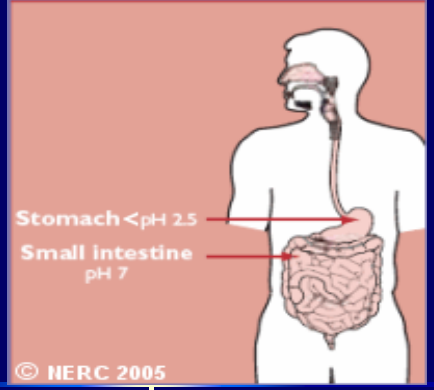
Definitions

Bioaccessibility

- Bioaccessibility: soluble fraction of a contaminant in the GI tract that is potentially available for absorption
- (In vitro) bioaccessibility estimator of In vivo oral relative bioavailability (RBA) *(validated method for As, Cd, and Pb, Rodriguez et al., 1999; Schroder et al., 2003, 2004)*

$$\%\ \text{Gastric bioaccessible metal} = \frac{\text{Soluble metal gastric } \varphi\ (\text{mg/kg})}{\text{Total metal in soil (mg/kg)}} \times 100$$

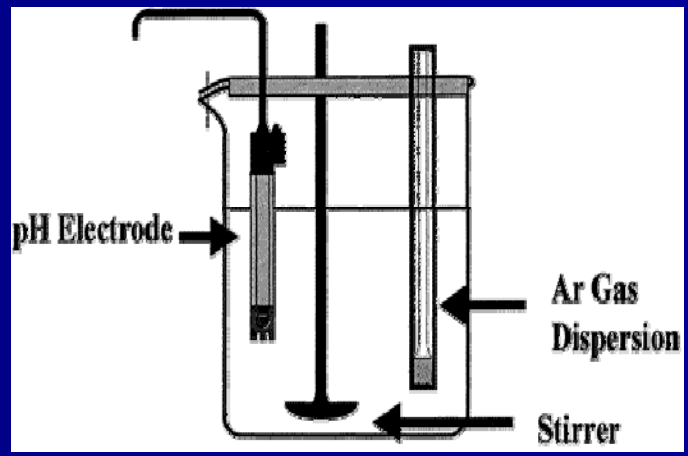
$$\%\ \text{Intestinal bioaccessible metal} = \frac{\text{Soluble metal intestinal } \varphi\ (\text{mg/kg})}{\text{Total metal in soil (mg/kg)}} \times 100$$

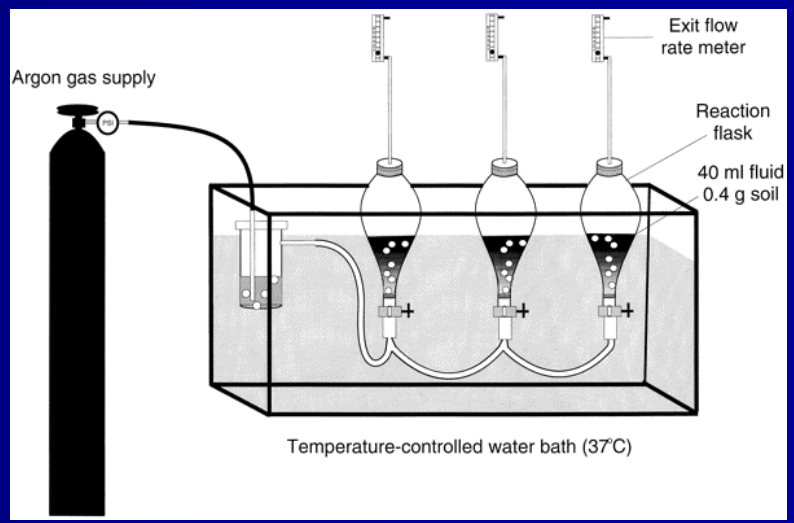


In vitro bioavailability

Some existing methods

- Gastric phase
  - Simulation of stomach conditions
  - Acidic conditions, mixing, 37°C
- Gastrointestinal phase
  - Simulation of stomach and intestine (pepsin, bile, pancreatin…)
  - Mixing, 37°C
  - IVG *(Rodriguez et al., 1999)* validated for As, Pb, Cd
  - PBET and SBRC *(Ruby et al., 1996)* validated for As, Pb





European regulation

Toys & bioaccessibility

- Directive 88/378/CEE: Safety of toys
  - Maximal bioavailability for 8 chemical elements : Sb, As, Ba, Cd, Cr, Pb, Hg, Se
- Standard CEN EN 71-3:1994: Safety of toys
  - Applied by 18 European countries
  - Toy material ground to < 500 µm
  - Extraction at 37°C, 2 h, pH = 1.5 (HCl)
  - Solid: liquid ratio = 1:50 (allows ratio up to 1:500)

In vitro *vs* In vivo

- Costs (200 $/sample vs > 30 000 $/sample)
  - Duration of the test (1 d vs 2-4 weeks)
  - Difficulties in the application
- Ethics (avoid the use of animals)
- Dose that has to be administered in vivo is too high
  - Concentrations in soils are not relevant
  - Volume of ingested soil higher than the one ingestible by a child
- Representativity
  - In vivo tests performed on a little number of samples
- Extrapolation : animal to human

Environment Agency, 2005. Escher and Hermens, 2004. Marschner et al., 2006. Pouschat and Zagury, 2006. Rodriguez et al., 1999. Ruby, 2004. Saikat, 2006

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Bioaccessibility of metals in soils

What we know

- Lead 
- Arsenic 
- Mercury 
- Cadmium 

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Validation of in vitro methods: what must be done

- Correlation In vivo – In vitro
  - Wide variety of soils (different origins)
- Inter-laboratories comparison (Round Robin with BARC)
- Rigorous QA/QC
  - Blanks, spiked samples, replicates, certified soil samples
- Sensitivity analysis of method
  - pH, extraction time, soil particle size
- Evaluation of limitations
- Comparison to existing protocols
- Submission to an independent scientific arbitrage.

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Bioaccessibility

Acceptance by authorities

Canada, under study (BARC)

US EPA, under validation for As, Cd, Hg / Accepted for Pb

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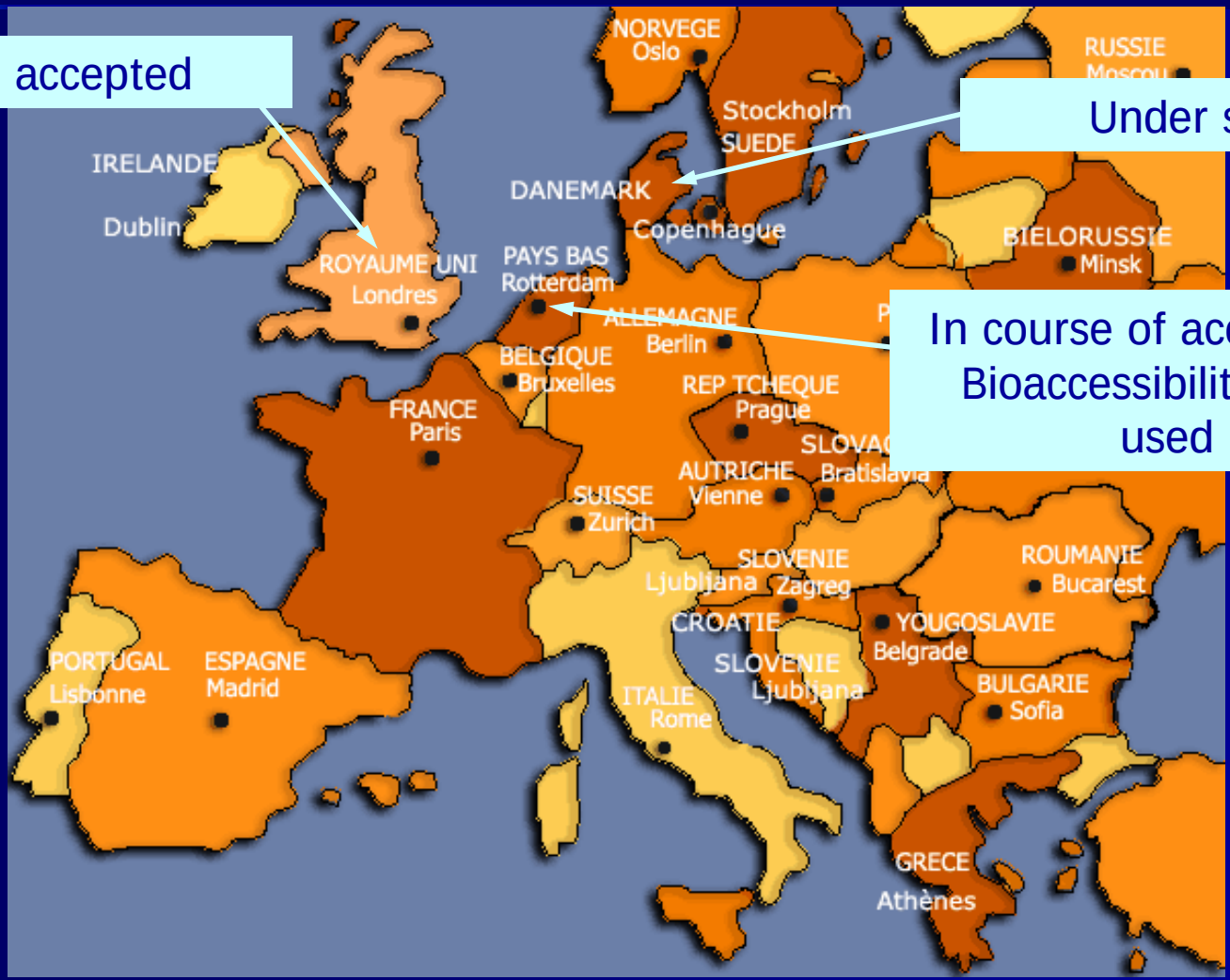
Bioaccessibility – Europe

Acceptance by authorities

Almost accepted

Under study

In course of acceptation Bioaccessibility of Pb used



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- Case study
  - Soils near CCA-treated utility poles (As, Cr, Cu)
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Case study

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Case studies: CCA-treated poles

Background

CCA: waterborne wood preservative composed of arsenic, chromium, and copper oxides (CCA-C : 47.5% CrO_3, 18.5% CuO, 34% As_2O_5).

Since Jan 1, 2004, US EPA (and Health Canada) do not allow CCA products for residential uses *(Katz and Salem, 2005)*

Contamination:

→ Wood treatment plants

→ **Near treated wood structures**

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CCA-treated poles
Objectives

- To assess the bioaccessibility of arsenic (and Cr and Cu) in a scenario of soil ingestion near CCA-treated utility poles
- To estimate an average As, Cu, and Cr daily intake (CDI adjusted) from ingestion of soil near CCA-treated utility poles.

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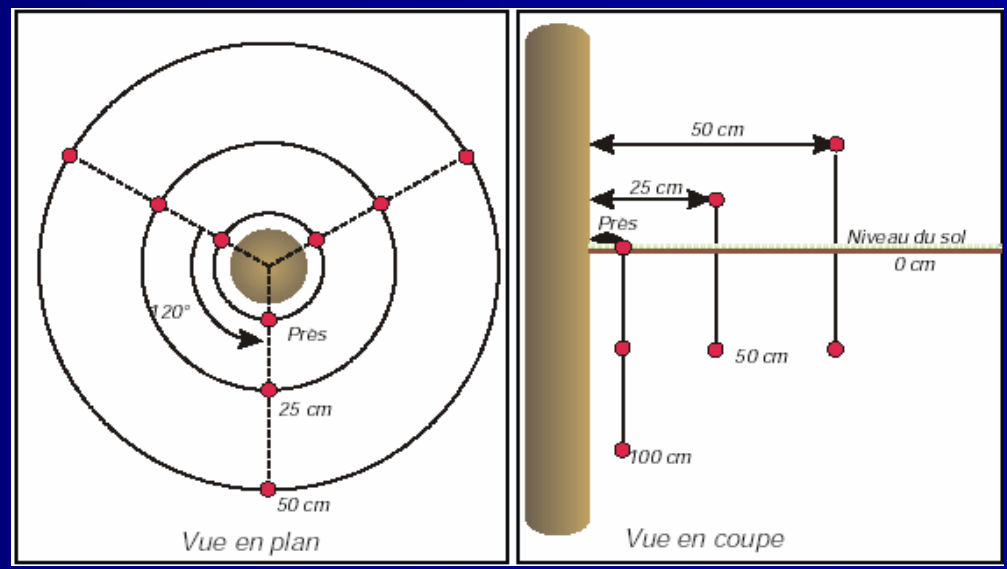
CCA-treated poles
Methodology

- Installation of 12 CCA-treated poles in 4 different soil types (3 poles/site)
- Sampling and characterization of soils after 6,18, and 36 months of service
- Bioaccessibility on composite surface samples collected near each wood pole
- Bioaccessibility on certified soils (SRM 2710 & 2711) and procedure blanks (QA/QC)

Photo 12 : Vue des trois poteaux, site de Kingsey-Falls (déc. 2002).



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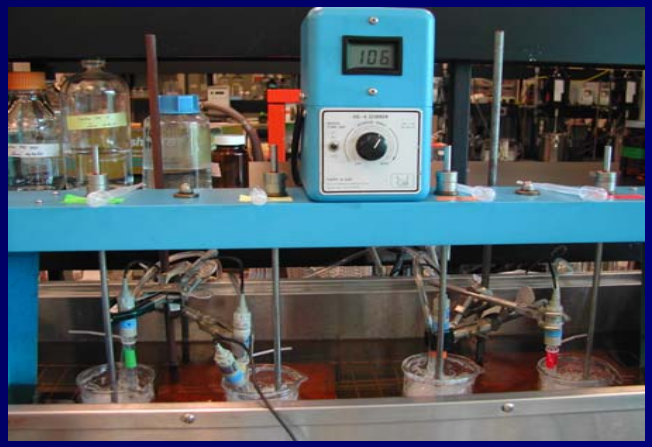
CCA-treated poles

Evaluation of bioaccessibility



- In vitro gastrointestinal extraction (IVG) of soils at 37°C, modeling a child digestive tract
- 1g of soil < 300 µm in 150 mL of solution
- Gastric phase, 1h, with porcine pepsin, pH = 1.8 (HCl)
- Intestinal phase, 1h, with porcine pancreatin and bile, pH = 5.5

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Results : CCA-treated poles

Soil characterization (around 12 wood poles)

Physicochemical characteristics of surface soils near CCA-trated utility poles (soil < 2 mm)										
Site & Type	Soil (n=3)	Sand %	Silt %	Clay %	pH	CEC meq/100g	TOC %	As (mg/kg)	Cu (mg/kg)	Cr (mg/kg)
1 Clayey	1A	21.9	25.1	12.2	6.10	17.3	2.6	170	720	97
	1B	23.8	32.4	17.8	6.17	29.8	1.6	94	390	57
	1C	16.8	36.5	17.4	6.37	23.0	2.9	77	210	43
2 Organic	2A	40.4	35.7	1.40	7.13	286	20	170	2 100	270
	2B	51.2	31.2	6.50	7.17	324	37	160	4 300	430
	2C	50.0	37.7	4.50	6.20	137	40	170	2 800	320
3 Sandy	3A	85.9	11.2	2.50	6.18	28.8	2.5	160	610	73
	3B	47.4	17.8	8.20	6.35	25.5	2.3	110	330	48
	3C	82.0	11.1	0.90	6.62	29.7	2.2	200	720	82
4 Sandy	4A	45.8	15.7	10.8	6.64	38.4	5.4	170	660	63
	4B	62.0	10.8	1.50	5.47	53.1	6.1	120	730	77
	4C	43.2	30.4	9.50	5.27	42.5	5.6	170	770	66
Mean	-	47.5	24.6	7.50	-	86.3	11	148	1 195	136
Std-dev		21.6	10.6	5.60	-	107	14	38	1 240	129
Minimum		16.8	10.8	0.90	5.27	17.3	1.5	77	210	43
Maximum		85.9	37.7	17.8	7.17	324	40	200	4 300	430
Certified	SRM 2710		-	-	5.20	-	3.2	538	2 689	15
	SRM 2711		-	-	8.00	-	1.3	95	97	17

Concentration of As homogenous in all soil types (no significant difference)

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Results: CCA-treated poles

As bioaccessibility

Arsenic bioaccessibility in soils (< 300 µm) collected near CCA-treated utility poles							
Soil (n=3)	As (mg/kg dry soil)	Soluble As (%)	Soluble As per site (%)	Bioaccessible As (gastric) (%)	Bioaccessible As (gastric) per site (%)	Bioaccessible As (intestinal) (%)	Bioaccessible As (intestinal) per site (%)
1A	225 ± 6	1.2 ± 0.1		25.7 ± 3.0		28.3 ± 2.2	
1B	131 ± 0	1.2 ± 0.1	1.1 ± 0.2	24.2 ± 2.9	23.6 ± 2.6	26.8 ± 2.8	26.7 ± 1.7
1C	58.0 ± 2.1	1.0 ± 0.1		20.7 ± 2.9		25.0 ± 2.7	
2A	219 ± 6	15 ± 1		56.2 ± 4.7		59.1 ± 7.0	
2B	172 ± 19	22 ± 2	24 ± 10	41.7 ± 3.8	53.8 ± 11	46.7 ± 2.8	57.4 ± 9.9
2C	153	35		63.6 ± 1.2		66.3 ± 2.3	
3A	144 ± 6	2.6 ± 0.1		40.5 ± 3.4		47.7 ± 0.8	
3B	37.4 ± 2.5	1.3 ± 0.2	2.7 ± 1.4	42.7 ± 2.2	43.2 ± 3.0	51.2 ± 2.5	50.8 ± 2.9
3C	231 ± 17	4.1 ± 0.4		46.3 ± 0.6		53.5 ± 1.3	
4A	251 ± 12	1.4 ± 0.1		26.4 ± 1.1		30.9 ± 1.3	
4B	173 ± 10	2.1 ± 0.1	1.9 ± 0.4	23.0 ± 1.9	24.3 ± 1.8	27.1 ± 2.6	27.9 ± 2.6
4C	238 ± 6	2.1 ± 0.1		23.5 ± 1.0		25.9 ± 0.5	
Mean	169	7.4		36.2		40.7	
Std-dev	69	11		14.4		14.9	
SRM 2710	538 ± 12	0.3 ± 0.0	0.3 ± 0.0	27.6 ± 0.4	27.6 ± 0.4	25.2 ± 0.3	25.2 ± 0.3
SRM 2711	95 ± 5	-	-	45.7 ± 6.3	45.7 ± 6.3	43.0 ± 5.6	43.0 ± 5.6
CRM 025	319 ± 12	-	-	71.3 ± 3.7	71.3 ± 3.7	64.8 ± 5.2	64.8 ± 5.2

Lower: fine-grained soils (loams). Max: organic and sandy soils

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CCA-treated poles

Results: Arsenic

- Bioaccessibility & soil properties:
  - Correlated (+) with TOC content ($r^2 = 0.36$, $p < 0.05$, n=12)
  - Correlated (+) with sand content ($r^2 = 0.52$, $p < 0.05$, n = 9)
  - Correlated (+) with water soluble As ($r^2 = 0.51$, $p < 0.01$, n = 12)
  - Correlated (-) with clay content ($r^2 = 0,43$, $p < 0.05$; n = 12)
  - NOT correlated with total As in soil samples.

- Mean in vitro bioaccessibility: 41 % (25 – 66 %)
- Other metals:
  - Cr: 8.5 % (0 – 33 %)
  - Cu: 54 % (19 – 89 %)

In vivo RBA in CCA-soils: around 50 %

(Casteel et al, 2003)

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CCA-treated poles
Arsenic Intake

- Intake of As from incidental ingestion of CCA-contaminated soil:
  - EPC = 169 mg/kg, RBA = 41 %
  - 0.19 µg/kg/d (0.04–0.36) < 0.3 µg/kg/d *(Oral MRL for chronic exposure (ATSDR, 2005))*
- Inorganic As intake from food + water for children: 0.4 – 0.6 µg/kg/d (Yost et al., 2004)
- As intake from CCA-contaminated soil ingestion much lower than As intake from water and food.

Pouschat and Zagury (2006), Environ. Sci. Technol.

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CCA-treated poles

Cu and Cr Intake

- Intake of Cu from incidental ingestion of CCA-contaminated soil:
  - EPC = 1200 mg/kg, RBA = 54 %
  - 9-90 µg Cu /d < 340-440 µg/d *(Recommended Dietary Allowance for Children (1-8 yr old) (NAS, 2001)*
- Intake of Cr from incidental ingestion of CCA-contaminated soil:
  - EPC = 136 mg/kg, RBA = 8.5 %
  - 0.2-2 µg Cr /d < 11-15 µg/d *(Adequate Intake for children (1-8 yr old) (NAS, 2001)*
- Cu and Cr intake from incidental CCA-contaminated soil ingestion is lower than recommended dietary values !

Pouschat and Zagury (2007), Pract. Period. Haz. Tox. Radioact. Waste Mngmt. ASCE

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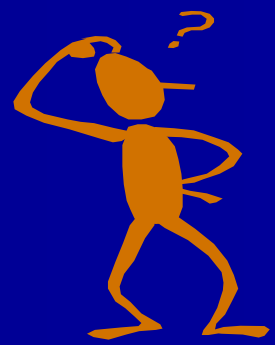
Conclusions

- Multiples advantages of in vitro tests
- Currently acceptable (As and Pb) in HHRA if fully validated (QA/QC, in vivo-in vitro, ...) and well supported by other techniques
- Metal intake from incidental ingestion of CCA-contaminated soils by young children is very low and the chances to observe adverse health effects appear limited.



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