Supplemental Material:

Assessing Performance of a Mouse Assay for Determination of Arsenic Bioavailability in Contaminated Soils

Authors: Karen D. Bradham [*,1], Gary L. Diamond[2], Michael F. Hughes[3], Stan W. Casteel[4], Julie Klotzbach[2], William Thayer[2], David J. Thomas[3]

[1] U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, Research Triangle Park, North Carolina 27711

[2] SRC, Inc., North Syracuse, New York 13212

[3] U.S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Integrated Systems Toxicology Division, Research Triangle Park, North Carolina 27711

[4] Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, Missouri 65205

*Corresponding author: bradham.karen@epa.gov, 109 TW Alexander Drive, MD-D205-05, Research Triangle Park, North Carolina; phone: (919) 541-9414; fax: (919) 541-3527; e-mail: bradham.karen@epa.gov.

Estimation of confidence limits on RBA:

The relative bioavailability (RBA) for arsenic in any test material is calculated as the ratio of the urinary excretion fraction (UEF) for the test material and the mean UEF for the sodium arsenate heptahydrate reference material (Equation 1).

$$RBA\% = \frac{UEF\ Soil}{UEF\ Arsenate} \times 100$$

Each UEF in equation 1 is derived from multiple estimates of UEF for groups of 3 mice housed together in a single metabolic cage (the unit of measure in the assay is data from a single cage). Therefore, estimating confidence limits on the RBA requires estimating the confidence limit on a ratio of mean values for UEF where each mean has an associated uncertainty that must be estimated from the sample distributions.

Fieller's theorem allows the calculation of a confidence interval for the ratio of two means where the underlying distributions of the numerator and denominator are normal (Fieller, 1954). Although, sample sizes for typical assays (N=4 cages) were too small to allow a rigorous evaluation of normality of most sample UEF distributions, normality could be evaluated for selected samples with larger sizes. The mean UEF of sodium arsenate-amended diets was estimated using 24 independent estimates obtained in repeated assays. The mean UEF for these assays was 61.9% ± 4.6 (SD); the distribution showed low skew (1.07) and the assumption of normality was not rejected by standard goodness-of-fit tests (K-S statistic $p>0.95$; Shapiro Wilk W $p=0.50$). Similarly, 12 independent estimates of UEF for diets amended with SRM Sample ID 14 also showed low skew (1.09). Here, the mean was 26.5±2.1% (SD) and the assumption of normality was not rejected (SD, K-S statistic p=0.99, Shapiro Wilk W p=0.54). Similarly, 12 independent estimates of UEF for diets amended with SRM Sample ID 15 (26.0%±1.9, skew = 0.17) and goodness of fit tests indicated that a standard distribution provided a reasonable model for the data (K-S statistic p=0.99; Shapiro Wilk W p=0.91). It is not surprising the UEF distribution would be symmetrical, because each UEF value is actually an average of dose and excretion data from 3 mice, housed together in a single cage.

The estimates of confidence limits based on Feiller's Theorem compared well with estimates based on bootstrap methods (Table SM-1). In the parametric bootstrap, normal distributions for the UEF values for the diets amended with soil or sodium arsenate were represented by their respective sample means and SD, and each was randomly sampled (with replacement) N times, where N was the UEF sample size. In the non-parametric bootstrap, the discreet distribution of UEFs for each material were randomly sampled with replacement, N times. Confidence limits based on the bootstrap were very similar to those estimated from Fieller's Theorem.

References

Fieller, EC. 1954. Some problems in interval estimation. *Journal of the Royal Statistical Society, B* **16** (2): 175–185. JSTOR 2984043

Table SM-1 Comparison of Confidence Limits Estimated from Fieller's Theorem and Bootstrap Methods

			Fieller's Theorem		BS - Normal[b]		BS - NonParam[c]	
Sample ID	N[a]	Mean RBA	LCL	UCL	LCL	UCL	LCL	UCL
1	4/24	39.9	36.2	43.8	36.8	43.1	37.5	42.4
2	3/24	14.5	11.2	17.8	12.7	16.3	13.0	15.8
3	4/24	26.7	22.8	30.7	23.8	29.7	24.2	28.7
4	4/24	48.7	43.4	54.2	44.4	53.1	45.8	52.4
5	4/24	49.7	45.0	54.5	45.8	53.7	47.0	53.0
6	4/24	51.6	47.0	56.3	47.7	55.5	48.3	54.4
7	8/24	11.2	10.6	11.8	10.5	11.9	10.6	11.7
8	4/24	24.0	20.9	27.2	21.6	26.4	22.3	26.0
9	4/24	26.3	23.4	29.4	23.9	28.7	24.5	28.2
10	4/24	35.2	30.9	39.6	31.9	38.6	33.0	38.2
11	4/24	20.9	15.9	26.0	17.5	24.4	17.8	23.2
12	4/24	35.0	31.2	38.9	32.0	38.1	32.4	37.1
13	4/24	33.2	27.7	38.7	29.3	37.2	30.5	36.6
14	12/24	42.9	40.5	45.4	40.0	45.8	40.5	45.0
15	12/24	42.1	39.8	44.4	39.4	44.9	39.8	44.1

BS, bootstrap, LCL, 95% lower confidence limit; RBA, relative bioavailability; SE, standard error; UCL,95% upper confidence limit

[a]Number of UEF estimates for the soil/number of Absolute Bioavailability (ABA) estimates for sodium arsenate.

[b]Bootstrap of N random draws from normal(mean, SD) distribution of ABAs

[c]Booststrap of N draws from discrete(mean) distribution of ABAs