northgate
environmental management, inc.

MEMORANDUM

From: Deni Chambers, Northgate
Renee Kalmes, Exponent

DATE: February 19, 2010/
Revised May 14, 2010

To: Brian Rakvica, NDEP

RE: Protocol: Bioaccessibility Method for Dioxin/Furans in Soil

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As requested by NDEP in their February 9, 2010 memorandum, the following protocol presents an *in vitro* bioaccessibility extraction test for dioxin/furans in soil to be implemented at the Tronox Henderson, Nevada Site (Site). The *in vitro* extraction method generally follows that reported in Ruby et al. (2002) and further described in Finley et al. (2009). The objective of this study is to provide site-specific information that will allow for an understanding of the likely relative bioavailability of dioxins/furans from site soils (on a TEQ basis). The data that emerge from this study will be evaluated to elucidate the nature of dioxins/furans in soils at the site, and will be interpreted in the context of available studies on the bioavailability of dioxins/furans, as has been presented in recent publications, including but not limited to Budinsky et al. (2008) and Finely et al. (2009).

As requested by NDEP, the dioxin/furan congener profile for relevant Site soil samples is presented in Attachment 1, because it provides the basis for identifying one dioxin/furan type at the Site.

Soil Sample Collection and Analysis

Soil sample collection will be targeted in areas of the Site where prior data indicate dioxin/furan TEQ concentrations generally between 1,000 and 3,000 parts per thousand and include a representative range of organic carbon content, while also considering the site conceptual model. Soil samples (0-1 feet below ground surface [bgs] in depth) will be collected from ten different locations at the Site, as follows:

Sample Location	Total Dioxin TEQ (ppt)	Fraction of Organic Carbon (%)
Two samples from Area 4		
SA 169	2,000	0.145
Near SA 84	1,200	0.629
Four samples from Area 2		
Near SA 41	2,237	1.25
Near SA 114	2,522	7.18
Near SA 150	3,052	0.274
Near SA 167	2,027	0.073
Four samples from Area 1		
Near SA 75	1,265	0.063
Near RSAH3	1,360	0.15
Near RSAL3	1,141	0.895
Near RSAK4	1,556	0.166

At each of these locations, one additional sample will be collected and archived in the event that additional analysis is needed (10 for initial analysis and 10 archived samples). Samples will be homogenized in the field prior to being transferred to appropriate sample containers. Samples will be collected in accordance with procedures outlined in the Quality Assurance Project Plan (QAPP) (AECOM 2009), including use of sample containers, preservatives, and holding times as specified in Table B-1 of the QAPP[1]. Standard Operating Procedures (SOPs) presented in BRC SOP-06 (Sample Management Procedures) and SOP- 34 (Investigated Derived Waste Management) will be followed.[2]

All bioaccessibility extractions and dioxin/furan analytical work (for soils and extraction fluid) will be conducted by Vista Analytical at their laboratory:

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El Dorado Hills, CA 95762-9622
(916) 673-1520

In the lab, the soils will be air-dried and sieved to the <250-µm particle size prior to being analyzed for dioxin/furan. All soil samples and extraction fluid samples will be analyzed for dioxins/furans by EPA Method 1613. (This method is technically identical to EPA Method 8290, but with different quality control limits. Method 1613 is generally recommended for use on biological tissue samples, and therefore was deemed more appropriate for evaluation of simulated gastric fluid, although the distinction is insignificant, given the similarities in the methods.) Data will be reported as individual congeners to allow for reporting of results in toxicity equivalency (TEQ). All samples will

[1] Quality Assurance Project Plan, Tronox LLC Facility Henderson Neveda. AECOM 2009. Revised July 20.
[2] Basic Remediation Company Standard Operating Procedures, BMI Common Areas, Clark County Neveda. SOP -06 and SOP -34. December 2008.

be analyzed for dioxin/furan content using isotope dilution gas chromatography-mass spectrometry, to ensure that the collected soils represent an appropriate dioxin/furan TEQ range for use in the bioaccessibility study. All 10 samples (and any additional archived samples) will also be analyzed for organic carbon content according to United States Environmental Protection Agency (USEPA) Method Lloyd-Kahn 9060.

Bioaccessibility Extraction Method

The extraction will be carried out in 1-liter (L) amber glass bottles with Teflon®-lined screw caps. The bottles will be partially immersed in a water bath to maintain a temperature of 37 °C throughout the extraction procedure. Slow mixing will be provided by a stainless-steel paddle stirrer mounted in a rheostat-controlled motor (Arrow Engineering Model 1750®), or on a shaking water bath. A stirring/shaking rate of 30 revolutions per minute (rpm) will be maintained during the *in vitro* extraction.

The method generally follows that published by Ruby et al., (2002), but scaled back to 90% to allow for the use of 1-L bottles. The test procedure involves extraction of 9 grams (g) of test soil (<250-µm size fraction) in 0.9 L of extraction fluid (1:100 soil:solution ratio[3]), using a sequential extraction procedure that simulates a stomach phase (pH 1.5 with various enzymes, proteins, and fatty acids for 1 hour) followed by a small-intestinal phase (pH 7.2 with additional enzymes for 4 hours). Subsequent to the small-intestinal incubation, the extraction solution will be centrifuged (to remove any soil particles), and the extraction fluid will be submitted for analysis according to USEPA Method 1613. The resultant data, in combination with the total concentrations of the target analyte(s) for each soil, will be used to calculate the fraction of chemical that is liberated from each test soil (i.e., fraction that is bioaccessible).

The extraction procedure will be conducted according to the following method:

- Stock solutions should be mixed as specified in Ruby et al. (2002) (and modified in Finley 2009). Text below provides the basic steps in mixing these solutions. The attached Table 4 provides a scale-up to provide adequate solution for the full suite of extractions to be undertaken in this effort, and the contents of each extraction vessel.

[3] The 1:100 soil:fluid ratio was initially implemented in bioaccessibility testing for metals in soil. Lower soil:fluid ratios (e.g., 1:5 or 1:25) were found to limit dissolution from soils, most likely by constraining dissolution kinetics. Insufficient data are available to accurately estimate the soil:fluid ratio that might occur in a child following inadvertent soil ingestion, although some have suggested that higher ratios (e.g., 1:1000) are representative of the ratio of total daily soil ingestion to total daily fluid production in the gastrointestinal tract (Richardson 2006). High soil:fluid ratios have potentially adverse impacts on detection limits as well as creating larger waste streams. Therefore, it is generally believed that the ratio needs to be adequately high to avoid constraints on dissolution kinetics. For lead, in vitro extraction methods using a 1:100 soil:fluid ratio result in bioaccessibility estimates that are directly predictive of the relative oral bioavailability of lead. The ratio of 1:100 was selected for use in this method (Ruby 2002), based on precedent and logistical considerations.

- Prepare 4 L of buffered stomach fluid by adding 60.06 g glycine (0.2 M; Sigma UltraPure®) to 4 L of Type II deionized (DI) water, and adjust to pH 1.5 with concentrated HCl (requires approx. 240 mL)
- Add 35.2 g of sodium chloride (NaCl, concentration of 150 mM in stomach fluid)
- Add 4.0 g of pepsin (activity of 800–2,500 units/mg, final concentration of 1.00 g/L in stomach fluid)
- Add 20 g bovine serum albumin (BSA; minimum 98 percent, final concentration of 5 g/L in stomach fluid)
- Add 10 g mucine (Type III, purified from porcine stomach; final concentration of 2.5 g/L in stomach fluid)
- Place 0.9 L of the stomach solution in each reaction vessel
- Add 5.4 mL of oleic acid (90%; Aldrich Chemical) to each extraction vessel
- Add 9 g of soil (<250 μm size fraction) to each reaction vessel
- Stir for 1 hour with paddle stirrer at 30 rpm to simulate stomach-phase extraction
- Bring reaction fluid in each vessel to pH 7.2 by adding sodium hydroxide (NaOH; 50 percent w/w, approximately 9 mL)
- Add 540 mg porcine pancreatin to each extraction vessel (activity equivalent to 8× U.S.P. specifications)
- Add 3.6 g of bovine bile (50 percent bile acids, mixture of free and conjugated acids) to each extraction vessel
- Stir for 4 hours with paddle stirrer at 30 rpm
- After 4 hours of small-intestinal extraction time, allow the solids to settle, and decant all of the fluid from each reaction vessel into four 250-mL centrifuge tubes. Centrifuge at 3,000 Gs for 10 minutes and collect the supernatant in a 1-L amber glass bottle. Record the volume of extraction fluid collected.

Mass Balance Testing

As a check on the recovery from the *in vitro* extraction, a mass balance test will be performed on the replicate extraction samples by adding the two following steps (outlined below) to the extraction protocol:

- Using DI water, wash the post-extraction soil from the reaction vessel onto a 1.0-μm glass-fiber filter. Wash any soil pellets in the centrifuge tubes onto the filter. Wash the filtered soil with 20 mL DI water. Add the filtrate to the extraction supernatant (in the 1-L amber glass bottle), and measure the volume of extraction fluid.

- Collect the post-extraction soil, and remove 2 g for determination of percent moisture. Ship the remaining post-extraction soil (wet) to the analytical laboratory for analysis.

Quality Control

In addition to the 10 site soils, a set of samples will be included to allow for an assessment of data quality. These will include (at a minimum):

- Triplicate testing of one site soil
- Extraction blank
- Extraction spike (to be representative of the congener mix found in site soils)

ATTACHMENT

1 Vista Analytical Services Dioxin/furan Analytical Results for Soil Samples

TABLES

1 Dioxin/Furan Congener for Select Soil Samples a)

2 Dioxin/Furan Congener Percentage of TEQ for Select Soil Samples a)

3 Dioxin/Furan Congener Percentage of TEQ for Select Supplemental (Depth) Soil Samples a)

4 Stock Solutions for Bioaccessibility Testing

Profile Set 1

Profile Set 2

ATTACHMENT 1: DIOXIN CONGENER PROFILE ANALYSIS

To evaluate the potential similarities or differences among dioxin/furan congener profiles from Site soils, a congener profile analysis was conducted for 37 soil samples collected from Areas I, II, and IV. Most of these samples represent concentrations of dioxin/furan TEQ within the approximate range of 1,000 to 3,000 ppt, which is the range of interest. However, for comparison purposes, some additional congener profiles for samples with higher reported dioxin/furan concentrations are included, and four (4) dioxin/furan samples collected near the former effluent pond berms SA 201-0.5B, RSAJ6-0.5B, RSAJ7-0.5B, RSAK3-0.5B) are included. Dioxin fingerprinting was not conducted for Area III samples, because all dioxin/furan TEQ results are below the BCL screening level of 1000 ppt.

Table 1 provides the dioxin/furan TEQ for the 37 samples and the individual congener results, while Table 2 presents the congener data as a percentage of the total TEQ for the 37 samples. Total organic carbon content of the samples is also presented in Table 2. As shown in the first set of fingerprint profile figures (Set 1), the congener fingerprints of all the samples are fairly consistent. With the exception of one sample (SA-129), dioxins generally account for less than 6% of the total TEQ and 2,3,7,8-TCDD constitutes a very minor component of each soil sample (less than 0.3%). In all samples, the hexa, hepta, and octa furans account for the vast majority (at least 75%) of the total dioxin/furan TEQ in each sample, with OCDF accounting for at least 40% of the total TEQ concentration. This pattern is essentially identical to that reported by Finley et al. (2009) for a magnesium facility in which chloride and metals were separated via an electrolytic process.

Table 3 provides the congener data as a percentage of the total TEQ for a set of supplemental soil samples in which dioxin/furan samples were collected at a depth of 1.0–1.5 ft bgs and 1.5–2.0 ft bgs. The second set of fingerprint profiles (Set 2) also shows that the fingerprint profiles for these samples are fairly consistent with depth.

Based on this evaluation, the dioxin/furan samples of interest have similar “fingerprints,” and there is little variability in this pattern among samples collected in Areas I, II, and IV, within high and low dioxin/furan TEQ concentrations or by depth. The fingerprint profiles will be confirmed based on the data collected as part of the bioaccessibility study.