

**Results of Bioaccessibility Study for Dioxin/Furans in Soil**  
**Tronox LLC**  
**Henderson, Nevada**

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## 1.0 OBJECTIVE

An in vitro bioaccessibility extraction test for dioxins/furans in soil was implemented for soils from the Tronox Henderson Site in Henderson, Nevada (Site). The objective of the study was to provide site-specific information to evaluate the relative oral bioavailability of dioxins/furans from site soils (on a toxicity equivalent [TEQ] basis). The data from the study were interpreted in the context of available studies on the bioavailability of dioxins/furans that have been presented in recent scientific publications.

The study was implemented according to the bioaccessibility protocol that was approved by the Nevada Division of Environmental Protection (NDEP) on February 23, 2010.<sup>1</sup> The protocol outlines the soil collection and analytical methods, bioaccessibility extraction procedures, and use of quality assurance/quality control (QA/QC) samples (Northgate 2010). The protocol also presents the dioxin/furan congener profile for the relevant Site soil samples, as it provides the basis for identifying one dioxin/furan source type at the Site.

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<sup>1</sup> Memorandum from D. Chambers, Northgate and R. Kalmes, Exponent to Brian Rakvica, NDEP. Protocol: Bioaccessibility Method for Dioxin/Furans in Soil. February 19, 2010. Letter from Shannon Harbour, NDEP, to Matt Paque, Tronox, approving protocol, February 23, 2010.



## **2.0 MATERIAL AND METHODS**

### **2.1 Soil Collection and Analysis**

Ten surface soil samples (0–1 feet below ground surface [ft bgs]) were collected on March 8, 2010, at the locations outlined in the protocol and shown on Figure 1. The soil samples were targeted in areas of the Site where prior data indicated dioxin/furan TEQ concentrations generally between 1,000 and 3,000 ppt, and that included a representative range of organic carbon content.

Samples were homogenized in the field prior to being transferred to appropriate sample containers. All samples were 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.<sup>2</sup> Standard operating procedures (SOPs) presented in BRC SOP-06 (Sample Management Procedures) and SOP-34 (Investigated Derived Waste Management) were followed.<sup>3</sup>

Soil samples were shipped to Vista Analytical Laboratory (Vista) in El Dorado Hills, California. Soils were allowed to air dry, and were then sieved to the <250 micron (µm) particle size fraction. All samples were analyzed for dioxin/furan content using isotope dilution gas chromatography-mass spectrometry according to Environmental Protection Agency (EPA) Method 1613. Method 1613 is technically identical to EPA Method 8290, but with different quality control limits. Additionally, a split of each soil sample collected in the field was submitted to Columbia Analytical Services in Kelso, Washington, and analyzed for organic carbon content according to EPA Method Lloyd-Kahn 9060. Organic carbon content was measured for both unsieved and sieved (<250 µm particle size fraction) portions from each sample, for comparison to prior Site data and for consistency with the soil particle fraction used in the bioaccessibility study.

### **2.2 Soil Collection and Analysis**

After soil samples were confirmed to meet the target total TEQ and organic carbon content concentration range, the in vitro soil extraction was conducted using procedures outlined in the bioaccessibility protocol (Northgate 2010). The extraction procedures were observed by Mr. Kurt Fehling (NDEP consultant) and Ms. Yvette Lowney (Exponent) on March 25th and 26th at

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<sup>2</sup> Quality Assurance Project Plan, Tronox LLC Facility Henderson Nevada. AECOM 2009. Revised July 20.

<sup>3</sup> Basic Remediation Company Standard Operating Procedures, BMI Common Areas, Clark County Nevada. SOP-06 and SOP-34. December 2008.



Vista. Only minor deviations from the protocol were observed; primarily that the extraction was scaled back to 90% to allow the full fluid volume required (extraction fluid and pH adjustments) to fit into 1 liter (L) glass bottles. Both the volume of initial extraction fluid and the mass of soil were scaled. This modification was made with the agreement of Mr. Fehling and Ms. Lowney.

In summary, the extraction was carried out in 1-L amber glass bottles with Teflon®-lined screw caps. The bottles were partially immersed in a water bath to maintain a temperature of 37 °C throughout the extraction procedure. Slow mixing was provided by placing bottles on a shaker inside the water bath. A shaking rate of 30 revolutions per minute (rpm) was maintained during the in vitro extraction. The test procedure involved extraction of 9 g of test soil (<250-µm size fraction) in 0.9 L of extraction fluid (1:100 soil:solution ratio), 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 incubation in simulated small-intestinal fluid, the extraction solution was centrifuged (to remove any soil particles), and the extraction fluid was analyzed according to EPA Method 1613.

As a check on the recovery from the in vitro extraction, several measures were included, to ensure our ability to assess the reliability of the extraction results. These included method blanks, matrix blanks, replicates, spikes, and a mass balance evaluation.



## 3.0 RESULTS

### 3.1 Soil Samples

The dioxin/furan congener and TEQ results for each of the soil samples, as well as the fraction organic carbon content (FOC) are summarized in Table 1. The analytical reports for the analyses of soil samples are provided in Appendices A and B. The FOCs of the unsieved and sieved samples ranged from 0.134% to 1.78%, and 0.125% to 2.35%, respectively. These results indicate that there is no significant difference in FOC between sieved and unsieved soil. The FOC ranges are also consistent with the general range of FOC in samples collected site wide. Based on a prior analysis of 251 site samples, the average FOC at the Tronox site is 0.44%, with a majority (93%) of samples ranging from 0.02% to 1%.

The dioxin/furan TEQ concentrations of the soil samples range from 817 pg/g to 3600 pg/g. The congener profiles, shown in Figure 2, are consistent between samples, as well as with profiles observed in prior Site soil samples (and presented in the bioaccessibility protocol). The congener profiles indicate that dioxins generally account for less than 6% of the total TEQ, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) constitutes a very minor component of each soil sample (less than 0.5%). 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 octachlorodibenzofuran (OCDF) accounting for at least 40% of the total TEQ concentration. Based on these data, the soil samples selected for use in the bioaccessibility study are representative of site soils both by congener profile and organic carbon content.

With regard to QA/QC soil samples, no target compounds were detected in the field blank. Results for the field duplicate (RSAK4009-0.0B-BIO-A) and primary sample (RSAK4-0.0B) were comparable. Low-level hepta- (4.36 pg/L) and octa- (6.26 pg/L) furan values were detected in the equipment blank; however, the associated soil concentrations were not affected. The matrix spike/matrix spike duplicate (MS/MSD) analysis resulted in sporadic recoveries due to target analyte concentrations greater than 4 times the spike found in primary sample SA169-0.0B-BIO-A (i.e., OCDF detected 45,500 pg/g, yielding zero recovery).



## 4.0 BIOACCESSIBILITY DETERMINATION

All bioaccessibility extraction testing proceeded as specified in the bioaccessibility protocol, with the modification (discussed above) of scaling down the process. All extractions and analytical work were performed by Vista at their facility in El Dorado Hills, California. Concentrations of dioxins/furans in the final extraction fluid are reported on Table 2. All 17 dioxin/furan congeners analyzed for were detected in the extraction fluid of all soil samples tested. Concentrations were dominated by the furan congeners rather than dioxins, with the highest concentrations from OCDF and 1,2,3,4,6,78-HpCDF. TEQ concentrations in the extraction fluid ranged from 1160 to 9010 pg/L. The analytical reports for the analyses of soil samples are provided in Appendix C.

The bioaccessibility of the dioxins/furans was calculated, on a congener-specific basis, by comparing the mass of each congener in the extraction fluid to the mass of each congener present in the soil that was subjected to extraction. Table 3 presents the bioaccessibility results, on a congener-specific basis and weighted to provide a total TEQ of the bioaccessible fraction of dioxins/furans in each soil. Percent bioaccessibility for total TEQ ranged from 5% (Sample RSAK4-0.0B-BIOA-DUP) to 31% (Sample SA114-0.0B-BIO-A). The sample with the lowest observed bioaccessibility was a replicate sample, and it poorly matched the results from the replicate. Specifically, despite very similar concentrations of dioxins/furans in the replicate soil samples, the bioaccessibility varied from 5% to 28% between the two samples. The 5% value appears low relative to all other samples evaluated. Despite no apparent anomaly in the sample extraction, or laboratory error, this sample was excluded from subsequent evaluations to ensure that conclusions were not biased low. The bioaccessibility across the nine remaining samples ranged from 10% to 31% on a TEQ basis, with an overall mean of 20%. On a congener-specific basis, bioaccessibility values ranged from 6% to 43%, with no clear pattern in extraction efficiency across the different soils.

### 4.1 Bioaccessibility QA/QC

As specified in the bioaccessibility protocol, several measures were included to ensure our ability to assess the reliability of the extraction results. These included method blanks, matrix blanks, replicates, spikes, and a mass balance evaluation. Results for these samples are provided in Tables 4a through 4c, and discussed below. Overall, the data indicate good precision and accuracy, that total recovery was good, and that the method was relatively free from contamination.





- **Method Blank:** A method blank (clean water carried through the process) was included to assess whether any contamination entered the extraction vessel and to assess analytical bias. Results for all congeners fell below analytical detection limits, indicating that the method is generally free from contamination.
- **Matrix Blank:** A matrix blank (extraction fluid with no soil or spike) was included on each of the two days that extractions were conducted. Results for these two quality control samples indicate that the extraction fluid was generally free from contamination with dioxins/furans. The exceptions were that 300 pg/L of OCDD were detected in each of these samples, and 40 pg/L of 1,2,3,4,6,7,8-HpCDD was detected in one. The specific source of these is unknown, but is likely to be associated with the biologically derived constituents of the extraction fluid (e.g., pancreatin, bile salts, etc.). Concentrations of these congeners in the soil extracts were much higher than detected in the matrix blank. No adjustments were made to the data based on the results from the matrix blank (e.g., to subtract out the “background” concentration). This could result in calculated bioaccessibility values that are biased slightly high, but given the low concentrations of dioxins in the matrix blank, the magnitude of this error would be small.
- **Matched Spike:** To quantitatively assess the ability of the laboratory to recover the various dioxin/furan congeners from the extraction matrix in the concentration range of relevance for the project, a “matched spike” was included, in which extraction fluid was spiked with the mass of each congener equivalent to the mass that would be introduced with the soil. The matched spike was calculated based on soil concentrations for soil sample SA167-0.0B-BIO. Table 4a depicts the spiked and measured concentrations of each congener from this sample. Results indicate consistently good recovery of each congener from the extraction fluid; for 13 of the 17 congeners, recovery exceeded 90% of the spiked concentration. Three of the remaining samples exhibited 89% recovery, and the lowest recovery was 87%. This indicates that dioxins/furans can be recovered from the extraction fluid in the concentration range of interest for this study, and that there is no low bias due to poor recovery.
- **Replicates:** To assess precision/reproducibility, bioaccessibility extraction testing was conducted in triplicate for one sample. Across the three replicates, TEQ concentrations in the extraction-fluid samples were 4470 pg/L, 4540 pg/L, and 5850 pg/L for the three samples, respectively. These data are summarized in Table 4b, and indicate a relative standard deviation (RSD) of 16% for the samples.
- **Mass Balance:** Samples were included in the extraction scheme to allow for evaluation of the total recovery of dioxins/furans from the soil. This was done by evaluating the mass of each congener in both the extraction fluid and the post-extraction soils. This was performed in triplicate on soil sample SA167-0.0B-BIO. The results for the extraction fluid are described in the prior bullet (regarding replicates). The TEQ concentrations found in the three post-extraction soils were 1250 pg/g, 1160 pg/g, and 1340 pg/g. Table



4c provides data regarding the mass balance evaluation. Results indicate that, for the three soil samples tested, recovery (i.e., mass found in the extract plus mass remaining in the soil) of the TEQ were 68%, 75%, and 95% for the three soils, respectively. On a congener-specific basis, for all three samples, the lowest recovery was observed for 1,2,3,6,7,8-HxCDF (50%–70%). Consistently across all three samples tested, the highest mass balance recovery was observed for 2,3,4,6,7,8-HxCDF and OCDD (90%–128%).

Overall, the quality control samples included in this evaluation were relatively comprehensive, and indicate that the bioaccessibility data appear to be of high quality, with good precision and accuracy, and will have low potential for bias due to analytical limitations or error.



## 5.0 DISCUSSION

Appendix A of EPA's Risk Assessment Guidance for Superfund, Part A (U.S. EPA 1989), indicates that adjustments to the absorption efficiency may be made as part of the risk assessment process, to ensure that the exposure estimate from the environmental exposure medium of concern is consistent with the toxicity value. In the case of dioxin, several studies have reported that dioxin can bind tightly to the soil, thereby greatly reducing the systemic uptake of ingested soil-bound dioxin. Because the EPA oral cancer slope factor for dioxin is based on liver tumor incidence in animals treated with dioxin in rat chow (Kociba et al. 1978), and the derivation of the Agency for Toxic Substances and Disease Registry (ATSDR) minimal risk level (MRL) is based on behavior displacement observed in monkeys exposed to dioxin in their diet (Schantz et al. 1992), it is appropriate and consistent with EPA guidance to account for the soil matrix effect by considering data on the relative oral bioavailability of dioxin from soils. A technical memorandum from NDEP consultants posted on the NDEP BMI website addresses use of an adjustment factor for arsenic bioavailability in soil for the same reasons (Copeland and Otani-Fehling 2008).

Oral bioavailability factors for dioxin have been determined by evaluating the fraction of ingested dioxin that accumulates in tissues of animals versus a control group dosed with a pure reference formulation (usually dioxin suspended in corn oil). Soil characteristics such as particle size, clay content, and total organic carbon content have been shown to influence the oral bioavailability of soil contaminants (Pu et al. 2004; Yang et al. 2005). Table 5 presents the soil characteristics and range of oral bioavailability in vivo estimates for dioxin (as a single congener or as total dioxin TEQ) from various published studies performed in rats and guinea pigs and relevant in vitro studies. Most studies have reported relative bioavailability and absolute bioavailability estimates of approximately 10% to 40% and 10% to 30%, respectively, with one Times Beach study reporting relative and absolute bioavailability values up to 70% and 50%, respectively (McConnell et al. 1984; Umbreit et al. 1986; Shu et al. 1988; Wendling et al. 1989; Wittsiepe et al. 2007; Budinsky et al. 2008; Finley et al. 2009).<sup>4</sup>

As reported by Ruby et. al (2002), the alternative in vitro bioaccessibility assay estimates the oral bioavailability of dioxins/furans in humans using a physiologically based extraction test to determine the fraction of dioxins/furans that would be solubilized ("accessible") in the human gastrointestinal tract and, therefore, would be available for absorption. The use of in vitro assays for estimating oral bioavailability has been accepted in regulatory decision making and risk

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<sup>4</sup> The absolute bioavailability is the ratio of the amount of the chemical absorbed compared to the amount ingested. The relative bioavailability is the ratio of the chemical in some medium compared to the absolute bioavailability of the chemical.



assessment (U.S. EPA 2006) and has potential practical and methodological advantages over in vivo studies.

Results of bioaccessibility assays using simulated human GI fluids have been compared to in vivo oral bioavailability studies. Both Budinsky et al. (2008) and Finley et al. (2009) reported lower mean TEQ extracts in gastric fluid studies than oral bioavailability measured in rat-dosed studies. In particular, Budinsky et al. reported that the oral bioavailability of dioxins/furans from soil is species dependent and noted that use of a juvenile swine model or an in vitro bioaccessibility assay is preferable to reliance on rodent models. Moreover, Budinsky et al. noted that the mean dioxin/furan TEQ bioavailability measured in soil-dosed swine (23%) was similar to the bioaccessibility results using simulated GI fluids (25%) compared to the relative oral bioavailability using rats (37%). Others have recognized the swine to be a better model than rats for the human gastrointestinal system because of the close similarity between human and swine physiology (Krishnan et al. 1994; Eklund et al. 2004). EPA has acknowledged the pig to be a better model for bioavailability assessment of soils contaminated by metals (U.S. EPA 2006; Casteel et al. 2006), and swine are a preferred model for human nutrition studies (Miller and Ulfrey 1987).

Additionally, while most published studies on bioavailability evaluated 2,3,7,8-tetrachlorinated dibenzo-p-dioxin (TCDD), Budinsky et al. (2008) and Finley et al. (2009) examined all 17 dioxin/furan congeners and reported bioavailability on a total TEQ basis, making these two studies particularly relevant to the Tronox Henderson site where dioxin TEQ data have been collected.

The Finley et al. 2009 publication reports an overall bioavailability of dioxins/furans in five soil samples on a total TEQ basis, ranging from 17%–50%, with a mean of 38%, using soil TEQ concentrations from 0.53 to 45.2 nanograms per gram (ng/g, or parts per billion). Finley et al. 2009 also reports that bioavailability was independent of initial soil TEQ concentrations over this 80-fold range of soil TEQ concentrations, in which the organic carbon content in soils was less than 1%. The soil mass and total TEQ were dominated by the furans, similar to the findings at the Henderson Site. With regard to the in vitro bioaccessibility portion of the study, Finley et al. reports a mean value of 29% for soils that were sieved to the <250 µm particle size, based on five samples with soil concentrations ranging from 0.7 to 101 ppb. Finley et al. indicated that the bioaccessibility of dioxin/furan congeners can be several-fold higher in finer soil particle size fractions relative to bioaccessibility from coarser particle size fractions. In interpreting the results of the Tronox study, it is important to note that all soils were sieved prior to extraction, and test results represent findings for the fine particle size fraction (i.e., <250 µm), which is believed to be a more relevant measure of the soil particles that may be ingested.



## 6.0 CONCLUSION

This study was undertaken to provide site-specific information regarding the in vitro bioaccessibility of dioxins/furans in soils at the Tronox site. EPA has used in vitro assays to estimate oral bioavailability in regulatory decision making and risk assessment (U.S. EPA 2006). The results of the extraction test indicate that the study data are of good quality, with bioaccessibility values that range from 10% to 31% across the nine soils evaluated, with a mean bioaccessibility value for the site soils of 20%, all expressed on a TEQ basis. These values are in the same range as those reported for in vivo relative bioavailability studies, particularly those based on swine, which is considered to be the preferred test species over rodents because of more similar gastrointestinal physiology. These values are at the lower end of the range of in vivo bioavailability results presented by Finley et al. (2009), which utilized soil with a dioxin fingerprint similar to that of the Tronox Henderson site, but was conducted using rats rather than swine. Finally, the average value of 20% from the Tronox study is in good agreement with the average in vitro value of 25% and the average in vivo value of 23% reported by Budinsky et al (2008).

Based on the results of this site-specific study and review of the pertinent literature, and to account for the potential uncertainty between in vitro and in vivo studies, we propose to use a bioavailability value of 31% in conducting a deterministic risk assessment at the Tronox site. This value represents the maximum reported in our study, rather than the average of 20%. Application of this value results in a site-specific risk-based remedial goal for dioxin TEQ of 3,200 ppt.



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