ORAL BIOACCESSIBILITY OF DIOXINS/FURANS FROM INDUSTRIAL SOILS USING A SIMULATED HUMAN G.I. TRACT

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Introduction

Polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) are common environmental contaminants with very high soil binding coefficients. Laboratory experiments with test animals indicate that the oral systemic absorption (bioavailability) of PCDD/Fs from soil is lower than for PCDD/Fs in lipophilic vehicles such as corn oil or other oil-based delivery media used in animal-feeding studies. For example, administration of tetrachlorodibenzo-*p*-dioxin (TCDD) in commonly used vehicles typically yields oral bioavailabilities ranging from 70%-90%[1-3], while the reported oral bioavailability of TCDD in soils ranges from 0.5-60% relative to reference oral formulations[4-8].

The relative bioavailability of soil-bound chemicals can be determined directly from feeding studies in which the concentration of the parent chemical or a metabolite is measured in tissues and/or via a "mass-balance" analysis involving measurements of chemicals excreted in the feces. However, there are anatomic and physiological differences between humans and common animal test species, which may confound the applicability of the results for human risk assessment purposes[9,10].

An *in vitro* bioaccessibility study permits derivation of a conservative estimate of the *in vivo* oral bioavailability of soil-bound chemicals in humans that can be used as an alternative or supplement to animal studies. In a bioaccessibility study, the soil is extracted with fluids that simulate the stomach and small intestine segments of the human GI tract. The amount of chemical present in the liquid phase following the extraction is used to determine the bioaccessible fraction. Because the extraction conditions are believed to be at least as harsh as those present *in vivo*, presumption of 100% absorption of the bioaccessible fraction yields a conservative estimate of bioavailability. The *in vitro* approach avoids animal use, has the advantages of simplicity and lower cost, and permits evaluation of many different variables (effect of soil type, soil particle size, chemical concentration, etc.) that simply is not practical with the more costly and time-consuming *in vivo* studies.

To date, there are no published bioccessibility measurements of PCDD/Fs in industrial soils. In this paper, we describe the bioaccessibility results for PCDD/Fs in soils near an operating facility in the U.S. The measured TEQ in the soils ranges from 0.7 µg/kg to 23.6 µg/kg and OCDF is the primary congener, comprising approximately 90% of the total PCDD/F mass. The purpose of this study is to supplement the existing bioaccessibility and bioavailability data for PCDD/Fs in contaminated media.

Materials and Methods

A total of eight surface soil samples (0-3 inches depth) were collected from an industrial site with historical PCDD/F discharges. The soils were analyzed for 17 polychlorinated dibenzo-*p*-dioxins and furans with EPA method SW 8290 using high resolution gas chromatography / high resolution mass spectrometry.

The soil samples were air dried and sieved in an attempt to isolate the fraction less than 250 microns in diameter. Two samples (#4 and #5) were coarse grained and could only be sieved to a < 500 micron fraction. One sample (#3) was coarse grained and gummy and was used unsieved.

The sieved fraction of each sample was subjected to the following *in vitro* extraction method:

The extractions were conducted in 1-liter Teflon bottles, which were immersed in a water bath at 37° C. Mixing was accomplished with a stir table oscillating at 30 revolutions per minute. A buffered solution was prepared by adding 60 grams of glycine (0.2 M; Sigma UltraPure®) to 4 L of Type II deionized (DI) water, and adjust to pH 1.5 with concentrated HCl (~240 mL). To this, 32.5 g of sodium chloride (NaCl, concentration of 150 mM in stomach fluid), 4.00 g of pepsin (activity of 800-2,500 units/mg, final concentration of 1.00 g/L in stomach fluid), 20 g bovine serum albumin (minimum 98 percent, final concentration of 5 g/L in stomach fluid), and 10 g mucine (Type III, purified from porcine stomach; final concentration of 2.5 g/L in stomach fluid) were added. Eight hundred mL of the gastric solution were placed in each Teflon bottle, and 4.8 mL of oleic acid (90%; Aldrich Chemical) were added. Eight grams of test soil were added, and the resulting mixture was stirred at 30 rpm on the mixing table for one hour.

After the 1-hour simulated gastric portion of the test, the solution in each bottle was adjusted to pH 7.2 using sodium hydroxide (50 percent w/w, approximately 10 mL). Finally, 480 mg pancreatin (activity equivalent to 8 × U.S.P. specifications) and 3.2 mg bovine bile (50 percent bile acids, mixture of free and conjugated acids) were added to each reaction vessel and stirred at 30 rpm for 4 hours to simulate small intestinal-phase extraction.

Each reaction vessel was centrifuged at 5000 ×*g* for 10 minutes. The supernatant was decanted into a graduated cylinder and total volume was measured. All eight samples were analyzed for 17 PCDD/F congeners at Alta Analytical Laboratories, Inc. (Alta) in El Dorado Hills, CA. Quality assurance samples included an extraction blank, a spiked extraction blank, and a triplicate analysis of one sample (#2) to assess reproducibility.

Results and Discussion

A majority of the soil TEQ concentration and extractant resulted from four congeners: 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, and 1,2,3,4,6,7,8-HpCDF. As shown in Figure 1, the average % congener contribution to total TEQ was very similar for soils vs. extractants.

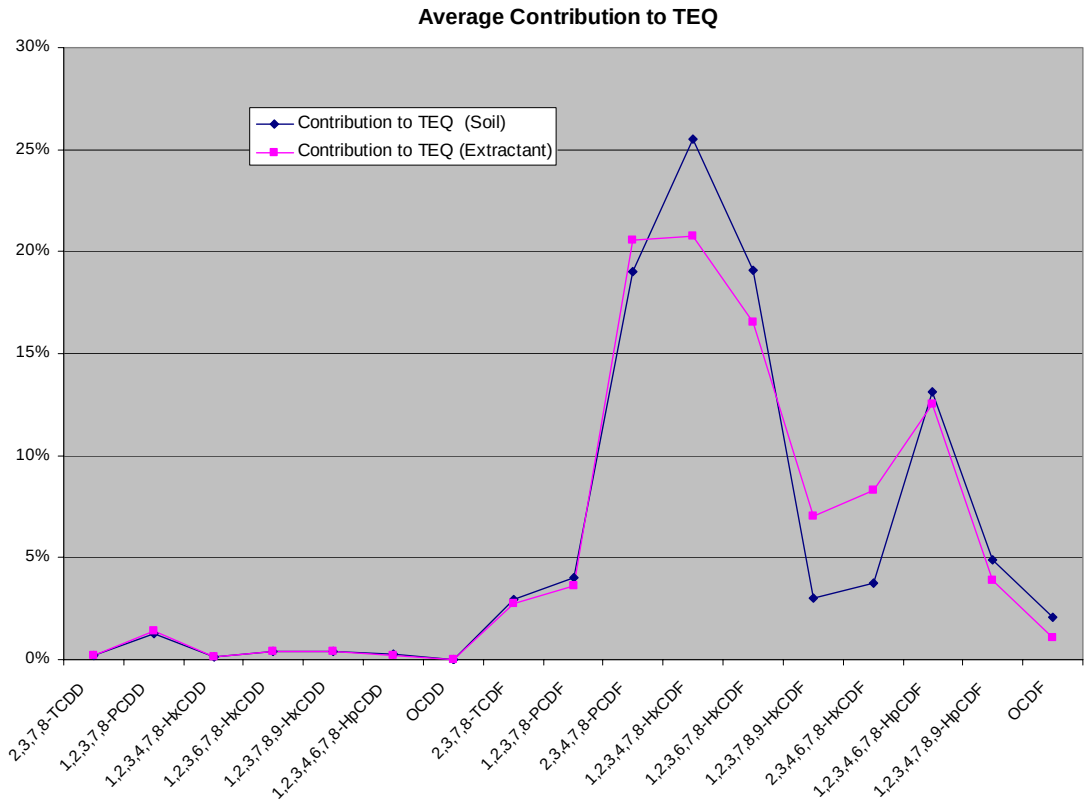

Figure 1 – Average contribution to TEQ (Soil and Extractant)

Bioaccessibility total TEQ results for the eight samples ranged from 9 to 46%, with an overall average of 22%. There did not appear to be any trends with respect to degree of bioaccessibility vs. initial soil concentration. The bioaccessibility values for TCDD ranged from 2 to 75%, (average of 24%). Bioaccessibility was lowest in samples #3, #4, and #5; these samples were either sieved to below the 500-micron particle-size level, rather than the 250-micron level (#4 and #5), or not seived at all (#3). This result suggests that, at least with the soils used in this study, bioaccessibility may decrease with increasing particle size. As indicated in Figure 2, the bioaccessibility of the two congeners 1,2,3,7,8,9-HxCDF and 1,2,3,4,6,7,8-HxCDF was clearly elevated relative to the other congeners

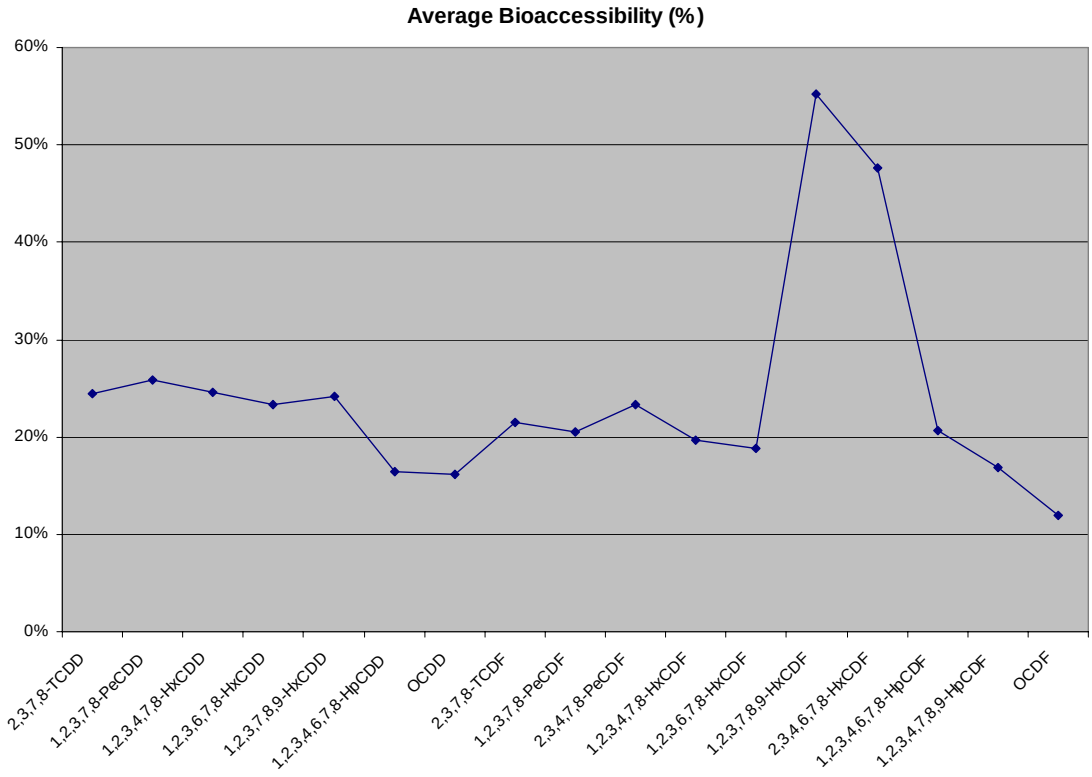

Figure 2 – Average Bioaccessibility of 17 PCDD/F congeners

The mean bioaccessibility measured in this study (22%) is within the range determined from *in vivo* TCDD bioavailability studies (0.5%-60%). This value agrees with values from other, similar studies and likely represents an upper bound estimate of oral bioavailability for these soils[1,11].

References

1. Diliberto J, Jackson J, Birnbaum L.. *Toxicol Appl Pharmacol* 1996;138:158.
2. Rose J, Ramsey J, Wentzler T, Hummel R, Gerhring P. *Toxicol. Appl. Pharmacol* 1976;36:206.
3. Piper W, Rose J, Gehring P. *Environ Health Perspec* 1976;5: 241.
4. Lucier G, Rumbaugh R, McCoy Z, Hass R, Harvan D, Albro P. *Fund Appl Toxicol* 1986;6:364.
5. McConnell E, Lucier G, Rumbaugh R, Albro P, Harvan D, Hass J, Harris M. *Science* 1984;223:1077.
6. Bonaccorsi A, di Domenico A, Fanelli R, Merli F, Motta R, Vanzati R, Zapponi A . *Arch Toxicol* 1984;S7:431.
7. Shu H, Paustenbach D, Murray F, Marple L, Brunck B, Dei Rossi D, Teitelbaum P. *Fund Appl Toxicol* 1988;10:648.
8. Umbreit T, Hesse E, Gallo M. *Science* 1986;232:497.
9. Casteel S, Evans T, Turk J, Basta N, Weis C, Henningsen G. *Int J Environ Health* 2001;203:473.
10. Casteel S, Cowart R, Weis C, Henningsen G, Hoffman E, Brattlin W, *Fundam Appl Toxicol* 1997;36:177.
11. Ruby M, Fehling K, Paustenbach D, Landenberger B, Holsapple M. *Environ Sci Technol* 2002;36:4905.