IN VITRO BIOACCESSIBILITY STUDY OF LOW CONCENTRATIONS (50-350 ppt TEQ) OF DIOXIN/FURANS IN WEATHERED SOILS

K. A. Fehling,[1] M. V. Ruby,[2] and D.J. Paustenbach.[3]

Exponent, 631 First Street, Suite 200, Santa Rosa, CA 95404,[1] , Exponent, 4940 Pearl East Circle, Suite 300, Boulder, CO 80301[2] , Exponent, 149 Commonwealth Drive, Menlo Park, CA 94025[3]

Introduction

The oral and dermal bioavailability of polychlorinated dibenzodioxins and furans (PCDDs/Fs) in soil has been studied in several *in vivo* rodent assays.[1-9] However, the uncertainties associated with scaling up animal data to humans limit the utility of these data for the purposes of setting site-specific soil cleanup goals. In addition, bioavailability is largely governed by the physical and chemical properties of the soil (e.g., organic content, particle size distribution, etc.), so results may differ substantially from those used in the published studies. Furthermore, differences in the soil PCDD/F concentrations and the presence of co-contaminants may influence bioavailability and confound the interpretation of the results.[1,2] Accordingly, for the purposes of setting site-specific soil cleanup goals for PCDDs/Fs, the collection of site-specific bioavailability data that are relevant to human exposures offers several advantages over the published literature, particularly if the data can be collected in a cost-effective and relatively simple manner.[10]

In this paper, we describe an *in vitro* extraction test for determining the percent of PCDDs/Fs in soil that may be liberated or solubilized in the human gastrointestinal tract, and therefore available for absorption (i.e., the bioaccessible fraction). A test of this type has been used to assess the bioaccessibility of various heavy metals in soils, and has been demonstrated to correlate with results from animal studies of lead bioavailability in soil.[10] This same approach has been used previously to measure the bioaccessibility of other lipophilic organic compounds in soil, such as polynuclear aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), and the test described herein was modeled on those efforts.[11,12] Most importantly, the *in vitro* test provides for the formation of bile salt micelles during the small intestinal phase of the extraction, which are known to facilitate desorption of hydrophobic organic compounds from soil.[11,12,13,14] Because bioaccessibility (i.e., solubilization from soil) is a precursor to bioavailability (i.e., systemic absorption of the solubilized compound), an accurate determination of bioaccessibility can be used to estimate bioavailability.

Material and Methods

The test soil contained PCDDs/Fs resulting from aerial releases of byproducts from manufacturing and waste combustion processes prior to approximately 1980. Soil concentrations used in this study ranged from 50 to 350 ppt. The general test procedure has been described by Ruby et al.[10] It involves the extraction of 10 grams (g) of test soil (< 250-μm size fraction) in 1 liter (L) of extraction fluid

(1:100 soil:solution ratio), using a sequential extraction procedure that simulates a stomach phase (pH 1.5 for 1 hour) followed by a small-intestinal phase (pH 7.2) with various enzymes, proteins, and fatty acids for 4 hours. Subsequent to the small-intestinal incubation, the extraction solution was centrifuged (to remove any soil particles) and analyzed for concentrations of dioxin and furan congeners. The resultant data, in combination with the total PCDDs/Fs data for each soil, was used to calculate the fraction of each dioxin and furan congener that is liberated from each test soil (i.e., is bioaccessible).

Six composite soil samples were collected for use in this study, with one sample evaluated in triplicate. Each composite soil sample was air-dried, and sieved to < 250 μm because this size fraction is most likely to adhere to skin and be ingested via hand-to-mouth activity.[15] Subsamples of the sieved material from each sample were analyzed for pH, total organic carbon, and particle size distribution (i.e., sand, silt, clay), and for total concentrations of the 17 dioxin/furan congeners. Each sieved sample was also subjected to the *in vitro* extraction test.

Extraction Method

One L amber glass bottles with Teflon-lined screw caps were partially immersed in a water bath to maintain a temperature of 37°C throughout the extraction procedure. Slow mixing was provided by a stainless-steel paddle stirrer mounted in a rheostat-controlled motor at a rate of 30 revolutions per minute (rpm). The extraction procedure was conducted according to the following method (all chemicals were obtained from Sigma Chemical Company):

Four L of buffered stomach fluid was prepared by adding 60.06 g glycine (0.2 molar [M]; Sigma UltraPure) to 4 L of Type II de-ionized (DI) water, and the pH was adjusted to 1.5 with concentrated hydrochloric acid (HCl) (approx. 240 mL). To this was added 35.2 g of sodium chloride (NaCl, 150 mM final conc.), 4.00 g of pepsin (activity of 800–2500 units/mg, 1.00 g/L final conc.), 20 g bovine serum albumin (BSA, 5 g/L final conc.), and 10 g mucine (Type III, from porcine stomach; 2.5 g/L final conc.). One L of the stomach solution was placed in each reaction vessel along with 6 mL of oleic acid (90 percent). Ten g of soil (< 250-μm size fraction) was added and the resulting solution was stirred for 1 hour.

The solution was then brought to pH 7.2 by adding sodium hydroxide (NaOH, 50 percent w/w, approximately 10 mL) and 600 mg porcine pancreatin (activity equivalent to 8 × U.S.P. specifications) and 4 g of bovine bile (50 percent bile acids, mixture of free and conjugated acids). This solution was then stirred for 4 hours with a paddle stirrer at 30 rpm.

After the 4 hour extraction time, the solids were allowed to settle, and all of the fluid from each reaction vessel was decanted into 250 mL bottles and centrifuged at 3,000 times gravity for 10 minutes. The supernatant was placed in 1 L amber glass bottles. All extract samples were shipped on ice under chain of custody to Alta Analytcial Laboratory for analysis of PCDDs/Fs by Method 8290.

Results and Discussion

Table 1. Results for the soil samples.

Sample Identification	Concentration pg/kg	Average Percent Extracted
CC-S-27	101	19%
CC-S-27S	337.6	19%
NEPP-S-08S	66.4	21%
CC-S-34S	126.8	25%
NEPP-S-08	48.5	33%
CC-S-32	64.3	34%
	Overall Mean:	**25%**

Table 2. Extracted percentages for each of the 17 congeners.

Congener	CC-S-27	CC-S-27S	NEPP-S-08S	CC-S-34S	NEPP-S-08	CC-S-32	Mean	St. Dev.
2,3,7,8-TCDD	15	17	20	24	48	39	27	13.3
1,2,3,7,8,-PeCDD	16	16	19	25	14	35	21	7.6
1,2,3,4,7,8-HxCDD	16	18	16	27	27	35	23	7.6
1,2,3,6,7,8-HxCDD	21	18	16	27	29	34	24	7.0
1,2,3,7,8,9-HxCDD	20	18	16	28	19	31	22	6.2
1,2,3,4,6,7,8-HpCDD	23	26	22	37	47	33	32	9.8
OCDD	20	20	17	28	51	28	27	12.7
2,3,7,8-TCDF	17	16	14	22	20	33	20	6.9
1,2,3,7,8-PeCDF	19	17	19	23	25	32	22	5.7
2,3,4,7,8-PeCDF	19	18	14	21	24	37	22	8.2
1,2,3,4,7,8-HxCDF	20	19	19	21	37	38	26	9.2
1,2,3,6,7,8-HxCDF	31	16	17	15	35	36	25	10.0
2,3,4,6,7,8-HxCDF	18	17	18	24	24	37	23	7.3
1,2,3,7,8,9-HxCDF	19	16	22	22	29	34	24	6.6
1,2,3,4,6,7,8-HpCDF	24	23	26	33	33	40	30	6.5
1,2,3,4,7,8,9-HpCDF	17	19	33	23	44	29	27	9.8
OCDF	12	32	43	30	56	29	34	14.6
Sample Average:	**19%**	**19%**	**21%**	**25%**	**33%**	**34%**		
Total TEQ Concentration (ppt):	**101.0**	**337.6**	**66.4**	**126.8**	**48.5**	**64.3**		

Our results indicate an overall mean bioaccessibility of 25 percent for the seventeen 2,3,7,8 substituted congeners. The relative differences for each of the congeners was not as great as originally anticipated (see Table 1). Interestingly, this methodology which attempts to mimic the human stomach (*in vitro*) yielded results which were consistent with those reported in the various published bioavailability studies of 2,3,7,8-TCDD in animals (*in vivo*). It should be noted that two additional samples were analyzed and the results indicated greater than 100% bioaccessibility. These kinds of results can occur at very low concentrations of PCDDs/Fs (< 25 ppt) due to "noise" in the analytical instrumentation or extraction procedures. Since other samples at greater concentrations (>25 ppt) yielded consistent results, these two samples are not presented in Table 1.

Although total TCDDs/Fs concentrations in the six samples spanned almost an order of magnitude (48.5 to 337.6 ppt TEQ), the bioaccessibility of TCDDs/Fs only varied from 19 to 34 percent, suggesting that the concentration of dioxins/furans in soil do not have a dramatic effect on the extent of bioavailability. Similar observations have been made in animal bioavailability studies that used multiple dose groups of varying concentrations.

Since this approach attempts to mimic the human gastrointestinal tract, we believe it is likely to be more representative of the actual behavior of ingested PCDDs/Fs in humans than the results that have been obtained using animal testing (all of which have used rodents). Because the experimental method is a reasonable surrogate for the human gastrointestinal tract, and because the results are similar to those obtained with animals, we believe that it is appropriate to use the *in vitro* extraction method to estimate the bioavailability of dioxins/furans in soil to humans . Based on our review of the literature, this appears to be the first such study to evaluate low environmental concentrations of PCDDs/Fs (about 50 to 350 ppt) in aged soils, and to determine a bioaccessibility for each of the 17 dioxin-like chemicals.

We recommend that data obtained from this kind of *in vitro* study should be used when conducting human health risk assessments of the dioxins/furans.

References

1. Shu, H., Paustenbach, D., Murray, F.J., Marple, L. Brunck, B., Dei Rossi, D. and Teitelbaum, P. (1988). Fund. Appl. Toxicol. 10, 648-654.
2. Hrudey, S.E., Chen, W., and Rousseaux, C.. (1996). Bioavailability. New York, CRC-Lewis Publishers.
3. McConnell, E.E., Lucier, G.W., Rumbaugh, R.C., Albro, P.W., Harvan, D.J., Hass, J.R., and Harris, M.W. (1984). Science 223, 1077-1079.
4. Poiger, H. and Schlatter, C. (1980). Food Cosmet. Toxicol. 18, 477-481.
5. Umbreit, T.H., Hesse, E.J., and Gallo, M.A. (1986). Science 232, 497-499.
6. Gallo, M.A. (1986). Pitot Committee Report, Dioxin update committee convened by J.A. Moore, Office of Pesticides and Toxic Substances. Environmental Protection Agency, July 1-2, 1986.
7. Kaminsky, L.S., DeCaprio, A.P., Gierthy, J.F., Silkworth, J.B., and Tumasonis, C. (1985). Chemosphere 14(6/7) 685-695.

8. Lucier, G.W., Rumbaugh, R.C., McCoy, Z., Hass, R., Harvan, D., and Albro., P. (1986). Fundam. Appl. Toxicol. 6, 364-371.
9. U.S.EPA. (2000). Exposure and human health reassessment of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Washington, DC, EPA/600/P-00/001Ab.
10. Ruby, M.V., Davis, A., Link, T.E., Schoof, R., Chaney, R.L., Freeman, G.B., and Bergstrom, P. (1993). Environ. Sci. Technol. 27(13), 2870-2877.
11. Hack, A., and Selenka, F. (1996). Toxicol. Let. 88, 199-210.
12. Guha, S., Jaffé, P.R., and Peters, C.A. (1998). Environ. Sci. Technol. 32, 2317-2324.
13. Hernell, O., Staggers, J.E., and Carey, M.C. (1990). Biochemistry, 29(8), 2041-2056.
14. Rahman, A., Barrowman, J.A., and Rahimtula, A. (1986). Can. J. Physiol. Pharmacol. 64, 1214-1218.
15. Calabrese, E.J., and Stanek, E.J. (1998). Environ. Law Reporter, News & Analysis 28:10660-10671.