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Oral Bioavailability of Polychlorinated Dibenzo-*p*-Dioxins/Dibenzofurans in Industrial Soils

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ABSTRACT

In this study, the oral bioavailabilities of numerous 2,3,7,8-PCDD/F congeners were evaluated in soil samples from an industrial site. The purpose of this study is several-fold: (1) to compare the soil bioavailability results of the different PCDD/F congeners; (2) to evaluate the consistency of the bioavailability results with those obtained in an *in vitro* bioaccessibility study with simulated GI tract fluids; and (3) to develop quantitative bioavailability measurements that are appropriate for use in a health risk assessment for this site. Soil samples containing PCDD/F toxic equivalent (TEQ) concentrations ranging from 0.53–45.2 ng/g were administered to female Sprague Dawley rats via oral gavage. Reference formulations of PCDD/Fs were administered intravenously or by oral gavage. The overall relative bioavailability of PCDD/Fs in the soil samples on a TEQ basis ranged from 17 to 51%, with a mean of 38%. The results of the *in vitro* bioaccessibility study were consistent with the bioavailability results (mean extracted TEQ of 22%). Because of the clear relationship between increasing chlorination and decreasing bioavailability and bioaccessibility observed in this study, we suggest that simply extrapolating results from one congener to another may be associated with a high degree of uncertainty.

Key Words: dioxin, TEQ, soil contaminant, PCDD/F bioavailability, bioaccessibility.

INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCCD/Fs) are the subject of numerous environmental multi-pathway risk assessments in the U.S. Potential PCDD/F exposures that occur via incidental soil ingestion are typically a primary focus of such analyses. Indeed, one of the first attempts to quantitate the amount of soil incidentally ingested by children and adults was conducted as part of the 1984 U.S. Environmental Protection Agency's (USEPA's) health risk assessment for Times

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Beach, Missouri (Kimbrough *et al.* 1984), a town where residential soils were contaminated by 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (TCDD). Since that time, it has been established (based primarily on oral animal studies) that TCDD can have a high degree of soil affinity and that soil binding may therefore greatly retard the systemic uptake of ingested soil-bound TCDD. Because the USEPA oral cancer slope factor for TCDD is based on liver tumor incidence in animals treated with TCDD in rat chow (Kociba *et al.* 1978), it is standard risk assessment practice to account for this "soil matrix effect" via the use of data from TCDD oral bioaccessibility or bioavailability studies. Oral bioavailability factors for TCDD are typically determined by measuring the fraction of ingested TCDD that accumulates in the tissues of soil-dosed rodents versus a control group dosed with a pure TCDD reference formulation (usually TCDD in corn oil). Bioaccessibility studies involve serial *in vitro* soil extractions using fluids and conditions that simulate the physical and chemical environment in the human stomach and small intestine; the amount of chemical in the liquid phase at the completion of the extraction is considered to represent the bioaccessible fraction. If the extraction conditions are representative of those present *in vivo*, the bioaccessible fraction can be considered an approximation of the systemically absorbed (bioavailable) fraction.

The oral bioavailability of soil contaminants can be influenced by numerous soil characteristics, including particle size, clay content, total inorganic carbon content, or total organic carbon (TOC) content (Pu *et al.* 2004; Stewart *et al.* 2003a; Stewart *et al.* 2003b; Yang *et al.* 2005). The oral bioavailability of TCDD has been shown to decrease as the contact time with soil increases (Poiger and Schlatter 1980). Because soil characteristics can vary substantially by location, site-specific evaluations of matrix effects are usually preferred for use in quantitative health risk assessments. The term "relative bioavailability" is a metric of the matrix effect and it is typically determined by measuring tissue chemical concentrations in animals orally dosed with chemical-contaminated soil versus animals orally dosed with the chemical only. As the influence of the matrix effect increases, the relative oral bioavailability value (usually reported as a percentage) decreases. The relative oral bioavailability of TCDD in site-specific soils has been evaluated in several published studies, and the values typically range from 10–40% (Bonaccorsi *et al.* 1984; Lucier *et al.* 1986; McConnell *et al.* 1984; Shu *et al.* 1988; Umbreit *et al.* 1986; Umbreit *et al.* 1988; Wendling *et al.* 1989; Wittsieppe *et al.* 2001; Ruby *et al.*, 2002; Wittsieppe *et al.* 2007). While TCDD has been evaluated fairly extensively, very few oral bioavailability studies have examined the sixteen other 2,3,7,8-substituted PCDD/F congeners that often must be considered in health risk assessments. This represents a potentially critical data gap, since: (1) it is unclear whether and to what degree the relative TCDD bioavailability results are applicable to the other PCDD/F congeners and (2) in some instances, such as in the study presented here, TCDD contributes very little to the total PCDD/F total toxic equivalent (TEQ) concentration of the soil.

In this study, we describe the results of bioaccessibility and bioavailability studies conducted with PCDD/F-containing soils collected from waste management areas at an operating facility in the United States. The soils contain measurable levels of a majority of the 2,3,7,8-substituted PCDD/Fs, and PCDFs constitute a large fraction of the total soil TEQ. The PCDD/Fs are generated via an electrolytic process in which chloride and metals are separated from a brine; the impacted soils

at the facility contain residual materials generated during this process. For the *in vivo* bioavailability evaluation, rats were orally dosed with soils of varying PCDD/F concentrations, and hepatic PCDD/F levels were compared to those measured in reference groups dosed with neat formulations of PCDD/Fs (either by oral gavage or intravenous injection). The orally dosed referenced animals are used to determine the relative oral bioavailability of the soil-bound PCDD/Fs (relative bioavailability is a specific measure of the soil matrix effect); the intravenously dosed reference animals are used to determine the "absolute bioavailability" of the soil-bound PCDD/Fs (absolute bioavailability is an aggregate measure of all factors that may inhibit distribution of ingested PCDD/Fs to tissues, including soil affinity and incomplete gastrointestinal uptake). The results of an *in vitro* bioaccessibility study are also presented; the methods are based on the work of previous investigators, and are intended to closely mimic the extraction conditions in the human GI tract (Hack and Selenka 1996; Holman 2000; Oomen *et al.* 2000; Ruby *et al.* 2002; Wittsiepe *et al.* 2001). Specifically, separate "stomach" and "small intestine" fluids (buffered to pH 1.5 and 7.2, respectively) are used to extract soil particles during heating (37°C) and agitation.

The purpose of this study is several-fold: (1) to compare the soil bioavailability results of the different PCDD/F congeners, (2) to evaluate the consistency of the PCDD/F bioaccessibility versus the relative bioavailability results, and thereby assess the predictive accuracy of the bioaccessibility findings, and (3) to develop quantitative TEQ relative bioavailability measurements that are appropriate for use in a health risk assessment for this site. As part of these analyses, we examine the influence of different soil particle sizes, soil TEQ concentrations, and degree of congener chlorination on PCDD/F bioavailability. The study is designed to minimize hepatic enzyme induction in the soil-dosed groups; such induction is a potentially confounding factor that has apparently influenced the results of some previous oral PCDD/F bioavailability studies (Budinsky *et al.* 2008).

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MATERIALS AND METHODS

Soil Collection and Analysis

Thirteen surface soil samples (0–6 inches in depth) were collected from different locations at an operating industrial facility in the United States and shipped to Alta Analytical Laboratory, Inc. (Alta Analytical, El Dorado Hills, CA) for preparation and analysis. Eight samples were used in the bioacessibility analyses; five samples were used in the bioavailability studies. The soils were allowed to air dry, and were sieved to the <250-μm particle size fraction. Two samples used in the bioaccessibility study (Samples 4 and 5) could only be sieved to a <500-μm fraction, and one sample (Sample 3) was not sieved; these three samples are referred to as the "coarse" samples. The remaining five bioaccessibility samples were successfully sieved to <250 μm, and are referred to as the "highly sieved" samples. All five bioavailability samples were sieved to <250-μm. All samples were analyzed for PCDD/F content using isotope dilution gas chromatography-mass spectrometry according to USEPA Method 1613, revision B.

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Bioaccessibility Determination

The soil extraction method used here is taken from Ruby *et al.* (2002). The extractions were conducted in 1-liter Teflon bottles that were immersed in a water bath at 37°C. All reagents were obtained from Sigma Chemical Company, unless otherwise noted. A 0.2-M buffered solution was prepared by adding glycine (Sigma UltraPure®; 60 g) to 4-liter Type II deionized water and adjusting the pH to 1.5 with concentrated hydrochloric acid (~240 ml). Sodium chloride (32.5 g; final concentration 150 mM), pepsin (800–2,500 units/mg; 4 g; final concentration 1 g/l), bovine serum albumin (BSA, minimum 98 percent; 20 g; final concentration 5 g/l), and mucine (Type III, purified from porcine stomach; 10 g; final concentration 2.5 g/l) were added. As noted in Ruby *et al.* (2002), the BSA serves as a representative protein because PCDD/Fs may partition into the protein phase during simulated human digestion. Similarly, the mucin (a viscous mixture of glycoproteins and enzymes present in the mammalian stomach and intestines) is added because it is believed that mucin may increase the fraction of PCDD/Fs liberated from soil (Ruby *et al.* 2002).

The resulting simulated gastric fluid (800 ml), oleic acid (90%; 4.8 ml; Aldrich Chemical), and an aliquot of the appropriate soil sample (8 g) was added to a Teflon bottle. The mixture was stirred at 30 revolutions per minute (rpm) on a mixing table for 1 h for the simulated gastric phase of the extraction. The pH was periodically checked during the gastric phase to ensure that it was still at pH 1.5. Next, the solution was adjusted to pH 7.2 using sodium hydroxide (50 percent w/w; approximately 10 ml). Pancreatin (8× USP; 480 mg) and bovine bile (50 percent bile acids, mixture of free and conjugated acids; 3.2 mg) were added to each reaction vessel, and the suspension was stirred at 30 rpm for 4 h for the simulated intestinal phase of the extraction. Following the second extraction, each sample was centrifuged at 5000 $\times g$ for 10 min. The supernatant was decanted into a graduated cylinder, and the total volume was measured. A method blank (all components except soil) was carried through this entire process to establish a background concentration of PCDD/Fs in the final extract.

The concentration of each 2,3,7,8-substituted PCDD/F congener was determined by isotope dilution high resolution gas chromatography/high resolution mass spectrometry. The soil samples were analyzed according to USEPA method 1613 (USEPA 1984) at Severn Trent Laboratories (West Sacramento, CA). The extracts were analyzed according to USEPA method 8290 (USEPA 1994) at Alta Analytical Laboratories, Inc. (El Dorado Hills, CA). Ruby *et al.* (2002) reported an average of approximately 100% recovery from a TCDD-spiked extraction fluid blank in their study. Soil sample #2 was processed in triplicate to assess the reproducibility of the method. The corrected concentration of each PCDD/F congener in the soil extract was calculated by subtracting the background concentration in the method blank from the measured concentration. Results in which the ratio of the measured and background concentrations of a specific congener was less than 3:1 were excluded from the subsequent bioaccessibility calculations.

The bioaccessibility (percent extracted) for each congener was calculated by comparing the total congener mass in the final extract to the total congener mass in the soil sample prior to extraction. Likewise, the overall bioaccessibility of the

PCDD/Fs in each soil sample was calculated by comparing the total TEQ mass in the final extract to that in the soil sample prior to extraction. The TEQ concentrations of each congener were calculated using the 2005 World Health Organization toxic equivalency factors (TEFs) for PCDD/Fs (Van den Berg *et al.* 2006).

Bioavailability Study

Reference dose formulations

Dose formulations for the intravenous and oral gavage reference groups were prepared by Alta Analytical. The relative concentrations of the various congeners in the reference formulations were based on the mean fractional contribution of each congener to the total TEQ concentration of the soil samples used in the bioavailability study. To reduce the potential for confounding due to differences in hepatic enzyme induction between reference groups versus soil-treated groups, the concentration of each congener in the reference formulations was selected with the intent of yielding comparable systemic exposures following administration of the soil or the reference formulations. Specifically, based on the expectation of incomplete absorption of PCDD/Fs from soil, the concentration of each congener in the "high-dose reference formulations" was reduced to yield approximately 30% of the maximum dose administered to animals in the soil-treated groups. The reference formulations were also prepared at two lower dose levels ("medium" and "low") to account for the wide range of total TEQ concentrations in the different soil samples. The target concentration of each congener in the mid- or low-dose reference formulations was 5- or 25-fold lower, respectively, than the corresponding concentration in the high-dose reference formulation. The reference formulations were prepared in 1:1:18 Alkamuls EL620/ethanol/0.9% sterile saline for intravenous administration or in corn oil for oral gavage administration.

Animal husbandry

All in-life aspects of the study were performed at Charles River Laboratories (Redfield, AR), a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The study protocol was reviewed and approved by the testing facility's Institutional Animal Care and Use Committee. Female Sprague Dawley rats were obtained from a Charles River Laboratory breeding facility, and were allowed to acclimate at the testing facility for more than one week prior to the first day of dosing. The animals were 15 weeks old and weighed 251–321 g on Day 1. The current USEPA TCDD cancer slope factor is based on feeding studies with female Sprague Dawley rats.

The animals were housed in stainless steel wire mesh cages and were provided feed and filtered tap water *ad libitum*. To reduce the possibility of changes in the background tissue concentrations of PCDD/Fs, the rats were maintained on the same feed (Purina Rodent Diet #5L79) at the breeding and testing facilities.

Dosing

Six animals were assigned to each group. One group of animals was not treated, and was utilized to assess background tissue PCDD/F levels. Three groups of animals

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were treated with the low, mid, or high concentration of intravenous reference formulations at a dose volume of 4 ml/kg via an intravenous infusion at a rate of 0.4 ml/h. The duration of the infusion was approximately 3 h, and was intended to mimic the rate of absorption of PCDD/Fs following oral dosing. The syringe, tubing, and needle assemblies were weighed before and after the infusion to allow the dispensed volume to be calculated. The dispensed volume was corrected to account for the dead space in the injection cap, which was determined prior to the start of the study. The intravenous reference formulations were mixed immediately prior to use. Analysis of samples collected from the bottom, middle, and top levels of each intravenous reference formulation confirmed that the formulations were homogeneous at the start and end of the 3-h infusion period.

Three groups of animals were treated with a single dose of the low-, mid-, or high-dose corn oil reference formulations by oral gavage at a dose volume of 4 ml/kg. The formulations were mixed immediately prior to use.

For each of the five soil samples, one group of six animals was treated with a single dose of an aqueous suspension of the soil sample by oral gavage. The plunger was removed from a 3-ml syringe, and the target dose of dry soil (2000 mg/kg) was added directly to the barrel. Deionized water (8 ml/kg) was added to the syringe immediately prior to dosing. The plunger was replaced, the syringe was shaken vigorously, and the suspension was immediately administered by oral gavage. The syringe and gavage needle used to dose each animal were dried and weighed. The amount of soil administered to each animal was calculated by subtracting the mass of the dry soil residue remaining in the syringe and needle after dosing from the mass of dry soil that was originally added to the syringe.

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Sacrifice and tissue collection

Each animal in the soil-treated or reference groups was euthanized 24 h (±15 min) after administration of the bolus oral gavage dose or the start of the intravenous infusion. Two samples of liver (approximately 1 g each) were collected from the left lateral and median lobes and shipped to Charles River Laboratories (Montreal, Canada) for microsomal CYP450 1A1/2 activity assays. The remainder of the liver was shipped on dry ice to Alta Analytical for PCDD/F concentration analysis by mass spectrometry (USEPA Method 1613, revision B). The gut was examined for gross lesions and the presence of soil. Tissues were similarly collected from animals in the untreated control animals, except that disposable instruments were used to reduce the possibility of contamination with PCCD/Fs from animals in the treated groups.

Hepatic enzyme activity assays

The potential induction of hepatic CYP1A1/1A2 was evaluated by measuring 7-ethoxyresorufin-*O*-deethylase (EROD) activity as previously described (Rodrigues and Prough 1991). Briefly, microsomal preparations from each liver were incubated in triplicate in the presence of an NADPH-generating system at 37°C, and metabolite formation was measured spectrofluorometrically. Protein concentration was determined using a modified Lowry method (Lowry 1951; Ohnishi and Barr 1978). The normalized EROD activity in each reference or soil-treated group was compared to that in the untreated control group using one-way analysis of variance (ANOVA),

followed by the Dunnett's multiple comparisons test. A p value less than 0.05 was considered to be statistically significant.

Calculations and data analysis

PCDD/F concentrations measured in the method blank were negligible, and were not considered further in the analysis. The concentration of each congener in the liver of animals treated with reference formulations or soil samples was corrected by subtracting the mean concentration in the liver of animals in the untreated control group. For the purpose of this correction, tissue concentrations below the lower limit of quantitation were conservatively considered to be zero.

To minimize the potential for erroneously low bioavailability estimates in the soil-treated animals due to differences in hepatic enzyme induction, each soil-treated group was paired with the reference group that had the next highest total TEQ concentration in the liver at the time of sacrifice.

Relative bioavailability values were obtained by comparing the hepatic congener mass following soil administration versus oral reference formulation administration whereas absolute bioavailability values were obtained by comparing the hepatic congener mass following soil administration versus the intravenous reference formulation administration. The fraction of the administered dose present in liver was calculated by comparing the total amount of congener present in the liver at the time of sacrifice to the mass of the congener that was administered to the animal:

$$\begin{aligned}&\text{Mass Congener Dosed} = (\text{Concentration of Congener in Soil}) \\ &\quad \times (\text{Mass Soil Dosed}) \\ &\text{Mass Congener in Liver} = (\text{Concentration of Congener in Liver}) \\ &\quad \times (\text{Mass of Liver}) \\ &\text{Fraction of Dose Present in Liver} \\ &\quad = (\text{Mass Congener in Liver})/(\text{Mass Soil Dosed})\end{aligned}$$

The relative and absolute oral bioavailabilities of each congener were then calculated by comparing the fraction of the administered dose that was present in the liver of each soil-treated animal to the mean fraction of the administered dose that was present in the liver of animals in a selected oral (relative bioavailability) or intravenous (absolute bioavailability) reference group:

$$\begin{aligned}&\text{Oral Bioavailability}_{\text{congener}} \\ &\quad = (\text{Fraction of Administered Dose in Liver}_{\text{soil-treated}})/ \\ &\quad\quad (\text{Mean Fraction of Administered Dose in Liver}_{\text{referencegroup}})\end{aligned}$$

Similarly, the overall oral bioavailability of PCDD/Fs in each soil sample was calculated by comparing the fraction of the administered TEQ dose that was present in the liver of each soil-treated animal to the mean fraction of the administered TEQ dose that was present in the liver of animals in a selected reference group:

$$\begin{aligned}\text{Oral Bioavailability}_{\text{TEQ}} = &(\text{Fraction of Administered TEQ in Liver}_{\text{soil-treatedanimal}})/ \\ &(\text{Mean Fraction of Administered TEQ in Liver}_{\text{referencegroup}})\end{aligned}$$

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Selection criteria were established to exclude results that were considered likely to lead to erroneous bioavailability determinations because of experimental error, analytical variability, or contributions from background concentrations of PCDD/Fs. Specifically, the results of tissue concentration measurements for individual congeners were only included in the calculation of bioavailability if all of the following criteria were met: (1) at least 50% of the soil in the syringe was dosed; (2) the calculated amount of soil dosed was not greater than 105% of the amount added to the syringe; (3) the measured (uncorrected) tissue concentration of the congener was ≥3.0 pg/g tissue; and (4) the measured (uncorrected) tissue concentration was at least 3-fold greater than the highest background concentration of that congener measured in untreated control group animals.

RESULTS

The total organic carbon (TOC) content of the soil samples varied little, and was less than 1% in all cases. Each of the seventeen congeners was detected in a majority of the soil samples. The range of PCDD/F TEQ concentrations of the soil samples used in the bioavailability and bioaccessibility studies were comparable and, as presented in Tables 1 and 2, soil total TEQ concentrations ranged from 0.7–22.8 ng/g in the bioaccessibility samples, and 0.53–45.2 ng/g in the bioavailability samples, respectively. The congener "fingerprints" of the samples were fairly consistent. For example, six PCDF congeners (2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, and 1,2,3,4,6,7,8-HpCDF) accounted for at least 75% of the total soil PCDD/F TEQ in each sample, while the PCDDs accounted for less than 5% of the total TEQ; 2,3,7,8-TCDD was a very minor component of each soil sample (less than 1% of the total TEQ concentration).

Bioaccessibility Results

Five PCCD/F congeners were detected in the method blank: 1,2,3,4,6,7,8-HpCDF (7.78 pg/ml), 1,2,3,6,7,8-HxCDD (8.52 pg/ml), 1,2,3,4,6,7,8-HpCDD (180 pg/ml), OCDF (27.6 pg/ml), and OCDD (1350 pg/ml). The concentrations of 1,2,3,4,6,7,8-HpCDF and OCDF in all sample extracts were several orders of magnitude higher than the background concentrations measured in the method blank. The bioaccessibility data validity criterion, which required that uncorrected sample concentrations be at least 3-fold greater than the measured concentration in the method blank, resulted in the exclusion of results for 1,2,3,4,6,7,8-HpCDD and OCDD in some samples. The exclusion of these results had an insignificant impact on the overall bioaccessibility evaluations. Further, use of different exclusion criteria (*e.g.*, 10:1) had little influence on the results.

With the exception of 1,2,3,4,7,8-HxCDF in soil sample #5, all 17 PCDD/F congeners were detected in the extracts of each soil sample. Percent bioaccessibility (total TEQ basis) ranged from 8% (sample #3) to 45% (sample #8), with an overall mean of 22%. Percent bioaccessibility was independent of initial soil TEQ ($r^2 = -0.55$ for soil TEQ vs. total TEQ in extract). As shown in Table 3, soil particle size appears to have influenced the degree of extraction of the PCDD/F congeners. Specifically, for any given congener, the mean % mass extracted for the highly sieved (<250 μm)

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Table 1. Toxic equivalent concentrations of 2,3,7,8-PCDD/Fs in soil samples used in oral bioaccessibility analyses.

	Toxic equivalent concentration (pg/g soil)[a]							
Congener	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
2,3,7,8-TCDD	2.5	1.8	11	28	9.5	43	22	2.4
1,2,3,7,8-PeCDD	10	7.8	120	300	85	170	110	17
1,2,3,4,7,8-HxCDD	0.97	0.74	13	30	ND	13	11	1.5
1,2,3,6,7,8-HxCDD	2.8	2.5	40	100	30	49	28	4.3
1,2,3,7,8,9-HxCDD	3.9	2.2	29	93	32	46	39	4.7
1,2,3,4,6,7,8-HpCDD	2.1	1.6	31	75	20	32	20	2.7
OCDD	0.19	0.15	2.9	6.9	1.7	3.3	2.3	0.13
2,3,7,8-TCDF	40	42	110	270	96	460	280	30
1,2,3,7,8-PeCDF	20	17	273	600	156	270	246	30
2,3,4,7,8-PeCDF	81	69	900	2310	780	1080	1500	228
1,2,3,4,7,8-HxCDF	200	150	3100	7400	1800	2700	3000	230
1,2,3,6,7,8-HxCDF	160	130	2300	4600	1300	2000	1700	260
1,2,3,7,8,9-HxCDF	25	17	420	810	230	260	240	39
2,3,4,6,7,8-HxCDF	27	28	350	830	200	360	440	74
1,2,3,4,6,7,8-HpCDF	120	140	1600	2500	880	1100	930	170
1,2,3,4,7,8,9-HpCDF	38	34	630	1200	370	520	470	53
OCDF	39	51	990	1620	330	960	510	33
Total TEQ (ng/g soil)	0.8	0.7	10.9	22.8	6.3	10.1	9.5	1.2

ND = not detected.
[a]Total toxic equivalent (TEQ) concentration of soil sample based on 2005 WHO toxic equivalency factors.

Table 2. Toxic equivalent concentrations of 2,3,7,8-PCDD/Fs in soil samples used in oral bioavailability analyses.

	Toxic Equivalent Concentration (pg/g soil)[a]				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
2,3,7,8-TCDD	16.4	173	209	9.77	ND
1,2,3,7,8-PeCDD	175	740	723	38.1	7.78
1,2,3,4,7,8-HxCDD	16.5	62.9	55.8	3.30	0.680
1,2,3,6,7,8-HxCDD	53.5	145	138	11.3	2.03
1,2,3,7,8,9-HxCDD	59.2	156	150	13.0	2.24
1,2,3,4,6,7,8-HpCDD	37.8	106	91.2	7.57	1.18
OCDD	3.03	9.06	7.11	0.807	0.126
2,3,7,8-TCDF	128	1430	1840	103	16.9
1,2,3,7,8-PeCDF	330	1209	1128	57.9	12.7
2,3,4,7,8-PeCDF	1689	7080	6540	312	69.6
1,2,3,4,7,8-HxCDF	3320	9900	7910	567	118
1,2,3,6,7,8-HxCDF	2850	8430	6410	467	102
1,2,3,7,8,9-HxCDF	1360	4000	3000	220	48.4
2,3,4,6,7,8-HxCDF	1230	3840	3090	210	41.5
1,2,3,4,6,7,8-HpCDF	2290	4970	3400	453	72.3
1,2,3,4,7,8,9-HpCDF	833	1970	1470	138	23.4
OCDF	621	975	624	188	15.8
Total TEQ (ng/g soil)	15.0	45.2	36.8	2.8	0.53

ND = not detected.
[a]Total toxic equivalent (TEQ) concentration of soil sample based on 2005 WHO toxic equivalency factors.

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samples (#1, 2, 6, 7, and 8) was several-fold higher than the mean % extracted from the coarse samples (#3, 4, and 5). As a result, the mean of the total TEQ extracted from the highly sieved samples (29%) was approximately 3-fold greater than the mean total TEQ extracted from the coarse samples (10%) (Table 3). The degree of congener chlorination also appeared to have some influence on extractability. As shown in Figure 1A, there was a strong correlation ($r^2 = 0.98$) between increasing chlorination and decreasing bioaccessibility for the PCDD congeners. Interestingly, a similar trend was not observed ($R^2 = 0.03$) for the PCDF congeners (Figure 1B), due in part to the relatively high bioaccessibility values for 1,2,3,7,8,9-HxCDF and 2,3,4,6,7,8-HxCDF. In any given sample, these two HxCDF congeners had extraction efficiencies that were roughly 2–3-fold higher than for all the other PCDD/F congeners.

The reproducibility of the assay was evaluated by performing triplicate extracts and analyses of soil sample #2, which was sieved to <250 μm and had a total TEQ concentration of 0.7 pg/g. The relative standard deviation (RSD) of the bioaccessibility values for the individual PCDFs, which accounted for a large majority of the total soil TEQ concentration, ranged from 10 to 16%. The RSD values for the PCDD congeners, which were present in lower concentrations in the soil samples, ranged from 6% to 18%. The mean RSD value for all PCDD/F congeners was 13%.

Table 3. Mean bioaccessibility of 2,3,7,8-PCDD/Fs in sieved and coarse soil samples.

Congener	% mass extracted from sieved samples[a] Mean		% mass extracted from coarse samples[b] Mean	
2,3,7,8-TCDD	45%	(24%)	12%	—
1,2,3,7,8-PeCDD	37%	(14%)	11%	(1%)
1,2,3,4,7,8-HxCDD	32%	(12%)	11%	(2%)
1,2,3,6,7,8-HxCDD	31%	(11%)	10%	(1%)
1,2,3,7,8,9-HxCDD	32%	(15%)	12%	(1%)
1,2,3,4,6,7,8-HpCDD	22%	(10%)	9%	(2%
OCDD	18%	(16%)	7%	(2%)
2,3,7,8-TCDF	31%	(16%)	6%	(1%)
1,2,3,7,8-PeCDF	28%	(13%)	8%	(1%)
2,3,4,7,8-PeCDF	31%	(11%)	10%	(0%)
1,2,3,4,7,8-HxCDF	27%	(15%)	8%	(2%)
1,2,3,6,7,8-HxCDF	25%	(10%)	9%	(1%)
1,2,3,7,8,9-HxCDF	93%	(43%)	24%	(4%)
2,3,4,6,7,8-HxCDF	50%	(21%)	23%	(4%)
1,2,3,4,6,7,8-HpCDF	27%	(13%)	11%	(2%)
1,2,3,4,7,8,9-HpCDF	22%	(10%)	9%	(1%)
OCDF	16%	(8%)	5%	(2%)
Mean Total TEQ[c]	29%	(4%)	10%	(4%)

[a]Samples 1, 2, 6, 7, 8 were sieved to the <250-μm particle size fraction prior to analysis.
[b]Samples 4 and 5 were sieved to the <500-μm particle size fraction prior to analysis. Sample 3 was not sieved.
[c]Based on 2005 WHO toxic equivalency factors.
() = Standard deviation; only 2 values available for 2,3,7,8-TCDD in coarse samples.

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Bioavailability Results

In the soil-dosed animals, on average less than 18% of the soil remained in the syringe barrel after dosing. Shaking the syringe prior to dosing helped to minimize clogging difficulties; it was necessary to disqualify the results from 2 of the soil-dosed animals due to syringe clogging that caused less than 50% of the target dose of soil to be administered (the 2 animals were from different soil groups). The reference formulations and soil suspensions were well tolerated by the test animals; no clinical signs of toxicity or other adverse reactions were observed, and no treatment-related gross lesions were observed in the liver or GI tract of the untreated control animals or animals treated with the oral reference or soil formulations. No test article was apparent in the GI tract of soil-treated animals at the time of necropsy; however, the presence of feed and other material may have made it difficult to detect diffusely distributed soil in the GI tract.

The PCDD/F concentrations measured in the oral and intravenous reference dose formulations are summarized in Table 4. With the exception of 2,3,7,8-TCDD, the concentration of each congener in the low-, mid-, or high-dose reference formulations was within 25% of the target concentration. Because of the relatively low

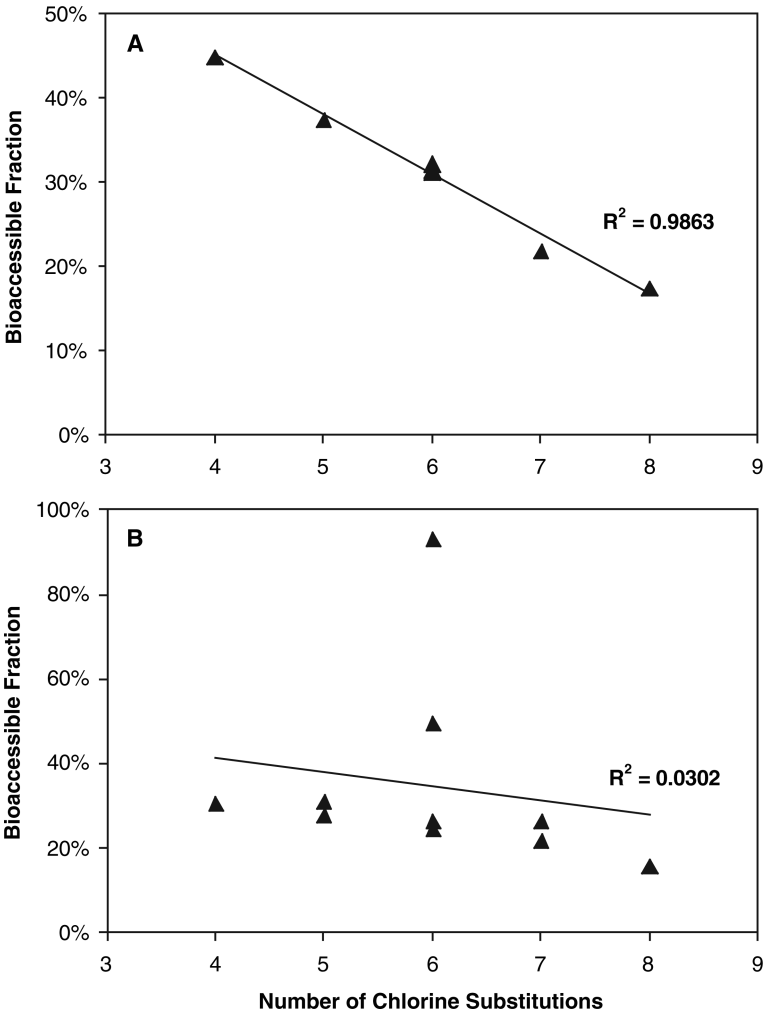

Figure 1. Relationship between extent of chlorination and bioaccessibility of polychlorinated dibenzo-*p*-dioxins (A) and (B) furans.

amount of 2,3,7,8-TCDD in the soil samples, the concentration of that congener was below the limit of quantitation in the low-dose oral reference formulations.

The presence of PCDD/Fs in laboratory rat feed and the liver of untreated rats has previously been characterized (Ruby *et al.* 2004). Eleven PCDD/F congeners were detected in one or more animals in the untreated control group (data not shown); however, these background concentrations were generally low, and only exceeded 1 pg/g in the case of 1,2,3,4,6,7,8-HpCDD (mean = 3.55 pg/g) and OCCD (mean = 14.78 pg/g). Corrections to the measured PCDD/F concentrations and selection criteria based on these background concentrations were made as described in the Methods section.

In the oral and intravenous reference animals, the PCDFs were detected in the liver at all doses; PCDDs were not detected in the low dose animals, but were

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Table 4. Mean bioaccessibility of 2,3,7,8-PCDD/Fs in sieved and coarse soil samples.

Congener	% mass extracted from sieved samples[a] Mean	% mass extracted from coarse samples[b] Mean
2,3,7,8-TCDD	45%	12%
1,2,3,7,8-PeCDD	37%	11%
1,2,3,4,7,8-HxCDD	32%	11%
1,2,3,6,7,8-HxCDD	31%	10%
1,2,3,7,8,9-HxCDD	32%	12%
1,2,3,4,6,7,8-HpCDD	22%	9%
OCDD	18%	7%
2,3,7,8-TCDF	31%	6%
1,2,3,7,8-PeCDF	28%	8%
2,3,4,7,8-PeCDF	31%	10%
1,2,3,4,7,8-HxCDF	27%	8%
1,2,3,6,7,8-HxCDF	25%	9%
1,2,3,7,8,9-HxCDF	93%	24%
2,3,4,6,7,8-HxCDF	50%	23%
1,2,3,4,6,7,8-HpCDF	27%	11%
1,2,3,4,7,8,9-HpCDF	22%	9%
OCDF	16%	5%
Mean Total TEQ[c]	29%	10%

[a]Samples 1, 2, 6, 7, 8 were sieved to the <250-μm particle size fraction prior to analysis.
[b]Samples 4 and 5 were sieved to the <500-μm particle size fraction prior to analysis. Sample 3 was not sieved.
[c]Based on 2005 WHO toxic equivalency factors.

present in the mid and high dose reference groups. The mean hepatic PCDD/F TEQ concentrations measured in the reference animals are summarized in Table 5. Both the oral and intravenous treatments demonstrated a strong dose–concentration relationship (r^2 of 0.99 for both).

Similarly, as shown in Figure 2, in the soil-dosed animals there was a strong correlation between initial soil PCDD/F TEQ concentration and measured liver PCDD/F TEQ concentration ($r^2 = 0.95$). All but one (sample #2) of the mean liver

Table 5. Hepatic 2,3,7,8-PCDD/Fs toxic equivalent concentrations in reference groups.

Reference formulation	Mean TEQ concentration in liver (pg/g tissue)[a] Low dose	Mid dose	High dose	Correlation coefficient
Oral	12.2	63.3	364	$R^2 = 0.99$
Intravenous	22.4	105	504	$R^2 = 0.99$

[a]Total toxic equivalent (TEQ) concentration in liver at time of sacrifice based on 2005 WHO toxic equivalency factors.

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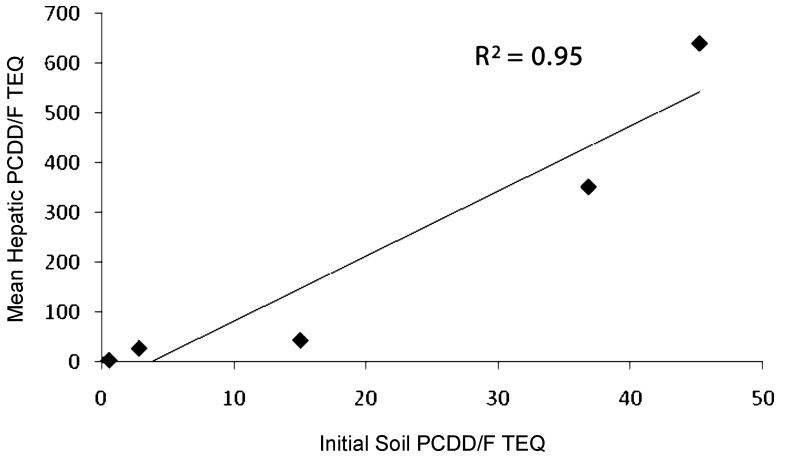

Figure 2. Relationship between initial soil PCDD/F TEQ versus hepatic PCDD/F TEQ.

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TEQ concentrations in the soil-dosed animals fell within the range of the liver TEQ concentration curves generated for the relative and absolute reference groups. As shown in Table 6, the PCDF congeners were generally detected in all of the soil-dosed animals; conversely, the PCDDs were essentially not detected, except in the animals dosed with the soil samples that contained the highest PCDD concentrations (samples #2 and 3). As shown in Table 6, on a TEQ basis, the oral bioavailability of the PCDD/Fs in the five soil samples ranged from 17–50% (mean of 38%). All of the congener bioavailability values in Table 6 are a percentage of mean mass measured in the soil-dosed animals relative to the mean mass measured in the oral reference animals (none of the numerical values are based on non-detects in the reference group). Similar to the bioaccessibility results, there was no correlation between initial soil TEQ and % bioavailability ($R^2 = 0.30$). As can be seen in Table 6, there was a general trend of decreasing bioavailability with increasing PCDF chlorination.

In Table 7 are summarized the mean hepatic PCDD/F TEQ concentrations for the soil-treated groups and also the % relative and absolute bioavailabilities determined for each soil sample. For each sample, the % relative bioavailability was higher than the % total bioavailability. In addition, for each sample, the congeners measured in the liver accounted for a vast majority (86% or greater) of the initial soil TEQ.

The hepatic EROD activities measured in the reference and soil-dosed groups are summarized in Figure 3. Mean hepatic EROD activities in the low-, mid-, and high-dose reference groups were not significantly elevated (relative to untreated controls). As shown in Figure 3, the mean hepatic EROD activity in the animals treated with soil samples #2 or 3 was significantly increased relative to the reference groups and the untreated controls.

DISCUSSION

In this study, the relative oral bioavailability of soil-bound PCDD/Fs was determined by comparing the fraction of administered PCDD/F TEQ present in the

Table 6. Relative oral bioavailability of 2,3,7,8-PCDD/Fs in soil samples.

	Relative oral bioavailability (%)				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
2,3,7,8-TCDD	ND	ND	ND	ND	ND
1,2,3,7,8-PeCDD	ND	100 ± 10	79 ± 12	ND	ND
1,2,3,4,7,8-HxCDD	ND	74 ± 6	52 ± 10	ND	ND
1,2,3,6,7,8-HxCDD	22 ± 5	67 ± 6	46 ± 7	82 ± NA	ND
1,2,3,7,8,9-HxCDD	ND	46 ± 4	32 ± 5	ND	ND
1,2,3,4,6,7,8-HpCDD	ND	32 ± 4	20 ± 4	ND	ND
OCDD	ND	23 ± 3	ND	ND	ND
2,3,7,8-TCDF	27 ± 5	76 ± 9	75 ± 7	81 ± 10	ND
1,2,3,7,8-PeCDF	26 ± 6	89 ± 9	69 ± 8	74 ± 9	61 ± 18
2,3,4,7,8-PeCDF	18 ± 4	50 ± 4	44 ± 6	52 ± 8	56 ± 15
1,2,3,4,7,8-HxCDF	23 ± 5	61 ± 6	42 ± 6	63 ± 8	47 ± 13
1,2,3,6,7,8-HxCDF	22 ± 5	59 ± 5	42 ± 6	62 ± 8	42 ± 11
1,2,3,7,8,9-HxCDF	5 ± 1	16 ± 2	13 ± 2	19 ± 3	23 ± NA
2,3,4,6,7,8-HxCDF	10 ± 2	32 ± 3	22 ± 3	28 ± 3	ND
1,2,3,4,6,7,8-HpCDF	13 ± 3	28 ± 3	18 ± 3	33 ± 4	19 ± 7
1,2,3,4,7,8,9-HpCDF	14 ± 3	34 ± 3	22 ± 3	39 ± 5	25 ± 8
OCDF	10 ± 3	21 ± 3	13 ± 2	27 ± 3	13 ± 5
Total TEQ[a]	17 ± 4	50 ± 4	39 ± 5	47 ± 6	36 ± 10

Results are shown as the arithmetic mean ± SD. NA = not applicable (indicated value based on data from 1–2 animals).
ND = not determined.
[a]Based on 2005 WHO toxic equivalency factors.

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livers of rats orally dosed with contaminated soil versus the fraction of administered TEQ measured in reference groups orally dosed with PCDD/Fs suspended in corn oil. This calculation assumes that elimination rates of the various congeners are the same in soil-dosed versus corn-oil dosed reference animals; it also assumes that the liver is a reasonable surrogate for whole-body concentrations of PCDD/Fs. Individual congener and total hepatic TEQ concentrations in the soil-dosed rats generally fell within the linear dose–response curve generated by the three reference groups (the low-, mid-, and high-dose reference groups). On a total TEQ basis, the mean relative oral bioavailability of the soil-bound PCDD/Fs ranged from 17–50%, (Table 6),

Table 7. Summary of bioavailability determinations.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Soil TEQ (ng/g soil)[a]	15.0	45.2	36.8	2.80	0.53
Mean TEQ in liver (pg/g tissue)	43.4	639	351	27.4	3.74
Reference group[b]	Mid Dose	High Dose	High Dose	Mid Dose	Low Dose
Overall relative bioavailability (%)	17 ±4	50 ± 4	39 ± 5	47 ± 6	36 ± 10
Overall absolute bioavailability (%)	11 ± 2	38 ± 3	30 ± 4	31 ± 4	26 ± 4

[a]Based on 2005 World Health Organization toxic equivalency factors.
[b]Reference group dose level used as the basis of bioavailability determinations.

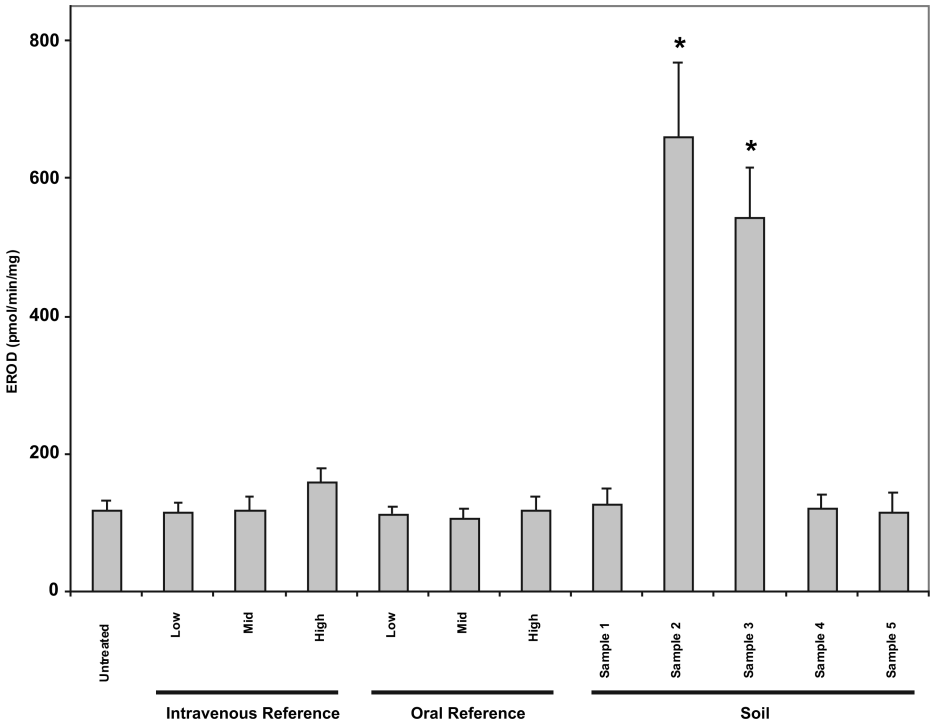

Figure 3. Hepatic 7-ethoxyresofurin-*O*-deethylase (EROD) activity in rats treated with polychlorinated dipenzo-*p*-dioxins/dibenzofurans in soil reference formulations. (*) indicates a statistically significant difference ($p < 0.05$) in hepatic EROD activity when compared to the untreated control group and reference formulations (using Dunnett's multiple comparisons test).

with a mean of 38%. The relative oral bioavailability of Sample #1 (17% TEQ) is much lower than the values derived for the other samples (36–50% TEQ), but the reasons for this are not clear. Bioavailability was independent of initial soil TEQ concentration over an 80-fold range of soil TEQ concentrations (0.53–45.2 pg/g), and for any given PCDD/F congener the fraction of the administered dose measured in the liver was similar across all dose groups.

Our findings are consistent with the recently published results of Budinsky *et al.* (2008), which, to our knowledge, is the only other rodent study that evaluated the relative oral bioavailability of numerous non-2,3,7,8-TCDD PCDD/F congeners in soils. The study design of Budinsky *et al.* (2008) is similar to ours in many aspects: female Sprague Dawley rats were orally dosed with sieved PCDD/F-containing soils (<250 μm), oral reference PCDD/F formulations in corn oil were employed, and measures were taken to minimize hepatic enzyme induction in the reference animals. Their study design differed in some respects: animals were fed soils mixed with rat chow (rather than gavage), the exposures occurred daily for 30 days (versus the single dose in the present study), and liver and adipose tissues combined were used

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to derive the bioavailability calculations. Budinsky *et al.* (2008) reported a relative TEQ oral bioavailability of 37% and 66% for urban and floodplain soils, respectively.

The soil PCDD/F mass and soil total TEQ were dominated by the PCDFs (Table 2), particularly 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-PeCDF, and 1,2,3,6,7,8-HxCDF, while the PCDDs were detectable in soil, but at relatively lower concentrations. Similarly, as can be seen in Table 6, the PCDFs were detected in the livers of soil-dosed animals, yet 2,3,7,8-TCDD was not detected in any soil-dosed animal, and the PCDDs in general were only measurable in animals dosed with soils that contained the highest PCDD concentrations (samples #2 and 3). The absence of measurable hepatic PCDDs in soil-dosed animals did not significantly influence the bioavailability TEQ estimates because the PCDDs contributed <5% to the total TEQ in any given soil sample.

Increasing degree of chlorination was associated with decreasing extractability. For example, as shown in Figures 1A and 1B, the bioaccessibility values for the PCDDs and PCDFs generally decreased linearly as a function of increasing chlorination. This finding suggests that PCDD/F soil affinity increases, and/or that water solubility decreases, as the degree of dioxin or furan chlorination increases. A similar trend was observed in the livers of the soil-dosed animals. Specifically, as can be seen in Table 6, for any given soil sample, the relative oral bioavailability of 2,3,7,8-TCDF and the PeCDFs was typically 2–3-fold higher than the relative bioavailability of OCDF and the HpCDFs. In the orally dosed reference animals, the percentage of administered OCDD and OCDF that accumulated in the liver (about 15–20%) was typically 2–4-fold lower than for all other PCDD/F congeners (30–71%), which is consistent with the fact that the more highly chlorinated congeners are relatively poorly absorbed systemically (Birnbaum and Couture 1988; Brewster and Birnbaum 1987; Van den Berg *et al.* 1985; Stephens *et al.* 1995; Diliberto *et al.* 1996). The PCDD/F hepatic levels in the intravenously dosed animals did not exhibit such a relationship (*i.e.*, relatively low hepatic OCDD/F levels). This finding is consistent with other parenteral studies that observed that all PCDD/F congeners were well absorbed into the liver (>90%) following non-oral administrations (Neubert *et al.* 1990; Abraham *et al.* 1989). These findings suggest that the increasing chlorination/decreasing hepatic concentration trend observed in the soil-dosed animals is not the result of relatively lower hepatic uptake or relatively faster hepatic metabolism/clearance of the highly chlorinated congeners. It is difficult to determine whether any such patterns were apparent in the aforementioned Budinsky *et al.* (2008) analysis because far fewer PCDD/F congeners were actually quantifiable in the liver tissues in that study. In short, in the soil-dosed animals, the relationship between increasing congener chlorination and decreasing hepatic concentration reflects a combination of: (1) the differential soil binding characteristics as observed in the bioaccessibility data and (2) preferential systemic (GI tract) uptake of the lesser-chlorinated congeners, as observed in the orally dosed reference animals.

As can be seen in Table 6, 1,2,3,7,8,9-HxCDF provides an obvious exception to this general trend. Indeed, this congener exhibits behavior that is anomalous even when compared to the other very closely related HxCDFs. Specifically, the relative bioavailability of 1,2,3,7,8,9-HxCDF (mean of 15% in all 5 soil samples) was clearly lower than the values measured for the other HxCDF congeners (*e.g.*, means of 47% and 45% for 1,2,3,4,7,8-, 1,2,3,6,7,8-HxCDF, respectively). Interestingly, the low relative bioavailability was likely not due to reduced soil extractability because,

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in fact, the bioaccessibility data indicate that this same congener had by far the *highest* measured degree of extractability of all the PCCD/F congeners, including the other HxCDFs (Table 3). A review of the reference group data provides some possible insight to these apparently contradictory findings: in the orally dosed groups, the % of dosed 1,2,3,7,8,9-HxCDF retained in the liver (mean of 40% for all dose groups combined) was similar to but somewhat lower than the percentages measured for the other HxCDF congeners (means of approximately 50–60% for all dose groups). This difference was more exaggerated in the intravenously dosed reference groups, where the % of dosed 1,2,3,7,8,9-HxCDF present in the liver (mean of 55% for all dose groups combined) was clearly below the means measured for the other HxCDFs (all of which were in a narrow range of 75–79%). This finding would seem to suggest that the relatively low hepatic levels of 1,2,3,7,8,9-HxCDF in the soil-dosed animals is due to a combination of low systemic absorption from the GI tract and low hepatic absorption/increased hepatic clearance. To our knowledge, the 1,2,3,7,8,9-HxCDF congener does not possess any unique physico-chemical properties that would yield such anomalous findings. These results, while curious, do not significantly influence the total estimated bioaccessible or bioavailable TEQ in this study because 1,2,3,7,8,9-HxCDF contributes very little to the soil or hepatic TEQ.

In summary, our observation that chlorination appears to influence PCDD/F extractability from soil, coupled with the observation that even congeners within a single homologue group may behave quite differently (in this case, the HxCDFs), suggests that extrapolation of bioaccessibility or relative bioavailability results from one congener to another may introduce significant uncertainty. This is particularly important, given the fact that almost all oral PCDD/F relative bioavailability studies with contaminated soils have examined only 2,3,7,8-TCDD. From a health risk assessment perspective, it would appear that there is a significant lack of relevant information regarding the potential systemic uptake of the non-2,3,7,8-TCDD congeners following incidental soil ingestion. Since, as our study indicates, (1) the behavior of 2,3,7,8-TCDD may not accurately predict the behavior of other congeners in either bioaccessibility or bioavailability studies and (2) 2,3,7,8-TCDD may not even be a primary congener of interest in some settings, we suggest that future research efforts should address this data gap.

The bioaccessibility study clearly demonstrated the importance of soil sieving and particle size considerations; the mean bioaccessible TEQ in fine particles (29%) was approximately 3-fold greater than the mean TEQ measured in coarse particles (10%) (see Table 3). The purpose of using sieved particles (<250 μm diameter) in the bioaccessibility (and bioavailability) study is to simulate the particle size distribution that adheres to skin, and is therefore most likely to be incidentally ingested via hand-to-mouth transfer (Dugan and Inskip 1985; Maddaloni *et al.* 1998). Higher percentages of PCDD/F extraction from smaller soil particles probably occurs because of increased surface area/volume considerations; that is, the smaller the particle, the greater the proportion of soil-bound chemical that is on the particle surface where (presumably) extraction occurs more easily (Lyytikainen *et al.* 2003). Hence, we suggest that bioaccessibility and bioavailability studies with soil-bound chemicals use sieved particles. As with the bioavailability results, the PCDD/F TEQ measured in the bioaccessibility extracts reflected the soil PCDD/F profile and were

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dominated by PCDFs. It is interesting to note that the mean TEQ measured in the bioaccessibility extracts (mean of 29% in sieved samples) was actually lower than the mean relative bioavailable TEQ measured in livers of the soil-dosed animals (38%). A very similar finding was reported by Budinsky *et al.* (2008), wherein *in vitro* soil extractions employing simulated human GI conditions (the same conditions used in the present analysis) yielded a mean PCDD/F TEQ bioaccessibility of 25%, yet the relative oral bioavailability as determined in soil-dosed rats was 37%. This result would seem counter-intuitive, as bioaccessibility assays are typically believed to represent maximal or "worst-case" estimates of oral bioavailability (because any additional reduction in bioavailability due to <100% systemic uptake of the desorbed congeners is not measured in a bioaccessibility analysis). In this study, we did not include a PCDD/F matrix spike of the simulated gastric fluid; we simply assumed 100% recovery (the true recovery is unknown). However, if the PCDD/F recoveries from the fluid were in fact much less than 100%, the bioaccessibility estimates would be biased low and this could explain why the bioaccessibility values were lower than the relative bioavailability values. On the other hand, as noted by Budinsky *et al.* (2008), it is possible that the rat model is simply not the most appropriate laboratory species for estimating human oral bioavailability of PCDD/Fs. Indeed, Budinsky *et al.* (2008) noted that the mean PCDD/F TEQ relative oral bioavailability measured in soil-dosed juvenile swine (23%) provided a much better match to the bioaccessibility results obtained with simulated human GI fluids (25%) than did the relative oral rat bioavailability data (37%).

PCDD/Fs are potent inducers of the metabolic enzymes CYP450 1A1 and 1A2 (Abraham *et al.* 1988; Santostefano *et al.* 1998; DeVito *et al.* 1997), both of which can be measured collectively via the EROD assay. Because these enzymes are responsible for the metabolism and clearance of certain PCDD/Fs, significant induction of these enzymes may confound PCDD/F bioavailability estimates. In particular, if significant enzyme induction occurs in the soil-dosed groups relative to the reference group, then bioavailability estimates may be biased low. As described in the draft USEPA Dioxin Risk Assessment Guidance document [*Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds*] (USEPA 2000), this potentially confounding factor can be minimized by collecting tissues prior to the onset of significant induction of hepatic enzyme activity, and by basing bioavailability determinations on comparisons of reference versus soil-dosed groups with similar hepatic PCDD/F concentrations. Both of these precautions were taken in this study. Specifically, animals were sacrificed within 24 h of dosing, and, as shown in Table 7, hepatic TEQs in soil- dosed animals were compared to reference groups with similar hepatic TEQs (as measured in the low-, mid-, and high-dose groups) to derive bioavailability estimates. As shown in Figure 3, mean hepatic EROD activities were not elevated in any of the reference groups, and also were not elevated in most of the soil-dosed groups (soil samples #1, #4, and #5). The observation of significantly elevated hepatic EROD activity in animals treated with soil samples #2 and 3 (Figure 3) was unexpected, because no such increases were observed in the high-dose reference groups that had similar, or even greater, hepatic concentrations of PCDD/Fs at the time of sacrifice (Table 7). It is possible that other soil components were responsible for the enzyme induction. Regardless, a strong correlation ($r^2 = 0.95$) was noted between soil PCDD/F TEQ and hepatic PCDD/F TEQ in the

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soil-dosed animals, suggesting that if any induction did occur in these two soil-dosed groups (soil samples #2 and 3), there was very little influence on hepatic PCDD/F retention.

There are some sources of uncertainty in the results and the accompanying interpretation that deserve mention. For example, it should be noted that a different set of soil samples was used in the bioaccessibility versus the bioavailability analyses, and therefore it is not entirely valid to make direct comparisons between the results of the two studies. Similarly, different soil samples were used in the coarse versus sieved comparison of the bioaccessibility analysis; a more direct comparison would involve isolation of coarse versus sieved particles from the same set of samples. Nonetheless, we believe the results illustrate the importance of using sieved particles in these types of studies. As noted earlier, some hepatic EROD induction was observed in the rats dosed with soil samples #2 and 3, which did not appear to significantly influence (*i.e.*, cause a decrease in) hepatic PCDD/F accumulation in these groups, and may have been due to soil components other than PCDD/Fs. It might be useful in these types of studies to include a "soil control" group, wherein rats are dosed with "clean" soils from a laboratory source.

This study is one of the few to evaluate the oral bioavailability of 2,3,7,8-substituted PCDD/Fs other than 2,3,7,8-TCDD in soil. The results presented here are consistent with those from other *in vitro* or *in vivo* studies that indicate that matrix effects can substantially reduce the absorption of PCDD/Fs from soil (Budinsky *et al.* 2008; Bonaccorsi *et al.* 1984; Umbreit *et al.*1988; Ruby *et al.* 2002; Poiger and Schlatter 1980; Shu *et al.* 1988). The results are also consistent with previous *in vivo* studies that indicate that the tetra- and penta-chlorinated PCCD/Fs are relatively well absorbed following oral administration, while the bioavailability of more highly chlorinated PCDD/Fs is substantially lower (Birnbaum and Couture 1988; Brewster and Birnbaum 1987; Van den Berg *et al.* 1985; Stephens *et al.* 1995; Diliberto *et al.* 1996). The bioaccessibility data indicated that, on a total PCDD/F TEQ basis, *in vitro* extractions using simulated GI fluids can provide a reasonably accurate alternative to the more costly bioavailability analyses. However, both the *in vivo* and *in vitro* methods indicate that attempts to extrapolate the results from one congener to another may be fraught with a high degree of uncertainty, because there were clear trends that are likely related to degree of chlorination.

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REFERENCES

Abraham K, Krowke R, and Neubert D. 1988. Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Arch Toxicol 62:359–68

Abraham K, Wiesmuller T, Brunner H, *et al.* 1989. Elimination of various polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) in rat faeces. Arch Toxicol 63:75–8

Birnbaum LS and Couture LA. 1988. Disposition of Octachlorodibenzo-p-dioxin (OCDD) in male rats. Toxicol Appl Pharmacol 93:22–30

Bonaccorsi A, di Domenico A, Fanelli R, *et al.* 1984. The influence of soil particle adsorption on 2,3,7,8-tetrachlorodibenzo-p-dioxin biological uptake in the rabbit. Arch Toxicol Suppl 7:431–4

Brewster DW and Birnbaum LS. 1987. Disposition and excretion of 2,3,7,8-pentachlorodibenzofuran in the rat. Toxicol Appl Pharmacol 90:243–52

Budinsky RA, Rowlands JC, Casteel S, *et al.* 2008. A pilot study of oral bioavailability of dioxins and furans from contaminated soils: Impact of differential hepatic enzyme activity and species differences. Chemosphere 70:1774–86

DeVito MJ, Diliberto JJ, Ross DG, *et al.* 1997. Dose-response relationships for polyhalogenated dioxins and dibenzofurans following subchronic treatment in mice. Toxicol Appl Pharmacol 147:267–80

Diliberto JJ, Jackson JA, and Birnbaum LS. 1996. Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) disposition following pulmonary, oral, dermal and parenteral exposures to rats. Toxicol Appl Pharmacol 138:158–68

Dugan MJ and Inskip MJ. 1985. Childhood exposure to lead in surface dust and soil: A community health problem. Public Health Rev 13:1–54

Hack A and Selenka F. 1996. Mobilization of PAH and PCB from contaminated soil using a digestive tract model. Toxicol Lett 88:199–210

Holman HYN. 2000. In Vitro Gastrointestinal Mimetic Protocol for Measuring Bioavailable Contaminants. United States Patent 6040188. Issued March 21, 2000

Kimbrough RD, Falk H, Stehr P, *et al.* 1984. Health implications of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) contamination of residential soil. J Toxicol Environ Health 14:47–93

Kociba RJ, Keyes DG, Beyer JE, *et al.* 1978. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. Toxicol Appl Pharmacol 46:279–303

Lowry OH, Rosebrough J, Farr AL, *et al.* 1951. Protein measurement with the Folin-Phenol reagents. J Biol Chem 193:265–75

Lucier GW, Rumbaugh RC, McCoy Z, *et al.* 1986. Ingestion of soil contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters hepatic enzyme activities in rats. Fundam Appl Toxicol 6:364–71

Lyytikainen M, Hirva P, Minkkinen P, *et al.* 2003. Bioavailability of Sediment-Associated PCDD/Fs and PCDEs: Relative importance of contaminant and sediment characteristics and biological factors. Environ Sci Technol 37:3926–34

Maddaloni M, Lolancono N, Manton W, *et al.* 1998. Bioavailability of soilborne lead in adults by stable isotope dilution. Environ Health Perspect 106:1589–94

McConnell EE, Lucier GW, Rumbaugh RC, *et al.* 1984. Dioxin in soil: Bioavailability after ingestion by rats and guinea pigs. Science 223:1077–9

Neubert D, Wiesmuller T, Abraham K, *et al.* 1990. Persistence of various polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) in hepatic and adipose tissue of marmoset monkeys. Arch Toxicol 64:431–42

Ohnishi ST and Barr JK. 1978. A simplified method of quantitating protein using the biuret and phenol reagents. Anal Biochem 86:193–200

Oomen AG, Sips AJ, Groten JP, *et al.* 2000. Mobilization of PCBs and lindane from soil during in vitro digestion and their distribution among bile salt micelles and proteins of human digestive fluid and the soil. Environ Sci Tech 34:297–303

Poiger H and Schlatter C. 1980. Influence of solvents and adsorbents on dermal and intestinal absorption of TCDD. Food Cosmet Toxicol 18:477–81

Pu X, Lee LS, Galinsky RE, *et al.* 2004. Evaluation of a rat model versus a physiologically based extraction test for assessing phenanthrene bioavailability from soils. Toxicol Sci 79:10–7

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Rodrigues AD and Prough RA. 1991. Induction of cytochromes P450IA1 and P450IA2 and measurement of catalytic activities. Meth Enzymol 206:423–31

Ruby MV, Fehling KA, Paustenbach DJ, *et al.* 2002. Oral bioaccessibility of dioxins/furans at low concentrations (50–350 ppt toxicity equivalent) in soil. Environ Sci Tech 36:4905–11

Ruby MV, Casteel SW, Evans TJ, *et al.* 2004. Rapid communication: Background concentrations of dioxins, furans and PCBs in Sprague Dawley rats and juvenile swine. J Toxicol Environ Health A 67:845–950

Santostefano MJ, Weng X, Richardson VM, *et al.* 1998. A pharmacodynamic analysis of TCDD-induced cytochrome P450 gene expression in multiple tissues: Dose- and time-dependent effects. Toxicol Appl Pharmacol 151:294–310

Shu H, Paustenbach D, Murray FJ, *et al.* 1988. Bioavailability of soil-bound TCDD: Oral bioavilability in the rat. Fundam Appl Toxicol 10:648–54

Stephens RD, Petreas MX, and Hayward DG. 1995. Biotransfer and bioaccumulation of dioxins and furans from soil: Chickens as a model for foraging animals. Sci Total Environ 175:253–73

Stewart MA, Jardine PM, Barnett MO, *et al.* 2003a. Influence of soil geochemical and physical properties on the sorption and bioaccessibility of chromium (III). J Environ Qual 32: 129–37

Stewart MA, Jardine PM, Brandt CC, *et al.* 2003b. Effects of contaminant concentration, aging, and soil properties on the bioaccessibility of Cr(III) and Cr(VI) in Soil. Soil and Sediment Contamination 12:1–21

Umbreit TH, Hesse EJ, and Gallo MA. 1986. Bioavailability of dioxin in soil from a 2,3,5-T manufacturing site. Science 232:497–9

Umbreit TH, Hesse EJ, and Gallo, MA. 1988. Bioavailability and cytochrome P-450 induction from 2,3,7,8-tetrachlorodibenzo-p-dioxin contained soils from Times Beach, Missouri, and Newark, New Jersey. Drug Chem Toxicol 11:405–18

USEPA(US Environmental Protection Agency). 1984. Method 1613, revision B: Tetra-Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRG/HRMS. Office of Water, Washington, DC, USA

USEPA. 1994. Method 8290. Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatrography/High-Resolution Mass Spectrometry (HRGC/HRMS). Office of Water, Washington, DC, USA

USEPA. 2000. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. Part II, Chapter I. EPA/600/P-00/001Bc. Office of Research and Development, Washington, DC, USA

Van den Berg M, de Vroom E, Van Greevenbroek M, *et al.* 1985. Bioavailability of PCDDs and PCDFs adsorbed on fly ash in rat, guinea pig and Syrian golden hamster. Chemosphere 14:865–9

Van den Berg M, Birnbaum LS, Denison M, *et al.* 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 93:223–41

Wendling J, Hileman F, Orth R, *et al.* 1989. An analytical assessment of the bioavailability of dioxin contaminated soils to animals. Chemosphere 18:929–32

Wittsiepe J, Schrey P, Hack A, *et al.* 2001. Comparison of different digestive tract models for estimating bioaccessibility of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) from red slag Kiesselrot. Int J Hyg Environ Health 203:263–73

Wittsieppe J, Erlenkamper B, Welge P, *et al.* 2007. Bioavailability of PCDD/F from contaminated soil in young Goettingen minipigs. Chemosphere 67:S335–S364

Yang JK, Barnett MO, Zhuang J, *et al.* 2005. Adsorption, oxidation, and bioaccessibility of As(III) in soils. Environ Sci Technol 39:7102–10

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