RELATIVE ORAL BIOAVAILABILITY OF POLYCHLORINATED DIBENZO-*P*-DIOXINS/DIBENZOFURANS IN SOIL

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ABSTRACT
This study examines *in vivo* bioavailability of polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs) in soils. The oral bioavailability of PCDD/Fs in soil samples collected from five representative locations at an industrial site was evaluated in rats. On a TEQ basis, the PCDD/F content of the soil was dominated by dibenzofurans while dibenzo-*p*-dioxins were responsible for less than 5% of the soil TEQ. Relative bioavailability determinations were based on comparisons of the concentration of PCDD/Fs in the liver of rats treated with an aqueous soil suspension to those in animals treated with a reference corn oil formulation. Eight to 16 PCDD/F congeners were detected at sufficient concentrations to be included in the bioavailability determinations for each soil sample. The bioavailability of individual PCCD/Fs was inversely correlated with degree of chlorination, and the mean values ranged from 15% for 1,2,3,7,8,9-HxCDF to 90% for 1,2,3,7,8-PeCDD. The overall relative bioavailability of PCDD/Fs in the soil samples (on a TEQ basis using the 2005 WHO toxic equivalency factors) ranged from 17 to 51% with a mean of 38%. The results of this study indicate that the oral bioavailability of PCDD/Fs can vary by congener or location and suggest that site-specific evaluations should be utilized in quantitative risk assessments.

INTRODUCTION
The relative bioavailability of 2,3,7,8-TCDD in soil collected from contaminated areas has been evaluated in several published studies.[1,2,3,4,5,6] The results from these studies vary widely but in general indicate that binding to soil reduces the oral bioavailability of 2,3,7,8-TCDD. The current study evaluated the relative bioavailability of PCDD/Fs in soil samples collected from various locations at an industrial site in the southwestern United States. OCDF was present in the highest concentration and accounted for at least 75% of the total PCDD/F content by mass. On a TEQ-weighted basis, six dibenzofuran 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 80% of the total soil PCDD/F content. 2,3,7,8-TCDD was a very minor component of the soil samples from this site and accounted for less than 1% of the total TEQ concentration.

MATERIALS AND METHODS
Soil Collection and Analysis. Five surface soil samples were collected from representative locations at the site, air dried and sieved to the < 250-µm particle size fraction. The sieved samples were analyzed for PCDD/F content using isotope dilution gas chromatography-mass spectrometry at Alta Analytical (El Dorado Hills, CA).

Reference Dose Formulation Preparation and Analysis. Dose formulations for the reference groups were prepared in corn oil in relative amounts based on the mean fractional contribution of each congener to the total TEQ concentration of the six soil samples. The reference formulations were prepared at three dose levels to allow for appropriate between-group comparisons in light of the wide range of soil TEQ concentrations evaluated in the study.

Animal Husbandry. The study protocol was reviewed and approved by the testing facility's Institutional Animal Care and Use Committee. Female Sprague Dawley rats were 15 weeks old and weighed 251 – 321 g on Day 1 following one week of acclimatization. The animals were housed in stainless steel wire mesh cages and were provided feed and filtered tap water *ad libitum*.

Dosing. Six animals were assigned to each group. One group of animals was not treated and was utilized for background PCDD/F determinations. Three groups of animals were treated with a single dose of the reference

formulations by oral gavage at a dose volume of 4 ml/kg. Six groups of animals were treated with a single dose of an aqueous suspension of the appropriate soil sample by oral gavage. Animals were not fasted prior to dosing.

Sacrifice and Tissue Collection. Each animal in the soil-treated or reference groups was euthanized 24 hours (± 15 minutes) after dosing. Two samples of liver 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 analysis of PCDD/F concentrations by mass spectrometry.

Hepatic Enzyme Activity Assays. The potential induction of hepatic CYP1A1/1A2 was evaluated by measuring 7-ethoxyresorufin-*O*-deethylase (EROD) activity.

Calculations and Data Analysis. The concentration of PCDD/Fs in the liver of reference group and soil-treated animals was corrected by subtracting the mean concentration of PCDD/Fs measured in the liver of animals in the untreated control group. For the bioavailability calculations, each soil-treated group was paired with a reference group that had similar liver TEQ concentrations. 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 the selected reference group.

RESULTS

- The oral bioavailability of the individual congeners was inversely correlated to the degree of chlorination
- The overall bioavailability value for each soil sample was determined primarily by the bioavailability of the penta-, hexa, or-hepta-chlorinated dibenzofurans
- The overall relative bioavailability of PCDD/Fs in the soil samples ranged from 17% (Sample 1) to 50% (Sample 2) (Table 1 and Figure 1).
- There was no apparent correlation between the overall oral bioavailability of PCDD/Fs and the total TEQ concentration of each soil.
- These results were consistent with those from previous studies which indicated that highly chlorinated PCDD/Fs are poorly absorbed from the GI tract.[7,8,9]
- These results were consistent with those from *in vitro* or *in vivo* studies which indicated that matrix effects can substantially reduce the absorption of PCDD/Fs from soil.[1,5,6,10,11]

CONCLUSIONS

- The affinity for the soil matrix reduced the absorbed TEQ fraction of PCDD/Fs by more than 60% when compared to the corn oil reference vehicle.
- Reliance on a default assumption of 100% oral bioavailability would be expected to result in substantial overestimates of the potential cancer or noncancer risks associated with exposure to PCDD/Fs in soil from this site.
- These values are also suitable for consideration in other environmental or experimental scenarios in which humans or animals are exposed to PCCD/Fs by the oral route.

Table 1. Relative Oral Bioavailability (% absorbed) of 2,3,7,8-Substituted Polychlorinated Dibenzo-*p*-Dioxins/Dibenzofurans in Soil Samples

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Mean**
Initial Soil TEQ (ppb)	**15.0**	**45.2**	**36.8**	**2.8**	**0.53**	
2,3,7,8-TCDD	ND	ND	ND	ND	ND	ND
1,2,3,7,8-PeCDD	ND	100±10	79±12	ND	ND	90±14
1,2,3,4,7,8-HxCDD	ND	74±6	52±10	ND	ND	63±15
1,2,3,6,7,8-HxCDD	22±5	67±6	46±7	82±NA	ND	54±26
1,2,3,7,8,9-HxCDD	ND	46±4	32±5	ND	ND	39±10
1,2,3,4,6,7,8-HpCDD	ND	32±4	20±4	ND	ND	26±9
OCDD	ND	23±3	ND	ND	ND	23±NA
2,3,7,8-TCDF	27±5	76±9	75±7	81±10	ND	65±25
1,2,3,7,8-PeCDF	26±6	89±9	69±8	74±9	61±18	64±24
2,3,4,7,8-PeCDF	18±4	50±4	44±6	52±8	56±15	44±15
1,2,3,4,7,8-HxCDF	23±5	61±6	42±6	63±8	47±13	47±16
1,2,3,6,7,8-HxCDF	22±5	59±5	42±6	62±8	42±11	46±16
1,2,3,7,8,9-HxCDF	5±1	16±2	13±2	19±3	23±NA	15±7
2,3,4,6,7,8-HxCDF	10±2	32±3	22±3	28±3	ND	23±10
1,2,3,4,6,7,8-HpCDF	13±3	28±3	18±3	33±4	19±7	22±8
1,2,3,4,7,8,9-HpCDF	14±3	34±3	22±3	39±5	25±8	27±10
OCDF	10±3	21±3	13±2	27±3	13±5	17±7
Overall Bioavailability[a]	**17±4**	**50±4**	**39±5**	**47±6**	**36±10**	

Notes: Results are shown as the arithmetic mean ± SD
NA = not applicable (indicated value based on data from 1 – 2 animals)
ND = not determined
[a] Overall bioavailability of PCDD/Fs in soil sample based on weighting of bioavailability values for individual congeners according to 2005 WHO toxic equivalency factors

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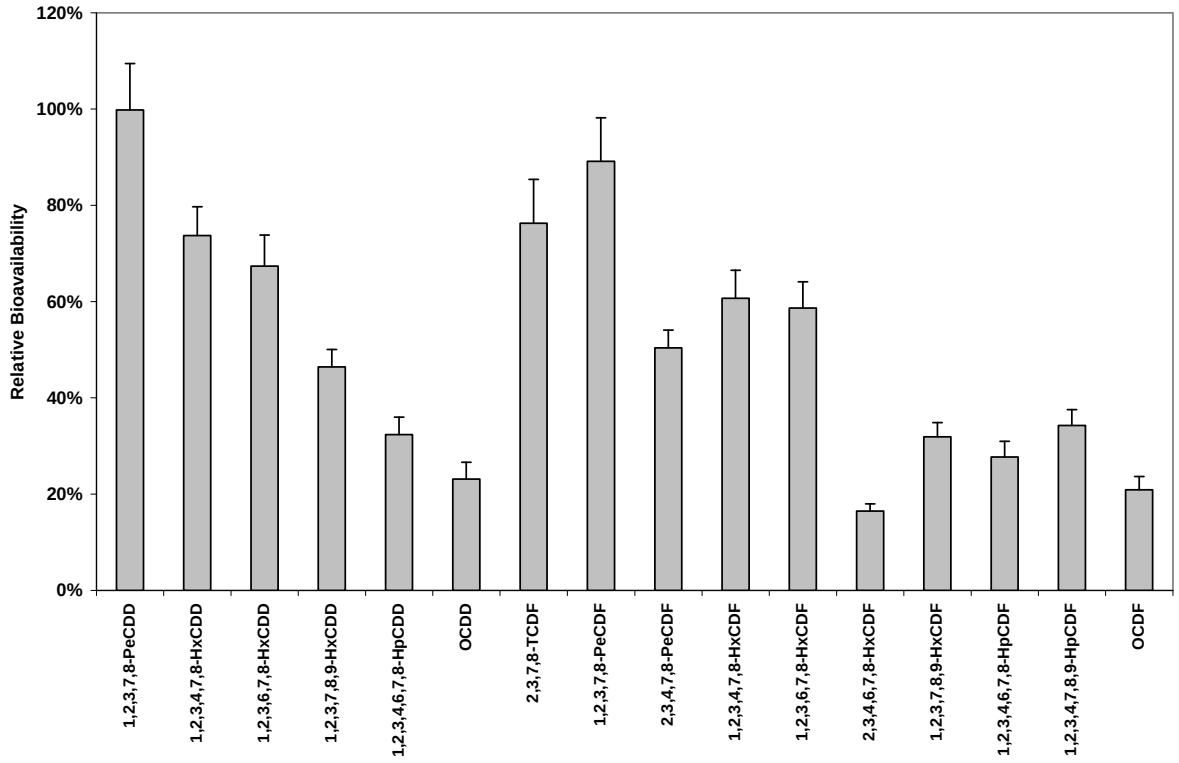

Figure 1. Relative oral bioavailability of polychlorinated dibenzo-*p*-dioxins/dibenzofurans in soil. The relative oral bioavailability of individual polychlorinated dibenzo-*p*-dioxins/dibenzofurans was calculated based on comparisons to groups which were treated with a reference formulation by oral gavage.