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A pilot study of oral bioavailability of dioxins and furans from contaminated soils: Impact of differential hepatic enzyme activity and species differences

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Abstract

An *in vivo* pilot study of the oral bioavailability of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in two soils with distinct congener profiles (one dominated by PCDDs, the other by PCDFs) was conducted in rats and juvenile swine. The pilot study revealed potential confounding of relative bioavailability estimates compared to bioavailability in spiked corn oil gavage for tetrachlorodibenzofuran (TCDF) in the rat study due to differential EROD induction between groups receiving soil and those receiving spiked control PCDDs/PCDFs. A follow-up study in rats with the furan-contaminated soil was then conducted with reductions in the spiked control doses to 20%, 50% and 80% of the soil-feed dose in order to bracket hepatic enzyme induction levels in the soil group. When hepatic enzyme induction was matched between the soil and spiked control groups, the apparent relative bioavailability for TCDF was reduced significantly. Overall, after controlling for hepatic enzyme induction, estimates of relative bioavailability in rats and swine differed for the two soils. In the rat study, the relative bioavailability of the two soils were approximately 37% and 60% compared to corn oil administration for the PCDD- and PCDF- dominated soils, respectively, on a TEQ basis. In swine, both soils demonstrated relative bioavailability between 20% and 25% compared to administration in corn oil. These species differences and experimental design issues, such as controlling for differential enzyme induction between corn oil and soil-feed animals in a bioavailability study, are relevant to risk assessment efforts where relative bioavailability inputs are important for theoretical exposure and risk characterization.

Keywords: Dioxin; Furans; Bioavailability; Soils; Swine; Rats

1. Introduction

Bioavailability directly impacts exposure potential to chlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) present in soil and sediment. Soil aging and the presence of organic carbon, such as black carbon, are believed to reduce oral bioavailability for hydrophobic compounds such as PCDD/Fs (Poiger and Schlatter, 1986; Alexander, 2000; Koelmans et al., 2006). Oral bioavailability studies of soils containing 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) exhibit a range of relative (RBA) and absolute bioavailability of approximately 10% to 40% and 10% to 30%, respectively (Bonaccorsi et al., 1984; McConnell

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et al., 1984; Lucier et al., 1986; Umbreit et al., 1986; Shu et al., 1988; Wendling et al., 1989; Wittsiepe et al., 2001; Wittsiepe et al., 2007). Van den Beg et al., 1987 reported congener-specific, hepatic retention differences for fly ash-bound PCDDs/Fs compared to liver retention from administration of oil extracts of municipal fly ash. For example, less than 20% of the dose of 2, 3, 4, 7, 8-pentachlorodibenzofuran (4-PeCDF) was detected in the liver following administration of crude fly ash relative to retention from an arachidis oil extract of the fly ash. In contrast to reduced bioavailability findings for soils and other solid matrices, TCDD-spiked corn oil exhibits a RBA of approximately 80% in laboratory rodents (Rose et al., 1976; Diliberto et al., 1996).

In mid-Michigan, soil remediation efforts are currently underway for two different soil locations: urban soils and floodplain soils and sediments. The urban soils reflect a dioxin-dominated toxic equivalency quotient (TEF) profile comprised mainly of 2, 3, 7, 8-TCDD and 1, 2, 3, 7, 8-PeCDD resulting from past incineration practices (Table 1). The floodplain soils exhibit a furan-dominated TEQ profile consisting of 2, 3, 4, 7, 8-PeCDF (4-PeCDF) and 2, 3, 7, 8-TCDF (TCDF) resulting from historic chloralkali production, i.e., late 1800s-early 1900s. Estimates of RBA and absolute bioavailability are to be used in the human health risk assessment being conducted as part of the overall remediation activities.

Initially, eight urban soils from this area, sieved to $a < 250$ μm fraction consistent with the size fraction believed ingested upon casual hand-to-mouth contact (Dugan and Inskip, 1985; Maddaloni et al., 1998; Casteel et al., 1997a and b; USEPA, 1999), demonstrated approximately 25% desorption (bioaccessibility) in an *in vitro* gastrointestinal tract model (Ruby et al., 2002). Bioaccessibility reflects the PCDD/F fraction available for systemic absorption under the conditions of the simulated gastrointestinal tract. Twenty-five percent Bioaccessibility is consistent with results reported by Wittsiepe et al. (2001) who reported TEQ-based bioaccessibility estimates ranging from 3.4% to 56% from Kieselrot (red slag from copper production). Twenty-five percent bioaccessibility (Ruby et al., 2002) is also within the range of oral bioavailability results from published studies of TCDD-contaminated soils (Bonaccorsi et al., 1984; McConnell et al., 1984; Lucier et al., 1986; Umbreit et al., 1986; Shu et al., 1988) and a soil comprised of mixed PCDD/F congeners (Wittsiepe et al., 2007).

Table 1
Soil profiles. Mean and coefficient of variability (CV) from triplicate sampling of the sieved (<250 μm) soil samples, TEQ, TEQ percent contributions, and other soil characteristics

Analyte	WHO_{2006}	TEF Urban soil				Floodplain soil			
		Mean (pg/g)	CV (%)	TEQ (pg/g)	% of TEQ	Mean (pg/g)	CV	TEQ (pg/g)	% of TEQ
2,3,7,8-TCDD	1.0	131	5.4	131	**49.7**	4.79	2.1%	4.79	0.74
1,2,3,7,8-PeCDD	1.0	66.9	1.9	66.9	**25.4**	5.13	4.8%	5.13	0.79
1,2,3,4,7,8-HxCDD	0.1	29.0	7.0	2.9	1.10	3.61	19%	0.361	0.06
1,2,3,6,7,8-HxCDD	0.1	73.5	5.6	7.35	**2.79**	21.0	22%	2.1	0.32
1,2,3,7,8,9-HxCDD	0.1	49.6	1.8	4.96	1.88	7.67	4.8%	0.767	0.12
1,2,3,4,6,7,8-HpCDD	0.01	1170	4.7	11.7	**4.43**	406	18%	4.06	0.62
OCDD	0.0001	13900	6.1	1.39	0.53	3960	13%	0.396	0.06
2,3,7,8-TCDF	0.1	33.6	12	3.36	1.27	2150	16%	215	**33.1**
1,2,3,7,8-PeCDF	0.03	25.7	3.6	0.771	0.29	1080	20%	32.3	**4.96**
2,3,4,7,8-PeCDF	0.3	36.1	4.7%	10.8	**4.11**	883	17%	265	**40.7**
1,2,3,4,7,8-HxCDF	0.1	55.1	4.7%	5.51	2.09	719	18%	71.9	**11.1**
1,2,3,6,7,8-HxCDF	0.1	29.5	5.6	2.95	1.12	164	17%	16.4	**2.52**
2,3,4,6,7,8-HxCDF	0.1	31.1	5.7	3.11	1.18	95.3	15%	9.53	1.46
1,2,3,7,8,9-HxCDF	0.1	12.3	6.1	1.23	0.47	137	22%	13.7	2.11
1,2,3,4,6,7,8-HpCDF	0.01	639	2.2	6.39	2.42	723	14%	7.23	1.11
1,2,3,4,6,7,8,9-HpCDF	0.01	30.4	5.5	0.304	0.12	68.3	19%	0.683	0.10
OCDF	0.0001	1230	2.2	0.123	0.05	1260	18%	0.126	0.02
TEQ, PCDD/F				261			649		
TEQ, PCB[a]				3.0			1.3		
Total TEQ				**264**				**651**	
Other parameters									
Solids, total (%)	99.2						98.9		
pH (s.u.)	5.77						7.69		
Carbon, total organic (%)	3.14						2.73		
Grain size (%)									
250 μm – 2 mm	31.1						42.1		
106–250 μm	44.9						26.8		
75–106 μm	11.4						8.78		
4–75 μm	12.1						21.4		
<4 μm	0.5						0.86		

The five congeners contributing most to total TEQ for each soil are bolded.
[a] Total TEQ for 12 TEQ-contributing congeners.

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Given the long weathering time of these soils and its potential impact on bioavailability (Alexander, 2000), a pilot study was conducted to assess *in vivo* bioavailability of PCDD/Fs in rats and juvenile swine. The initial pilot study compared the proportion of administered compounds retained in adipose tissue and livers in animals fed the local soils versus animals receiving the corn oil spiked with the appropriate PCDDs/Fs daily for 30 days. A spiked feed component of the study was also conducted in the rats, but this aspect of the pilot study is not reported here. The overall objective of the pilot study was to test the feasibility of analyzing dioxins and furans in liver and adipose tissue samples after feeding of the two local soils, which contained substantially lower concentrations of dioxins and furans than tested in previously published oral bioavailability studies for TCDD. Other objectives of the pilot oral bioavailability study were to compare rats to juvenile swine as experimental models; to evaluate the variability observed in tissue concentrations and RBA estimates and thus evaluate the number of animals needed to obtain robust results; to assess CYP1A1 and CYP1A2 induction and its impact on bioavailability estimates; and to evaluate the possibility of limiting future studies to just one tissue, such as liver. A follow-up to the pilot study was subsequently conducted in rats only to address potential confounding on bioavailability estimates attributed to differential hepatic enzyme induction primarily observed in rats receiving corn oil spiked with furans. These two studies, the pilot study on feasibility and the follow-up study to address the impact of differential enzyme induction, are described below.

2. Methods

This study was completed in two parts. The first part consisted of the pilot study from which unexpectedly high RBA estimates in rats, large differences between rat and swine RBA estimates, and differential EROD induction among the rat treatment groups were observed. A follow-up to the pilot study was subsequently completed to control for and to evaluate the impact of differential enzyme induction (EROD) on RBA estimates in rats and the rat to swine comparisons. An overview of the dose groups used in the pilot and follow-up studies is provided in Table 2.

Table 2
Dose groups for pilot and follow-up studies

Animal	Group Name	Description
Pilot Study		
Rats ($n = 10$; tissues from pairs of animals)[a]	Urban soil/feed	Soil sample from urban location mixed with granulated feed at 5% by weight
	Oil reference for urban soil	Corn oil spiked with the top five TEQ-contributing congeners in the same relative proportions as found in the soil sample, administered via daily gavage
	Floodplain soil/feed	Soil sample from floodplain location mixed with granulated feed at 5% by weight
	Oil reference for floodplain soil	Corn oil spiked with the top five TEQ-contributing congeners in the same relative proportions as found in the soil sample, administered via daily gavage
Swine ($n = 5$ animals per group)	Urban soil	Soil sample administered in dough balls twice per day
	Oil reference for urban soil	Corn oil spiked with the top five TEQ-contributing congeners in the same relative proportions as found in the soil sample, administered in gelatin capsules wrapped in dough balls
	Floodplain soil/feed	Soil sample administered in dough balls twice per day
	Oil reference for floodplain soil	Corn oil spiked with the top five TEQ-contributing congeners in the same relative proportions as found in the soil sample, administered in gelatin capsules wrapped in dough balls
Follow-up study		
Rats ($n = 5$ for soil groups, 7 for corn oil gavage groups)	Feed control	Undosed control group, fed clean feed, no gavage
	Oil control	Undosed control group, fed clean feed, gavaged with unspiked corn oil
	Soil group	Floodplain soil blended with diet, nominal daily dose rate X
	Oil reference 0.2X	Reference group, with corn oil spiked at 20% of calculated PCDD/F dose administered from soil
	Oil reference 0.5X	Reference group, with corn oil spiked at 50% of calculated PCDD/F dose administered from soil
	Oil reference 0.8X	Reference group, with corn oil spiked at 80% of calculated PCDD/F dose administered from soil

[a] A spiked-feed treatment group was included in the study to evaluate how rat chow would impact absolute and relative oral bioavailability. The results from this treatment group are not included in this manuscript.

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2.1. Initial pilot study: Oral bioavailability in rats and juvenile swine

Two animal models were selected for the study. Sprague Dawley rat females were selected because the TCDD cancer slope factor is based on a study using these rats (US EPA, 2006) and previously conducted dioxin bioavailability studies have utilized rats (Lucier et al., 1986; Shu et al., 1988). Juvenile swine were selected because the anatomy, physiology, and small intestine absorption mechanisms are similar between swine and humans (Weis and LaVelle, 1991; Casteel et al., 1998). This similarity has led to increasing use of swine as models of human oral bioavailability (Casteel et al., 2006; US EPA, 2006).

2.2. Soil dosing material preparation, animals and treatment groups

Rat Study. Four month old, 250 g Sprague Dawley female rats (Harlan Ind., Indianapolis, IN) were placed in individual steel cages and provided Purina laboratory rodent diet 5001 and de-ionized water *ad libitum* over a one-week quarantine period and 2 additional days prior to dosing. Ten rats were randomly assigned to each dose group.

The two soil samples were mixed with the diet (Nutritional International Rodent Lab Diet) at Wil Laboratories to provide a 5% wt/wt soil-feed mixture from each soil for the rats (5% represents the maximum amount of soil that rats are assumed to tolerate in their food). The soils consisted of one floodplain soil of approximately 650 parts per trillion (ppt) TCDD toxic equivalency (TEQ; based on the World Health Organization 2005 toxic equivalency factors reported by Van den Berg et al., 2006) and an urban soil sample, CC-S-27, 260 ppt TEQ, that was previously tested in the bioaccessibility study of Ruby et al. (2002) (congener data for each soil shown in Table 1). Soil samples were homogenized and sieved to 250 μm prior to incorporation into rat chow. Triplicate samples of the two soil-feed mixes were submitted to Alta Laboratories for analyses (EPA method 8290) to evaluate homogeneity of the mixture. Coefficients of variation for the congeners of interest ranged from 2.3% to 12% for the urban soil-feed mixture and from 4.5% to 14% for the floodplain soil-feed mixture. Analysis of samples taken at the completion of the study showed no appreciable loss or change in concentration over the 30 day in-life portion of the study (data not shown).

The two gavage reference materials (one for the urban soil-feed mix and one for the floodplain soil-feed mix) for the rat study were prepared in corn oil/acetone (99:1) by Alta Laboratories, and were formulated to deliver the same dioxin/furan doses of the five highest TEQ-contributing congeners as the soil/diet blends at the anticipated feed intake rate (23 g/d). The five dioxin/furan congeners contributing most to the TEQ of each soil were spiked into acetone (20 ml), and the concentrations of the five congeners in the spiked acetone was measured to confirm that analytical concentrations were close to target concentrations. Subsequently, 8.26 ml of this acetone was added to 817.7 ml corn oil (Spectrum Chemicals & Laboratory Products, National Formulary [NF] grade; analysis of the corn oil indicated negligible dioxin/furan concentrations. The two corn oil/acetone reference materials were then assayed for concentrations of the five target congeners. Relative percent differences (RPDs) between target and pre-dosing measured concentrations were generally in the range of 3–13%, except for 1, 2, 3, 6, 7, 8-HxCDD, which was present at a concentration approximately 40% greater than the target concentration. As this compound contributed less than 5% of the total TEQ of the soil and reference oils, this variation was considered acceptable for use in the study. The gavage reference mixtures were stored in amber glass bottles sealed with Teflon-lined lids, and were used within 60 days of preparation. The rats were given 50 g of the soil-feed mixtures every two days; remaining feed was weighed and the mass of spilled feed was estimated in order to estimate actual feed intake. The spiked corn oil materials were administered by daily gavage (1 ml/d) throughout the 30-day study.

Swine study. Juvenile swine (6 week-old *Sus scrofa* from Chinn Farms, Clearance, Missouri) were obtained and acclimated to the facility. Five swine were assigned to each treatment group. For the swine pilot study, the test soil doses were delivered by placing 1 g of the soil (either the urban or floodplain soil) in the center of a 10 g moistened dough ball (Zeigler Bros. Swine Diet) and offered to the swine. The swine were fasted for two hours prior to dosing, because previous studies conducted in this animal model indicated that a 2 h fast ensures eager acceptance of the 10 g dough ball containing the dose. Soil-containing dough balls were prepared every 3–4 days. Five dough balls (containing a total of 5 g of test soil) were given twice daily, at 9 a.m. and 4 p.m., for a total dose of 10 g soil/day. Immediately after each dosing, the animals were given one-half of their standard ration of swine feed. The two dose groups receiving the soil doses had their feed rations reduced by 80 g/day to compensate for the amount of food contained in the feed balls given these animals during dosing, relative to the corn oil dosed animals. Dosing and feeding continued twice daily for 30 consecutive days.

The dosing materials for the two swine reference oil groups were prepared in corn oil/acetone (99:1) by Alta Laboratories, and were spiked such that 2 ml of the corn oil/acetone mixture would deliver an equivalent dose of the five target congeners to 5 g of the test soil to which it was matched. To create these reference mixtures, the five dioxin/furan congeners contributing most greatly to TEQ in each soil were spiked into acetone (20 ml), and the concentrations of the five congeners in the spiked acetone were measured to confirm that analytical concentrations were close to target concentrations. Subsequently, 10 ml of this acetone was added to 990 ml corn oil (Spectrum Chemicals & Laboratory Products, National Formulary [NF] grade;

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analysis of the corn oil indicated negligible dioxin/furan concentrations. The two corn oil/acetone reference materials were then assayed for concentrations of the five target congeners. Relative percent differences (RPDs) between target and measured concentrations were in the range of 1–21%, which was considered acceptable for use in the study. The swine reference mixtures were stored in amber glass bottles sealed with Teflon-lined lids, and were used within 60 days of preparation.

For dosing, 1 ml of corn oil/acetone mixture was placed in each gel capsule (Torpac, 1.2 ml volume), and these were embedded in the center of a 10 g ball of moistened swine feed immediately prior to dosing. Two dough balls (containing a total of 2 ml of reference mixture) were given twice daily, at 9 a.m. and 4 p.m., for a total dose of 4 ml reference mixture/day. Immediately after dosing, the animals were given one-half of their standard ration of swine feed.

The administered dosages for the various treatment groups are shown in Table 3.

2.3. PCDD/Fs tissue analysis

Rats and swine were sacrificed at the end of 30 days of dose administration in the pilot study. Rat liver and adipose tissue was harvested for dioxin and furan analyses by combining two rats in each group, i.e., 5 combined liver and 5 combined adipose tissue samples for PCDD/Fs analyses. A previous preparation study of background concentrations of PCDDS/Fs in the liver and fat of Sprague Dawley females (Purina Laboratory Rodent Diet 5001) and juvenile swine (Ziegler, Bros., Inc., Gardners, PA) fed clean diets under the conditions of the study exhibited negligible concentrations of PCDDs/Fs. TCDD and 4-PeCDF concentrations in all samples of liver and fat were below detection limits in this background study (0.0594 pg/g and 0.0908 pg/g, respectively) (Ruby et al., 2004). High resolution gas chromatography/mass spectrometry analysis of dioxin and furan in tissue samples was conducted by Alta Laboratories, El Dorado Hills, California using EPA Method 8290.

2.4. EROD and MROD activities

EROD and MROD activities in hepatic tissue samples were measured using standard operating procedures of Michigan State University (SOP250 MSU-ATL SOP 250 version 1). Briefly, liver microsomes were prepared from each liver sample, and the protein levels and EROD/MROD enzymatic activities were fluorometrically measured according to the MSU Standard Operating Procedure (SOP) No. 250 (v 1.1), titled *Protocol for Liver Microsome Preparation, and Microsomal Protein Measurement and AROD Assays in the same 96-Well Plate*.

2.5. Relative bioavailability estimates

Relative bioavailability was estimated by comparing the fraction of the administered dose retained in the liver and adipose tissues combined between the soil-feed treated animals and the spiked corn oil reference groups similar to a method used by Wittsiepe et al. (2001), Wittsiepe et al. (2007). Three assumptions were made in the calculations: (1) the whole-body elimination rate for each congener could be approximated as a first-order process, (2) the elimination rate for each congener is the same in the soil-dosed vs. the spiked corn oil-dosed reference animals, and (3) the majority of each congener retained in the body would be distributed in the liver and adipose tissues in both soil- and corn oil-dosed reference groups, with the distribution to other tissues representing a similar proportion of the total retained dose in both soil and reference groups. The RBA of a compound from soil administration compared to administration of a spiked reference material

Table 3
Average daily administered doses for five target compounds, initial pilot study, pg/kg bw/d

	Rats				Swine			
	Soil		Reference corn oil		Soil		Reference corn oil	
	Mean ± SD	TEQ	Mean ± SD	TEQ	Mean ± SD	TEQ	Mean ± SD	TEQ
Urban soil and reference oil								
2,3,7,8-TCDD	302 ± 17	302	511 ± 14	511	70 ± 2	70	81 ± 4	81
1,2,3,7,8-PeCDD	172 ± 10	172	295 ± 8	295	36 ± 1	36	37 ± 2	37
1,2,3,6,7,8-HxCDD	247 ± 14	24.7	423 ± 12	42.3	39 ± 1	3.9	48 ± 2	4.8
1,2,3,4,6,7,8-HpCDD	4820 ± 270	48.2	5310 ± 140	53.1	621 ± 21	6.2	619 ± 29	6.2
2,3,4,7,8-PeCDF	100 ± 6	30	158 ± 4	47.4	19 ± 1	5.7	20 ± 1	6
Total TEQ		577		949		122		135
Floodplain soil and reference oil								
2,3,7,8-TCDF	6430 ± 370	643	8840 ± 1700	884	1120 ± 45	112	1080 ± 36	108
1,2,3,7,8-PeCDF	3920 ± 230	117.6	4400 ± 840	132	561 ± 23	16.8	647 ± 21	19.4
2,3,4,7,8-PeCDF	3370 ± 200	1011	3590 ± 680	1077	460 ± 18	138	550 ± 18	165
1,2,3,4,7,8-HxCDF	2630 ± 150	263	3040 ± 580	304	375 ± 15	37.5	438 ± 14	43.8
1,2,3,6,7,8-HxCDF	649 ± 38	64.9	798 ± 150	79.8	85 ± 3	8.5	108 ± 4	10.8
Total TEQ		2100		2477		313		347

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($RBA_{soil:ref}$) is the ratio of the absolute absorption fractions (f_{abs}) of the compound from the two media:

$$RBA_{soil:ref} = \frac{f_{abs,soil}}{f_{abs,ref}}$$

Under the assumption of first-order pharmacokinetics, the amount of a congener retained in the body as the proportion of administered dose following a period of administration, is a function of the absorption fraction and the elimination rate. If the elimination rate for a congener is constant between the soil and reference spiked corn oil groups, the relative bioavailability can be estimated as a ratio of the fractions of administered dose retained in the two groups:

$$RBA = \frac{FR_{soil}}{FR_{reference}}$$

where FR_{soil} and $FR_{reference}$ are the ratios of the total amount of congener retained in the body at the end of the study to the total amount administered for the soil and reference groups, respectively. To estimate the amount of each congener retained in the animal at the end of the study, the concentration of each congener in liver and adipose tissue was multiplied times the weight of the organ or tissue (liver or fat) to obtain the mass of the congener retained after 30 days of ingestion in those two tissues. This total was used as a surrogate for total body retention, as previous studies have demonstrated that approximately 70% to 90% of body distribution of PCDD/Fs are to these tissues (Hurst et al. 2000; Diliberto et al., 2001). Liver weights for both rats and swine were obtained at sacrifice and for rats, adipose tissue weights were estimated as a function of body weights at sacrifice using the relationship from Brown et al. (1997) based on data for male Sprague Dawley rats developed by Bailey et al. (1980); as cited by Brown et al., 1997). Three swine were dissected to estimate percent body fat composition in the juvenile swine in the study for use in calculating the mass of congeners retained in adipose tissue.

2.6. Follow-up study: Controlling for Hepatic enzyme induction

The follow-up rat study was performed as described for the pilot study with a few modifications. Only the floodplain soil sample was evaluated in the follow-up study. The oil reference doses were based on the assumption that rats would consume 18 g/d, based on the results of the pilot study. Because elevated hepatic TEQ concentrations and EROD and MROD activities were observed in the rats administered the spiked corn oil gavage treatment compared to the soil groups in the pilot study, the follow-up study used graded spiked corn oil dosages at 20%, 50% and 80% of the nominal soil-feed dose based on feed intake rates measured during the pilot study. In addition, since it was found that analytical methods were sufficiently sensitive that sufficient tissues could be obtained from individual rats, rats were not pooled in the second part of this study, thereby reducing animal use ($n = 5$ per group). Two control groups (clean feed and corn oil) were included to provide baseline EROD and MROD activity not obtained in the first part of the study. These control animals also provided concurrent background measurements of PCDD/Fs in liver and adipose tissue (adipose tissue and livers from 5 animals were pooled to provide one composite, background PCDD/F measurement). An overview of the dose groups is provided in Table 2.

Hepatic TEQ concentrations and EROD and MROD among the dosing groups was compared with ANOVA followed by Dunnett's multiple comparison test at an overall 95% confidence level, to identify the oil reference groups with hepatic TEQ and EROD and MROD activities that were not statistically significantly different from those of the soil group. The fraction of administered dose retained for each congener was evaluated for all individual animals across oil reference groups using multivariate linear regression to identify any relationship between fractions retained and hepatic TEQ concentration, EROD, or MROD activity. Among the animals receiving the three different furan dosages in spiked corn oil, a statistically significant relationship between the fraction of any specific congener retained and the enzyme activity or hepatic TEQ concentration would indicate a dependency of elimination rate on that parameter for that congener.

RBA of the congeners of interest in the soil was assessed by comparing the fraction retained between the soil group and the corn oil group that best matched the soil group's hepatic EROD activity and hepatic TEQ concentrations. A TEQ-weighted estimate of RBA was obtained by weighting the individual congener RBA estimates by their respective contribution to the TEQ concentration of each soil (van den Berg et al., 2006).

3. Results

3.1. Pilot study

No notable differences were noted in feed consumption among rats or body weight gains among rats or swine across all treatment groups (data not shown). However, total feed consumption by rats was lower than anticipated based on the literature values. Thus, administered doses of the target congeners to rats were lower in the soil/feed mixture groups than anticipated, and as a result, the corn oil gavage dose groups received greater doses (Table 3). Tissue concentrations and fraction of administered dose retained in liver and adipose tissues are presented in Table 4. Hepatic TEQ and EROD and MROD activities are presented in Table 5.

In rats, hepatic EROD (but not MROD) activity was statistically significantly elevated in each reference oil group compared to the paired soil/feed group. This was probably due to both the higher administered doses in the reference oil groups and to the likelihood of greater

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Table 4
Average concentrations and percent of administered dose retained for each target congener by tissue for rats and swine from the initial pilot study

Target congener	Rat tissue concentration, pg/g [% of administered dose]				Swine tissue concentration, pg/g [% of administered dose]			
	Soil		Reference corn oil		Soil		Reference corn oil	
	Liver	Adipose	Liver	Adipose	Liver	Adipose	Liver	Adipose
Urban soil and reference material								
2,3,7,8-TCDD	10.4 ± 0.8 [4.2 ± 0.3]	15.1 ± 1.6 [12.0 ± 1.6]	60.6 ± 3.3 [13.9 ± 0.9]	69.6 ± 3.4 [31.9 ± 1.7]	0.2 ± 0.1[a] [0.4 ± 0.1]	0.6 ± 0.3[a] [2.8 ± 1.3]	0.8 ± 0.2 [1.0 ± 0.3]	4.0 ± 0.4 [16.5 ± 1.6]
1,2,3,7,8-PeCDD	13.0 ± 1.2 [9.3 ± 0.6]	8.1 ± 0.8 [11.3 ± 1.6]	66.8 ± 3.5 [26.5 ± 0.9]	31.5 ± 2.1 [25.0 ± 1.6]	0.1 ± 0.1[a] [0.4 ± 0.2]	0.4±0.2[a] [4.0 ± 1.8]	0.4 ± 0.2 [1.2 ± 0.4]	1.9 ± 0.3 [17.3 ± 2.0]
1,2,3,6,7,8-HxCDD	33.3 ± 2.8 [16.6 ± 1.2]	6.6 ± 0.7 [6.5 ± 0.8]	135.6 ± 5.6 [37.6 ± 1.5]	21.1 ± 1.9 [11.7 ± 1.1]	0.2±0.1[a] [0.7 ± 0.4]	0.9±0.5[a] [7.3 ± 4.2]	0.9 ± 0.3 [1.9 ± 0.6]	2.7 ± 0.3 [18.8 ± 2.1]
1,2,3,4,6,7,8-HpCDD	350.0 ± 26.4 [8.9 ± 0.6]	30.2 ± 3.2 [1.5 ± 0.2]	1198.0 ± 51.2 [26.5 ± 0.9]	93.0 ± 8.3 [4.1 ± 0.5]	8.9 ± 3.0 [1.9 ± 0.6]	8.5 ± 2.2 [4.6 ± 1.1]	17.1 ± 6.6 [2.9 ± 0.8]	16.6 ± 3.8 [3.9 ± 1.8]
2,3,4,7,8-PeCDF	22.2 ± 1.6 [27.3 ± 1.7]	1.7 ± 0.2 [4.2 ± 0.6]	95.9 ± 6.9 [71.0 ± 2.7]	5.8 ± 0.6 [8.6 ± 0.8]	0.7 ± 0.2 [4.4 ± 1.2]	0.2±0.1[a] [4.2 ± 2.4]	1.8 ± 0.3 [9.6 ± 1.5]	1.1 ± 0.1 [17.5 ± 1.6]
Floodplain soil and reference material								
2,3,7,8-TCDF	338.4 ± 15.9 [6.5 ± 0.6]	128.7 ± 17.9 [4.9 ± 1.0]	599.3 ± 38.8 [7.2 ± 0.4]	231.5 ± 10.3 [5.5 ± 0.3]	0.2 ± 0.04 [0.01 ± 0.003]	0.9 ± 0.2 [0.3 ± 0.004]	0.6 ± 0.2 [0.05 ± 0.02]	3.8 ± 0.7 [1.2 ± 0.2]
1,2,3,7,8-PeCDF	268.8 ± 14.8 [8.4 ± 0.7]	52.4 ± 6.5 [3.2 ± 0.5]	588.5 ± 43.3 [14.2 ± 0.8]	125.0 ± 13.2 [6.0 ± 0.7]	0.2 ± 0.02 [0.02 ± 0.003]	0.6 ± 0.2 [0.3 ± 0.2]	0.2 ± 0.1 [0.03 ± 0.01]	2.2 ± 0.3 [1.2 ± 0.2]
2,3,4,7,8-PeCDF	1084.8 ± 97.2 [39.4 ± 2.1]	43.8 ± 4.1 [3.1 ± 0.4]	2535.0 ± 148.2 [75.0 ± 3.6]	103.5 ± 11.3 [6.1 ± 0.7]	11.0 ± 1.4 [2.7 ± 0.1]	5.8 ± 0.8 [4.2 ± 0.5]	53.4 ± 10.8 [10.4 ± 0.02]	24.5 ± 4.0 [15.0 ± 2.7]
1,2,3,4,7,8-HxCDF	668.4 ± 56.8 [31.2 ± 1.7]	32.0 ± 3.4 [2.9 ± 0.3]	1557.5 ± 60.8 [54.5 ± 1.7]	79.2 ± 10.6 [5.5 ± 0.8]	7.6 ± 1.2 [2.3 ± 0.2]	7.6 ± 0.8 [6.8 ± 0.6]	28.1 ± 5.9 [6.9 ± 1.4]	24.4 ± 2.7 [18.8 ± 2.4]
1,2,3,6,7,8-HxCDF	166.8 ± 13.2 [32.7 ± 2.2]	7.2 ± 0.5 [2.8 ± 0.3]	436.8 ± 6.8 [58.2 ± 3.2]	19.3 ± 2.7 [5.1 ± 0.7]	2.5 ± 0.3 [3.3 ± 0.2]	1.7 ± 0.1 [6.5 ± 0.4]	9.6 ± 2.1 [9.5 ± 2.0]	5.3 ± 0.6 [16.7 ± 2.1]

Mean ± SD. $n = 5$ (paired tissues for rats, 10 total rats per dose group; 5 individual swine per dose group) except as follows: $n = 4$ for the rat floodplain soil reference corn oil group; $n = 4$ for swine in the floodplain soil group.
[a] Tissue concentrations were below detection limits for some animals in group; ND = 1/2 detection limit was used to calculate mean values for group.

Table 5
Hepatic EROD and MROD activities and TEQ concentrations (WHO_{2005}, five target congeners) from initial pilot study

Animal	Administered material	Hepatic TEQ, pg/g	Liver microsomal activity, pmol/mg/min	
			EROD	MROD
Rat	Urban soil	36.8 ± 3	83 ± 14	101 ± 16
	Urban reference oil	181.7 ± 10	169 ± 53*	108 ± 9
	Floodplain soil	450.9 ± 38	319 ± 39	168 ± 28
	Floodplain reference oil	1037.5 ± 58	444 ± 34**	163 ± 64
Swine	Urban soil	0.6 ± 0.2	25 ± 3	114 ± 24
	Urban reference oil	2.0 ± 0.6	25 ± 16	95 ± 53
	Floodplain soil	4.3 ± 0.6	28 ± 14	97 ± 23
	Floodplain reference oil	19.8 ± 4.1	35 ± 3	123 ± 39

Mean ± SD.
* $p < 0.05$ compared to paired soil.
** $p < 0.01$ compared to paired soil.

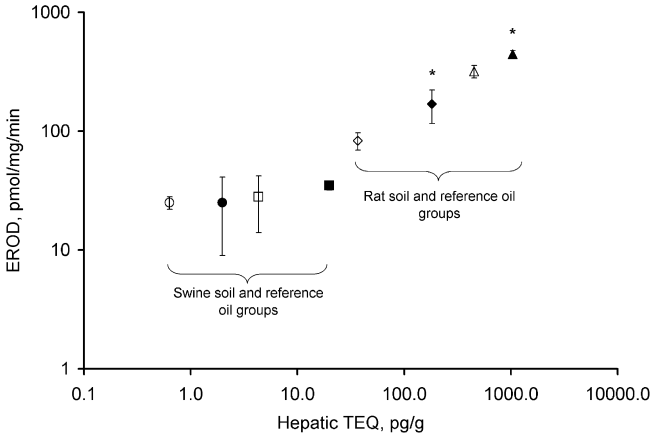

Fig. 1. EROD activity (mean ± 1 SD) as a function of hepatic TEQ (WHO_{2005}, five target congeners) for swine and rats by experimental group, pilot study. Open symbols, soil-administered groups; filled symbols, reference oil-administered groups.○: Swine, urban soil and reference; □: Swine, floodplain soil and reference; ◇: Rat, urban soil and reference; Δ: Rat, floodplain soil and reference. ∗: indicates statistically significant difference compared to paired soil group.

absorbed doses from corn oil compared to soil administration, both of which contributed to higher hepatic TEQ concentrations in the reference oil groups compared to the paired soil/feed groups (Fig. 1). No significant differences in hepatic EROD or MROD activity were observed among the swine treatment groups, even though hepatic TEQ was significantly different among dose groups.

Several congeners were non-detectable in some of the liver and adipose tissue samples of the swine given the urban soil (Table 4). This is attributed to the lower concentrations of dioxins present in the urban soil compared to the floodplain soil, the limited amount of soil administered via dough balls, and rapid growth resulting in dilution of tissue concentrations over the 30 days of administration. The corn oil-treated swine, on the other hand, had more

Table 6
Estimated relative and absolute bioavailability by congener and weighted by TEQ for two soils in rats and swine

Congener	% of Soil TEQ	Mean estimated relative bioavailability[a]			Estimated absolute bioavailability[b]			Estimated bioaccessibility (*in vitro* assay)[c]
			Swine			Swine		
		Rat (SD)	1/2 DL[d] (SD)	DL[d] (SD)	Rat	1/2 DL[d]	DL[d]	
Urban soil								
2,3,7,8-TCDD	49.7	0.35 (0.04)	0.18(0.08)	0.22 (0.04)	0.28	0.15	0.18	0.17
1,2,3,7,8-PeCDD	25.4	0.40 (0.03)	0.24(0.10)	0.34 (0.06)	0.32	0.19	0.27	0.16
1,2,3,6,7,8-HxCDD	2.8	0.47 (0.03)	0.38 (0.21)	0.45(0.14)	0.37	0.31	0.36	0.18
1,2,3,4,6,7,8-HpCDD	4.4	0.34 (0.02)	0.55(0.13)	0.55(0.13)	0.27	0.44	0.44	0.26
2,3,4,7,8-PeCDF	4.1	0.40 (0.02)	0.32 (0.09)	0.41 (0.13)	0.32	0.25	0.33	0.18
	TEQ-Weighted:	0.37	0.23	0.29	0.30	0.19	0.23	0.17
Floodplain soil								
2,3,7,8-TCDF	33.1	0.89 (0.12)	0.22 (0.04)	0.23 (0.04)	0.72	0.18	0.18	–
1,2,3,7,8-PeCDF	5	0.58 (0.05)	0.30 (0.13)	0.34 (0.08)	0.46	0.24	0.27	–
2,3,4,7,8-PeCDF	40.7	0.52 (0.03)	0.27 (0.02)	0.27 (0.02)	0.42	0.22	0.22	–
1,2,3,4,7,8-HxCDF	11.1	0.57 (0.03)	0.35 (0.02)	0.35 (0.02)	0.46	0.28	0.28	–
1,2,3,6,7,8-HxCDF	2.5	0.56 (0.04)	0.37 (0.02)	0.37 (0.02)	0.45	0.30	0.30	–
	TEQ-Weighted:	0.66	0.27	0.27	0.54	0.22	0.22	

[a] RBA estimates for soil compared to corn oil reference material based on liver plus adipose tissue measurements.
[b] Assuming an absolute availability from corn oil of 80%.
[c] As estimated for the same urban soil sample in an *in vitro* assay by Ruby et al. (2002).
[d] Non-Detects (ND) were treated as 1/4 the detection limit or as the detection limit.

reliably detectable congener concentrations presumably due to the greater bioavailability from the corn oil.

Table 6 presents the RBA and absolute bioavailability estimates for both soils in rats and juvenile swine. The RBA estimates were converted to absolute bioavailability estimates by assuming 80% absorption from corn oil (Rose et al., 1976; Hurst et al., 2000; Diliberto et al., 2001). Also included in this table are the *in vitro* bioaccessibility results for the same urban soil sample as reported in Ruby et al. (2002). The TEQ-weighted urban soil RBA estimates for the rat and swine based on the spiked corn oil comparisons were 37% and 23%, respectively (Table 6). These results show small congener-specific differences in RBA without any apparent pattern. With one exception, the estimates for RBA were lower in swine than in rats for congeners in the urban soil. The estimates of absolute bioavailability of the urban soil sample were similar to the estimates of bioaccessibility derived for this same soil using the *in vitro* assay (Ruby et al., 2000).

For the floodplain soils, rat and swine TEQ-weighted RBA estimates were 66% and 27%, respectively. TCDF showed an apparent RBA approaching 90% from the floodplain soil in rats. The rat RBA estimates for furans were nearly 2-fold greater than those estimated for dioxins from the urban soil sample and 2-fold or more greater than the RBAs estimated in swine, which did not vary substantially across soils.

The differential EROD activity between rat reference oil groups and corresponding soil groups may have resulted in faster hepatic elimination of TCDF and possibly some of the other furans (Brewster and Birnbaum, 1987; McKinley et al., 1993; Tai et al., 1993). Greater EROD-mediated hepatic clearance in the reference corn oil groups compared to the paired soil/feed groups would violate the assumptions used to calculate RBA. The unexpectedly high RBA estimates (i.e., the approximate 90% RBA for TCDF) observed in the pilot study could be due to CYP1A1-mediated clearance differences between treatment groups. Because no differential EROD or MROD induction among dose groups was observed for swine, it was hypothesized that a second experiment, in which graded doses of congeners in the reference corn oil gavage solutions were administered to rats at fractions of the estimated administered dose in soil, might reduce or eliminate the apparent differences between rats and swine in RBA estimates for the PCDF-dominated floodplain soil. Therefore, a follow-up study administering the floodplain soil in rats controlling for EROD induction was conducted.

3.2. Follow-up study

Rats gained comparable weight among the various treatment groups. The estimated daily dosages for the soil/feed, 0.2X, 0.5X and 0.8X corn oil groups were 1.5, 0.30, 0.74 and 1.21 ng/kg/day, respectively, on a TEQ basis (Table 7). Table 8 presents the hepatic TEQ, and EROD and MROD activities for all dose groups. The retention and distribution of congeners among liver and adipose tissues for the 3 graded reference oil groups and the soil/feed group are shown in Fig. 2. Liver and adipose TEQ concentrations in animals ingesting clean feed or corn oil were low and consistent with our previous study (Ruby et al., 2004). EROD activities in the soil-feed and the 0.5X and the 0.8X corn oil groups were statistically significantly greater than the EROD activity in the control groups; EROD activity in the soil/feed group was statistically elevated over all other groups except the 0.8X reference corn oil group. No statistically significant differences among groups in

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Table 7
Average daily administered doses of target congeners in floodplain soil or reference corn oil solutions to rats in the follow-up study, pg/kg bw/d

Congener	Soil		Reference corn oil solutions					
			0.2X		0.5X		0.8X	
	Mean ± SD	TEQ	Mean ± SD	TEQ	Mean ± SD	TEQ	Mean ± SD	TEQ
2,3,7,8-TCDF	5200 ± 170	520	959 ± 38	96	2360 ± 44	236	3830 ± 78	383
1,2,3,7,8-PeCDF	3240 ± 110	97	662 ± 26	20	1590 ± 30	48	2650 ± 54	80
2,3,4,7,8-PeCDF	2770 ± 91	831	594 ± 23	178	1480 ± 28	444	2400 ± 49	720
1,2,3,4,7,8-HxCDF	2190 ± 72	22	436 ± 17	4	1080 ± 20	11	1760 ± 36	18
1,2,3,6,7,8-HxCDF	537 ± 18	5	129 ± 5	1	313 ± 6	3	509 ± 10	5
Total TEQ, pg/kg bw/d		1475		300		742		1205

Soil was administered mixed in feed; reference corn oil doses were administered via daily gavage. $n = 5$ per group.

Table 8
Hepatic TEQ (WHO_{2005}, five target congeners), EROD, and MROD activity by dosing group for follow-up study of floodplain soil in rats

Dose group	Hepatic TEQ, pg/g	Liver microsomal activity, pmol/mg/min	
		EROD	MROD
Control feed	1.7[a]	33 ± 6	26 ± 2
Control feed + clean corn oil	1.9[a]	41 ± 7	27 ± 2
Reference corn oil, 0.2X	143 ± 11*	54 ± 8*	33 ± 4
Reference corn oil, 0.5X	329 ± 28*	81 ± 18*	35 ± 10
Reference corn oil, 0.8X	635 ± 44	106 ± 17	42 ± 7
Soil/feed mixture, dose X	648 ± 41	110 ± 24	35 ± 6

Mean ± SD.
[a] Tissue concentrations determined on a composite of tissues taken from 5 animals.
* Test for significant difference between oil reference groups and soil/feed mixture group using ANOVA followed by Dunnett's multiple comparison test at an overall 95 percent confidence level (overall $\alpha = 0.05$).

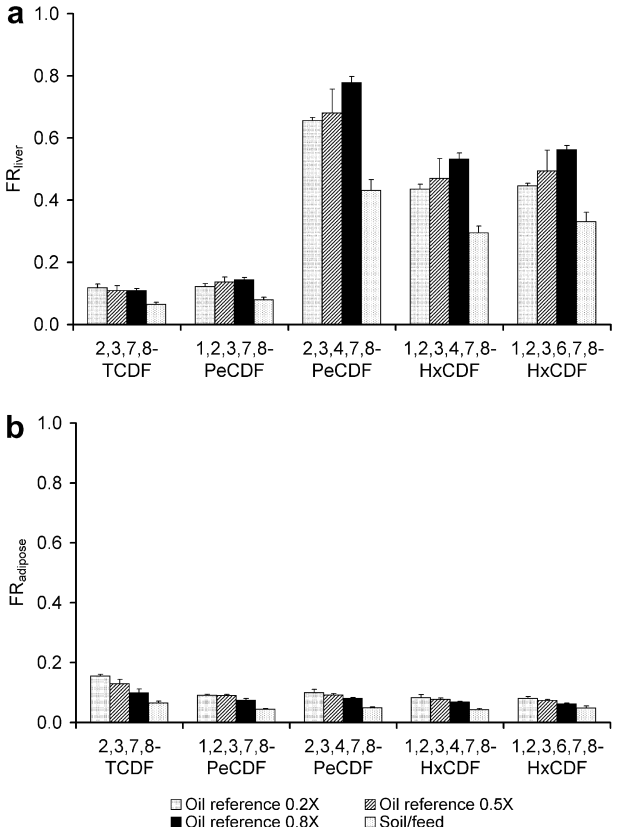

Fig. 2. Fraction of administered dose of target congeners in follow-up study retained in (a) liver, and (b) adipose tissue.

MROD activity were observed. The reductions in corn oil-administered doses to 80% of the nominal soil dose resulted in hepatic TEQ concentrations and EROD activities that were comparable to those in the soil/feed group.

The relationship between fraction of administered dose retained in liver and adipose tissue and hepatic TEQ, EROD activity, and MROD activity among the three graded reference corn oil groups was assessed using multivariate linear regression (Table 9). Statistically significant effects of hepatic TEQ or hepatic EROD activity were observed for several congeners. For 2,3,4,7,8-PeCDF and the two HxCDF congeners, a small but significant increase in fraction of administered dose retained was observed as hepatic TEQ increased. This might be accounted for by the slight increase in MROD activity (which was not statistically significant) since these congeners are preferentially sequestered in the liver by CYP1A2. Protein levels of CYP1A2 were not available to provide further information on this observation. For TCDF, the fraction of administered dose retained was significantly inversely related to hepatic EROD activity, resulting in a significant decrease in retained fraction over the increasing corn oil gavage dose groups (Fig. 3). The inverse relationship between retained fractional dose of TCDF and EROD increase probably reflects greater metabolic clearance of TCDF with increasing hepatic EROD activity, consistent with previous data on the inducibility of metabolism of TCDF (Tai et al., 1993; McKinley et al. 1993).

RBA estimates for the furan congeners in the follow-up study are shown in Table 10. The 4-PeCDF congener RBA estimates ranged from around 56% to 62% based on the 0.5X and 0.8X corn oil dosages. The estimated of RBA for TCDF from soil declined from approximately 90% in the pilot study to around 54% to 62% once EROD activity was matched in the reference oil group (Fig. 4). However, the other four furan congener RBA estimates were not significantly altered when EROD induction was controlled for suggesting that these congeners do not experience signifi-

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Table 9
Mulitvariate least squares regression analysis of the relationship between fraction of administered dose retained and hepatic TEQ (WHO_{2005}, five target congeners), EROD, and MROD activity for five target congeners (floodplain soil pattern) administered in corn oil at three graded doses (see Table 5 for dose levels)

	2,3,7,8-TCDF		1,2,3,7,8-PeCDF		2,3,4,7,8-PeCDF		1,2,3,4,7,8-HxCDF		1,2,3,6,7,8-HxCDF	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Intercept	3.1E−01	<0.0001	2.4E−01	<0.0001	7.6E−01	<0.0001	5.2E−01	<0.0001	5.4E−01	<0.0001
Hepatic TEQ, per pg/g	−2.9E−05	NS	5.1E-05	NS	3.5E−04	<0.01	2.7E−04	<0.01	3.3E-04	<0.01
EROD, per pmol/mg/min	−1.1E−03	<0.01	−4.9E−04	NS	−1.6E−03	NS	−1.2E−03	NS	−1.4E−03	NS
MROD, per pmol/mg/min	7.7E−04	NS	3.0E−05	NS	1.1E−03	NS	8.8E−04	NS	6.2E−04	NS
p for model:[a]	<0.0001		NS		<0.05		<0.05		<0.01	

Analysis was conducted using individual hepatic TEQ (WHO_{2006}, sum of five target congeners), EROD, and MROD activities for each animals across the three reference corn oil groups ($n = 5$ per group, 15 total). Values reported are regression coefficients, β, change in fraction of administered dose retained for each congener per unit change in hepatic TEQ or enzyme activity, and *p*-values for significance.

[a] *F*-test for model significance.

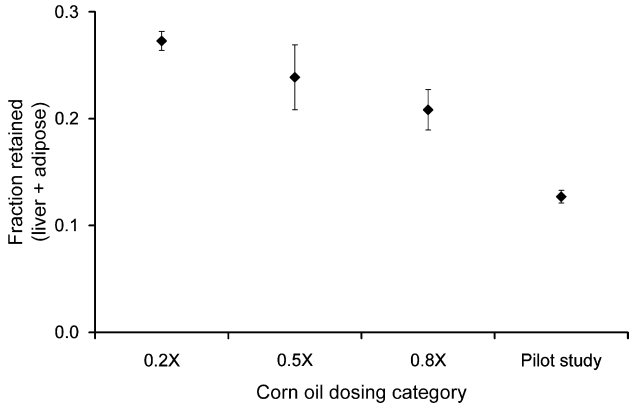

Fig. 3. Fraction of administered dose of 2,3,7,8-TCDF retained in liver and adipose tissue after 30 days of administration via corn oil gavage, combined results from pilot and follow-up study, mean ± 1 SD.

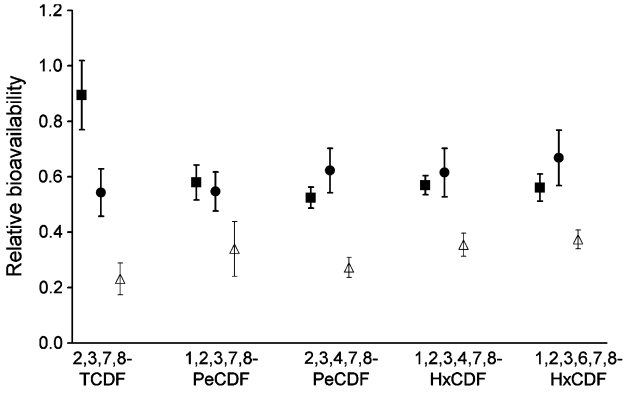

Fig. 4. Estimated relative bioavailability of five target congeners in rats and swine, soil administration compared to corn oil vehicle administration. Results presented from the rat pilot study (filled squares) and from the rat follow-up study with relative bioavailability compared to the 0.5X corn oil gavage group (filled circles) and from the swine pilot study (open triangles, non-detects replaced with the limit of detection). The estimate of RBA for TCDF in the follow-up study was substantially lower after hepatic EROD activity was matched between soil/feed and corn oil gavage groups. The estimates of RBA for swine were consistently less than half of the estimates derived from rats.

cant metabolic clearance at these modestly increased hepatic EROD activities, or they are not active substrates for CYP1A1. Fig. 4 also shows the RBA estimates for swine for the floodplain soil (calculated using the limit of detection for congeners that were non-detects). While matching the rat reference oil group on hepatic TEQ and EROD activity did reduce the outlying estimate of RBA for TCDF, the marked species differences in RBA estimates for the same soil persisted.

4. Discussion

This study provides RBA estimates for two soils with long-term weathered residue patterns resulting from entirely different processes (incineration deposition vs. dis-

Table 10
Mean relative and absolute bioavailability estimates for the floodplain soil in rats from the follow-up study

Congener	Contribution to soil TEQ	Relative bioavailability[a]		Absolute bioavailability[b]	
		0.8X reference	0.5X reference	0.8X reference	0.5X reference
2,3,7,8-TCDF	33.1%	0.62	0.54	0.50	0.43
1,2,3,7,8-PeCDF	5.0%	0.57	0.55	0.46	0.44
2,3,4,7,8-PeCDF	40.7%	0.56	0.62	0.45	0.49
1,2,3,4,7,8-HxCDF	11.1%	0.56	0.62	0.45	0.49
1,2,3,6,7,8-HxCDF	2.5%	0.61	0.67	0.49	0.53
TEQ-Weighted:		0.58	0.58	0.46	0.46

[a] RBA estimates for soil compared to corn oil reference material based on liver plus adipose tissue measurements from either the 0.8X or the 0.5X dose groups.

[b] Assuming 80% absolute bioavailability from corn oil (Diliberto et al. 2001; Hurst et al. 2000).

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charge of residue from a chloralkali process). PCDD/F concentrations in these soils were two to three orders of magnitude lower than in soils assessed in previous bioavailability studies. Most previous studies in this area have focused almost entirely on TCDD; in this study, soils comprised of a mix of PCDD/F congeners, likely to be encountered in soils from other locations, was assessed. Finally, this study presents the first comparison of oral bioavailability of PCDD/Fs between two experimental models. Three key findings from this study are relevant for assessing oral bioavailability of PCDD/Fs for risk assessment and in future studies:

1. Differential enzyme activity among experimental groups can influence clearance rates for at least one congener, even over relatively narrow dose ranges. If bioavailability is being assessed based on comparison of retained fraction of administered dose between the soil and a reference vehicle administration of compounds (as, for example, in Wittsiepe et al., 2007), such differential enzyme activity can substantially impact the estimate of RBA for selected compounds. CYP1A1 is responsible for the metabolism of 2,3,7,8-TCDF in rats (Tai et al., 1993; McKinley et al., 1993; Olson et al., 1994). 4-PeCDF can also induce its own metabolism due to CYP1A1 induction (Brewster and Birnbaum, 1987). Other congeners including TCDD and 1-PeCDF, show does-dependent reduction in bioaccumulation although the specific metabolic pathways have yet to be elucidated (DeVito et al., 1998; Diliberto et al., 2001; Jackson et al., 1998). On the other hand, induction of CYP1A2 protein results in dose-dependent sequestration of certain congeners, especially 4-PeCDF which is the most significant TEQ-contributing congener in the floodplain soil (Diliberto et al., 1999; NTP, 2006). Thus, both EROD and MROD activities govern the potential hepatic bioaccumulation of certain dioxins and furans and this bioaccumulation is the critical mass-balance factor for determination of RBA in this study.

2. *Hepatic EROD induction itself cannot be used as a surrogate for estimating bioavailability.* For the mixture of congeners tested in the follow-up study, hepatic EROD activity in the soil/feed group was similar to that in the reference oil group given 80% of the same dose; however, on a mass-balance basis, the RBA of the soil compared to the reference oil vehicle was approximately 60% rather than 80%. Previous studies of soils with contamination patterns dominated by TCDD have relied upon hepatic EROD activity as a direct surrogate for bioavailability (for example, Lucier et al., 1986) but under the conditions of this study of soils with a mixture of congeners, this approach would substantially overestimate the bioavailability of the tested soil.

3. The results from this study suggest that the oral bioavailability of PCDD/Fs from soil is species-dependent. Clear differences in estimated RBA were observed between rats and swine for both soils. There were some differences in protocol. Soils were administered to rats in a homogeneous mixture with feed. On the other hand, soil was administered to swine in small bolus doses administered wrapped in dough that is more comparable to incidental soil ingestion that occurs via hand-to-mouth activity in adults and children. It is possible that the mechanical mixing of soils with the relatively lipid-rich feed to insure soil-feed homogeneity and subsequent storage time before administration to rats resulted in some mobilization of the PCDD/F congeners from the relatively lipid-poor soil matrix into the feed lipids. Laboratory experiments that will include evaluation of the fast-desorption kinetics as a function of exposure to lipids present in rat chow are underway to investigate this possibility. However, the overall parallel design of the study generally suggests that such differences may not be responsible for the observed species differences.

Another possibility is that the absolute bioavailability of dioxins and furans in corn oil differs between the two species. If absolute bioavailability from corn oil is greater in swine, relative bioavailability estimates would appear lower for the same absolute bioavailability. However, the degree of difference observed for the floodplain soil, greater than 2-fold, is higher than could be accounted for even if absolute bioavailability from corn oil was 100% in swine.

Species differences in gastrointestinal absorption of metals have been clearly identified; for studies of metals bioavailability the swine is clearly preferred over the rat (Weis and Lavelle, 1991; Casteel et al., 2006; US EPA, 2006). This preference stems from physiological differences between rats and humans in mechanisms governing metals uptake and from the substantial physiological similarities between juvenile swine and children (Glauser, 1966; Shulman et al., 1988; Groner et al., 1990; Krishnan et al., 1994; Casteel et al., 1998; Swindle and Smith, 1998). However, this is the first demonstration that oral bioavailability of persistent organic compounds such as PCDD/Fs may also be species dependent. This observation raises two important questions: What are the mechanisms responsible for the observed differences? And, if there are species differences, which is the more relevant model for humans with respect to oral bioavailability of these compounds? If the species differences in oral bioavailability observed in this study reflect true differences, use of a juvenile swine model (preferred in biomedical research as a model for human physiology) or an *in vitro* bioaccessibility test designed to mimic human digestive tract characteristics (for example, as described by Ruby et al., 2002) would be preferable to reliance on the laboratory rodent model.

One potential criticism of this study was the decision not to use an intravenous route of administration as the reference for estimating absolute bioavailability of the PCDD/Fs in soil. Because of the relatively low TEQ concentrations in the test soils, administration over several weeks was necessary to ensure detectable tissue concentrations. Long term repeated intravenous administration of reference compounds to rodents was deemed impractical, as was any attempt to measure blood concentrations of the intravenously-administered compounds in order to assess

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area under the curve. In addition, a recent study found differences in distribution and retention of other persistent lipophilic halogenated hydrocarbons following intravenous administration compared to other routes of exposure (Qiao and Riviere, 2001). Finally, toxicity criteria for TCDD and related compounds are generally based on studies conducted using an oral route of exposure, and estimates of relative bioavailability compared to an oral vehicle would therefore be directly applicable in risk assessments. Therefore, an oral gavage dose in oil, the absorption of which has been characterized previously in Sprague Dawley rats (Rose et al., 1976), was used as the reference dose for estimating relative and absolute bioavailability.

The urban soil with residues due to incinerator deposition and dominated by PCDDs studied here demonstrated TEQ-weighted RBAs of about 25% and 37% in swine and rats, respectively. The RBA of the PCDF-dominated soil from the floodplain area was similar in swine, about 25%, but was found to be substantially higher in rats, about 58% on a TEQ-weighted basis. Further evaluation and research is underway to determine the impact, if any, of soil characteristics such as grain size, total organic carbon content, or the extent of black carbon on RBA for both soils [Koelmans et al., 2006]. For instance, the floodplain soil may reflect graphite electrode particulate with furan constituents present in the tar-like binder used to bind the graphite particles together. Hence, RBA may be more reflective on the size of these graphite particles and not on native soil characteristics. Overall, the study results presented here provide important information for consideration in design of future studies of bioavailability of PCDD/F and related compounds from soil and the use of these RBA values for deriving theoretical exposure estimates attributed to direct soil contact.

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