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Oral Bioaccessibility of Dioxins/Furans at Low Concentrations (50−350 ppt Toxicity Equivalent) in Soil

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Animal studies have indicated that the oral bioavailability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in environmentally contaminated soil could range from 0.5 to 60%. To estimate the oral bioavailability of TCDD, and the 16 other 2,3,7,8-substituted dioxin/furan congeners, this study used a physiologically based extraction test, designed around the anatomic and physiologic characteristics of the human digestive tract. This test measures the fraction of dioxins/furans in soil that would be solubilized in the gastrointestinal tract (i.e., that would be bioaccessible) and therefore available for absorption. Eight soils from Midland, MI, were evaluated in this study and exhibited TCDD concentrations of 1.7−139 pg/g (ppt) and total TEQ concentrations of 6−340 ppt. Bioaccessibility of dioxins/furans from these soils ranged from 19 to 34% (averaged across the 17 2,3,7,8-substituted dioxin/furan congeners), with an average of 25%. The total organic carbon in these soils was low—ranging from 1 to 4%—particularly for the soil series from which they were collected. Bioaccessibility of individual congeners did not appear to be correlated with degree of chlorination. Even though these dioxin/furan concentrations are much less than studied previously, these results are consistent with those from animal studies at other sites, which have generally yielded values of 20−60% relative bioavailability for TCDD in soil.

Introduction

An understanding of the oral bioavailability of dioxins/furans in soil is important when one is trying to establish the extent to which humans may be at risk from exposure to these compounds in the environment. Like other hydrophobic organic compounds (HOCs), polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs/Fs) bind to certain soil constituents, becoming progressively less available over time. This process of sequestration (often referred to as "weathering") is believed to result in decreased bioavailability of PCDDs/Fs in environmentally contaminated soils (*1*, *2*). During the 1980s, a number of animal studies were conducted using soils from high-profile hazardous waste sites (*3*−*7*). These studies suggested that the relative bioavailability (where relative bioavailabitity is the oral absorption of TCDD from soil relative to its absorption from a readily available dosing vehicle, such as corn oil) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in soil could vary by 2 orders of magnitude (from 0.5 to 60%), but was generally in the range of 20−60% (Table 1). Since that time, the interest in performing animal studies has waned, most likely due to the costs and technical limitations inherent to such studies.

All of the in vivo studies of TCDD in soil were performed in rodents or lagomorphs (rats, guinea pigs, and rabbits; Table 1), and the fact that these animals have significant anatomic and physiologic differences from humans (*8*) limits their applicability for evaluating human exposures. One alternative to in vivo studies in rodents or lagomorphs is to estimate the oral bioavailability of PCDDs/Fs in humans using a physiologically based extraction test to determine the fraction of PCDDs/Fs that would be solubilized in the human gastrointestinal tract and, therefore, would be available for absorption (i.e., the fraction that is bioaccessible—"bioaccessible" is used herein to describe the fraction of a dioxin/furan congener in soil that is solubilized in the gastrointestinal tract, and is therefore available for absorption.). This approach relies on the observation that contaminants in soil must be liberated in the gastrointestinal tract in order to be absorbed (i.e., they must enter the fluid phase); direct absorption of particulate material does not appear to be an important mechanism for the bioavailability of contaminants from soil (*9*). This is consistent with the work of Diliberto et al. (*10*), who observed that the fraction of TCDD liberated from soil controls the extent of oral bioavailability in rats. Thus, measuring the fraction of PCDDs/Fs that can be liberated from soil under gastrointestinal conditions can provide an estimate of the bioavailable fraction. This approach is becoming increasingly popular, and in vitro assays for estimating oral bioavailability have been developed for polycyclic aromatic hydrocarbons (PAHs, refs *11* and *12*), polychlorinated biphenyls (PCBs, refs *11* and *13*) and PCDDs/Fs (*14*) in soil.

Because an in vitro extraction test can be designed around the anatomic and physiologic characteristics of humans, it is potentially the most accurate and practical approach to estimating bioavailability in humans, short of actual human studies. However, because an in vitro approach does not measure absorption into actual tissues, it will be reliable only if liberation from soil in the gastrointestinal tract is the limiting step in the oral bioavailability process for PCDDs/Fs in soil.

An additional benefit of the in vitro approach is that it can be used to examine dioxin/furan concentrations in soil that are representative of the low end of environmental exposures. The in vivo studies cited above used soils containing 2−2,280 μg/kg TCDD (Table 1), whereas this study used soils containing 1.7−139 ng/kg TCDD (data not shown), which is ∼3 orders of magnitude lower than the concentrations used in the animal studies. Given that regulatory soil screening levels for TCDD range from 4 to 40 ng/kg, the soil concentrations used in this study are more relevant for human exposures to TCDD in soil. (The U.S. EPA Region III Risk-Based Concentrations (RBCs) for residential and industrial exposure scenarios are 4.3 and 38 ng/kg TCDD, respectively. The Region IX Preliminary Remediation Goals (PRGs) for

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TABLE 1. Bioavailability Studies of 2,3,7,8-TCDD in Environmentally Contaminated Soils[a]

study	test species	site	particle size (μm)	TCDD concn in soil (μg/kg)	rel bioavail[b,c] (%)
Lucier et al. 1986 (*5*)	Spague-Dawley rats	Minker Stout site, MO	<250	880	34 (22, 45)[d]
McConnell et al. 1984 (*4*)	guinea pigs	Times Beach, MO	<250	770	16[e]
		Minker Stout site, MO		880	26[e]
Bonaccorsi et al. 1984 (*3*)	albino rabbits	Seveso, Italy	30−74	81	32[f]
Shu et al. 1988 (*7*)	Sprague−Dawley rats	Times Beach, MO	<420	1.9, 28.6, 723[e]	60 (54−63)[g]
Umbreit et al. 1986 (*6*)	guinea pigs	salvage yard, NJ	n/a[g]	320	24
		2,4,5-T manufacturing site, NJ	n/a[g]	2,280	0.5

[a] All bioavailability estimates based on TCDD concentrations in liver of test animals. [b] Relative to TCDD dissolved in corn oil (*4, 5, 7*); TCDD dissolved in alcohol−water (1:1) (*3*); or clean soil "contaminated" with TCDD in corn oil/acetone (9:1) immediately prior to dosing (*6*).[c] Range of values observed for different soils given in parentheses. [d] Calculated from the results for the 1 and 5 μg/kg TCDD dose groups in Lucier et al. (*5*). The low-dose group (1 μg/kg) produced the 22% relative bioavailability estimate. [e] Calculated from the 3 μg/kg dose groups (soil and oil dosed) in McConnell et al. (*4*). [f] Calculated from the results for the 80 ng TCDD/day Seveso soil and alcohol-water dose groups. [g] Three different soils were studied, resulting in an average relative bioavailability of 61%. n/a, not available.

these exposure scenarios are 3.9 and 27 ng/kg TCDD, respectively.) Finally, the in vitro system can be used to evaluate all 17 of the 2,3,7,8-substituted dioxin/furan congeners—work that has not been attempted in animals.

Oral Bioavailability of PCDDs/Fs. In Sprague−Dawley rats, 84% of a single oral dose of TCDD in corn oil (1.0 μg/kg) was absorbed (*15*). When dosing was continued for 7 weeks, absorption of TCDD was 89 and 83% for doses of 0.1 and 1.0 μg/kg per day, respectively. At a greater dose (e.g., 50 μg/kg) of TCDD in corn oil, oral absorption decreased to 70% in Sprague−Dawley rats (*16*). In addition, Diliberto et al. (*10*) dosed Fischer 344 rats with 32 μg/kg TCDD in an Emulphor/ethanol/water mixture and observed 88% oral absorption. These results indicate that TCDD dosed to rats in a lipophilic vehicle will be absorbed to the extent of 70−88%. In a human volunteer, a single dose of TCDD in corn oil (1.1 ng/kg) was almost completely absorbed (>87%) (*17*). These results suggest that oral absorption of TCDD, when dosed in a corn oil matrix, is similar in rats and humans and generally exceeds 80%.

The oral bioavailability of TCDD decreases when it is associated with a soil matrix (as opposed to an oil matrix), and this effect becomes more pronounced over time. Poiger and Schlatter (*18*) observed that the oral bioavailability of TCDD in rats was inversely related to the length of time that the TCDD had been in contact with soil. Furthermore, when TCDD was mixed with an aqueous suspension of activated carbon, absorption in the rats was almost totally eliminated (200-fold decrease in bioavailability from the TCDD in contact with soil for 8 days).

The oral bioavailability of TCDD may be reduced further when it is present in site soils, as opposed to having been freshly added to soil in the laboratory. Studies using different animal models (Sprague−Dawley rats, albino rabbits, guinea pigs) have measured the oral bioavailability of TCDD from environmentally contaminated soils, based on accumulation of TCDD in the livers of the test animals (Table 1). These studies measured the absorption of TCDD from soil relative to its absorption from a readily available form, such as TCDD dissolved in corn oil, to derive a relative bioavailability value for TCDD in soil. The nine soils dosed during these experiments produced relative bioavailability estimates ranging from 0.5 to 63%, with an average of ∼35% (*3*−*7*). Within this data set, the 0.5% relative bioavailability observed by Umbreit et al. (*6*) for soil from a 2,4,5-T manufacturing site appears to be an outlier, because all of the other values range from 16 to 63% (Table 1). (Note that the critical toxicity study upon which the cancer slope factor for TCDD is based (*19*) used TCDD in rat chow, which should result in an absorption similar to, though possibly slightly less than, that of TCDD in oil.)

These results indicate that the oral bioavailability of PCDDs/Fs in soil varies; this is believed to depend on a number of factors, such as soil type (composition and chemistry), time of contact between PCDDs/Fs and soil (i.e., extent of aging), concentration of PCDDs/Fs in soil, size of the PCDDs/F dose, and the specific congeners present (*20*).

Basis for the In Vitro Test System. The testing protocol used in this study was designed to reflect the conditions and chemistry of the human gastrointestinal tract. Test parameters were selected to maximize the liberation of PCDDs/Fs from soil, while maintaining conditions that could realistically occur within the human gastrointestinal tract. The in vitro test used in this study was based on one developed for metals in soil (*21*, *22*) that was modified based on the design of in vitro test methods for HOCs in soil (*11*−*14*). Table 2 summarizes the key parameters of these existing in vitro test systems, along with the test used in this study. Of the previous studies, that of Wittsiepe et al. (*14*) is the most relevant, because the authors studied the bioaccessibility of PCDDs/Fs from two samples of "red slag" from a copper production process. Average bioaccessibility (i.e., average value for all dioxin/furan congeners analyzed in the study), based on the fraction of total toxicity equivalent (TEQ) extracted from the 100−200-μm size fraction, ranged from 11 to 52%. The greater bioaccessibility values were associated with tests in which a lipid source, either whole milk powder or grape seed oil, was added to the extraction. This is consistent with earlier research on PCBs, which demonstrated that both bile salts and a lipid source should be present to effectively liberate PCBs from soil (*11*, *13*).

The <250-μm size fraction of the test soils was used for this study, because this is the fraction of soil that is most likely to adhere to human hands and become ingested during hand-to-mouth activity (*23*). As a result, this size fraction has become the standard for use in oral bioavailability studies to estimate human exposures from incidental soil ingestion (*24*−*26*).

Although most of the chemistry that would liberate PCDDs/Fs from soil is believed to occur in the small intestine (i.e., partitioning of PCDDs/Fs into bile salt micelles, as discussed below), it is possible that processes occurring in the gastric environment could contribute to the release of PCDDs/Fs from soil. Therefore, an acidic stomach phase (pH 1.5 in HCl, to be representative of fasting conditions (*27*), which should most effectively liberate PCDDs/Fs from soil) was used as the first phase of the in vitro extraction. This was followed by incubation under conditions representative of the small intestine (neutral pH with pancreatic enzymes and bile salts present). Extraction times in the gastric (1 h) and small-intestinal (4 h) phases were representative

TABLE 2. Comparison of In Vitro Extraction Tests for HOCs in Soil

test parameter[a]	Hack and Selenka 1996 (*11*)	Holman 2000 (*12*)	Oomen et al. 2000 (*13*)	Wittsiepe et al. 2001 (*14*)		this study
compounds studied	PCBs, PAHs	PAHs	PCBs, lindane	PCDDs/Fs		PCDDs/Fs
soil size fraction	not indicated	ground to <1 mm	not indicated	<100 μm and 100−200 μm		<250 μm
				test A	test B	
soil/solution ratio	1:120	1:50	1:65	1:60	1:120	1:100
gastric pH	2.0	no gastric phase	1.0	2.0	2.0	1.5
gastric enzymes/ other substances	pepsin/ NaCl, mucin, whole milk powder	no gastric phase	pepsin/ BSA, mucin, urea	pepsin/ NaCl, BSA, mucin, glucose, urea, whole milk powder	pepsin/ mucin, whole milk powder, or grape seed oil	pepsin/ glycine, NaCl, BSA, mucin, oleic acid
gastric time (h)	2	not applicable	2	3	2	1
small-intestinal pH	7.0	6.5	5.5	7.5	7.0	7.2
small-intestinal enzymes/other substances	trypsin, pancreatin/ bile	bile salt mixture, lipid mixture	pancreatin/ bile	pancreatin/ bile	pancreatin, trypsin/ bile	pancreatin/ bile
small-intestinal time (h)	6	4	2	3	6	4

[a] All tests performed at 37 °C with gentle mixing.

of stomach residence and small-intestinal transit times, respectively (*28*, *29*).

In the human gastrointestinal system, ingested lipids (such as the triglycerides, which make up 95% of the human lipid diet (*30*)) are hydrolyzed into absorbable forms (fatty acids and monoglycerols) by gastric and pancreatic lipases. In the small intestine, these fatty acids combine with bile salts to form mixed micelles, which consist of a core of the hydrophobic lipids surrounded by a shell of lipoproteins (i.e., bile salts). These bile salt micelles can traverse the mucine layer adjacent to the intestinal wall and be absorbed across the intestinal epitheleum. This is the process by which ingested lipids are absorbed by humans. It is believed that these bile salt micelles in the small intestine provide a lipid sink into which HOCs, such as PCDDs/Fs, can partition (i.e., they are transferred from soil directly into the micelle) and that the HOCs are then absorbed across the intestinal mucosa along with the micelle (*11*−*13*, *31*). For this reason, bioaccessibility tests for HOCs have generally included some form of lipid material along with bile salts. Various researchers have used different lipid sources, including powdered whole milk (*11*, *14*); a mixture of oleic acid, monoolein, diolein, and lecithin (*12*); oleic acid alone (ref *32*; this study); and grape seed oil (*14*), in combination with bile salts to provide for the presence of bile salt micelles.

A representative protein was also added to the in vitro extraction test, because some HOCs (PCBs and PAHs in particular) are believed to partition into the protein phase during simulated human digestion (*11*, *13*). It is prudent to assume that PCDDs/Fs might behave in a similar manner, so bovine serum albumin (BSA) was added as a representative protein in the in vitro extraction fluid. In addition, mucin (a viscous mixture of glycoproteins and enzymes present in the mammalian stomach and intestines) was added to the extraction test, because researchers have observed that the presence of mucin increases the fraction of PAHs and PCBs that is liberated from soil (*11*).

Materials and Methods

The test soils used in this study contained PCDDs/Fs that originated from historical aerial releases by manufacturing and waste combustion processes, rather than from spills of PCDD-contaminated materials directly to soils. The aerial releases occurred primarily prior to 1980, while the test soils were collected in 2001. Eleven soil samples were collected from Midland, MI, for this study, each of which was a composite of 20 discrete soil cores. Eight of the samples were collected from a depth of 0−3 in. and three from 0 to 1 in. (Table 3). The latter three were collected from the same locations as three of the 0−3-in. samples, and their sample numbers indicate this by the addition of "-01" to the sample numbers of the 0−3-in. samples. For example, sample C27-01 is the 0−1-in. sample collected at the same location as sample C27 (0−3 in.).

TABLE 3. Soil Parameters and Testing Matrix for PCDD/F Bioaccessibility Study

sample	soil depth (in.)	pH (su)	TOC (%)	analysis of PCDDs/Fs in pre-extraction soil	post-extraction soil[a]	post-extraction fluid
C01	0−3	7.9	0.91	yes	no	yes
C02	0−3	8.0	0.81	yes	no	no
C24	0−3	8.1	0.87	yes	no	yes
C27	0−3	5.8	1.53	yes	yes	yes
C29	0−3	7.9	1.63	yes	no	no
C32	0−3	7.9	1.62	yes	yes	yes
C34	0−3	8.0	1.97	yes	no	no
N08	0−3	[e]	[e]	yes	yes	yes
C27-01	0−1	5.9	3.94	yes	yes	yes
C34-01[b]	0−1	7.6	2.74	yes	yes	yes
N08-01	0−1	[e]	[e]	yes	no	yes
extraction blank[c]	n/a	n/a	n/a	n/a	n/a	yes
82 ppt spike[d]	n/a	n/a	n/a	n/a	n/a	yes

[a] Analysis of soil for PCDDs/Fs performed after extraction procedure. [b] This soil sample was evaluated in triplicate in vitro extraction tests. [c] No soil added to the in vitro test system. [d] Duplicate tests performed on an 82 ppt spike of 2,3,7,8-TCDD in the in vitro test system. [e] Insufficient material was available to perform soil characterization analyses. n/a, not applicable.

Each soil sample was homogenized in a stainless steel mixing bowl, air-dried to constant weight, and sieved to <2 mm to obtain the bulk soil fraction. The <2-mm material was then sieved to obtain <250-μm material (referred to as the "fine" fraction) and 250−2000-μm material (referred to as the "coarse" fraction). The <250-μm fraction of each sample (with the exception of samples N08 and N08-01, for which there was insufficient material) was analyzed for pH, total organic carbon (TOC), and particle size distribution (sand, silt, clay). The <250- and the 250−2000-μm size fractions of each soil were analyzed for PCDD/F congeners

TABLE 4. Relative Mass of Dioxins/Furans in Coarse and Fine Fractions

	fine fraction[a]		coarse fraction[b]		
sample	concn[c] (pg/g)	weight (g)	concn[c] (pg/g)	weight (g)	PCDD/F mass in fine fraction (%)
C01	5.75	732.2	10.5	74.6	84.3
C02	20.3	897.7	30.2	28.9	95.4
C24	24.3	480.5	27.3	377.5	53.1
N08	48.5	60.0	48.0	567.4	9.6
C29	56.7	433.9	34.2	406.1	63.9
C32	64.3	524.4	57.7	427.0	57.8
N08-01	66.4	60.2	60.3	404.7	14.1
C34	91.7	508.5	48.8	358.3	72.7
C27	101	772.8	89.1	74.5	92.2
C34-01	127	388.9	134	214.8	63.2
C27-01	338	345.0	514	156.0	59.2

[a] Fine fraction is <250 μm. [b] Coarse fraction is 250−2000 μm. [c] All concentrations are reported in ppt (pg/g) TEQ.

by Alta Analytical Laboratories, Inc. (Alta) in Sacramento, CA.

The <250-μm fraction of the test soils was also subjected to the in vitro extraction procedure described below (all chemicals from Sigma Chemical Co., unless indicated otherwise).

The extractions were conducted in 1-L amber glass bottles, which were immersed in a water bath to maintain the extraction fluid at 37 °C. Mixing was provided by stainless steel paddle stirrers at a rate of ∼30 rpm.

Four liters of buffered stomach fluid was prepared by adding 60.06 g of glycine (Sigma UltraPure; concentration of 0.2 M) to 4 L of type II deionized (DI) water, and adjusting the pH to 1.5 with concentrated HCl (∼240 mL). To this was added 35.2 g of sodium chloride (concentration of 150 mM), 4.0 g of pepsin (activity of 800−2500 units/mg), 20 g of BSA, and 10 g of mucine (type III, from porcine stomach). The stomach solution (950 mL) was placed in each reaction vessel, and 6 mL of oleic acid (90%; Aldrich Chemical) was added. Ten grams of test soil (<250-μm size fraction) was added, and the resulting mixture was stirred for 1 h (30 rpm) to simulate stomach-phase extraction.

After 1 h, the solution was adjusted to pH 7.2 ± 0.2 using sodium hydroxide (50% w/w, ∼10 mL), after which, 600 mg of porcine pancreatin (activity equivalent to 8× USP specifications) and 4 g of bovine bile (50% bile acids, mixture of free and conjugated acids) were added to each reaction vessel. This solution was stirred (30 rpm) for 4 h to simulate small-intestinal-phase extraction.

After the 4-h extraction, the solids were allowed to settle, and the extraction fluid was decanted into four 250-mL bottles and centrifuged at 3000*g* for 10 min. The supernatant from each test was then combined in a 1-L amber glass bottle, preserved with 0.008% $Na_2S_2O_3$, and shipped to Alta for analysis of PCDDs/Fs.

For the five mass balance tests, the postextraction soil was collected by washing the soil pellets from each of the four 250-mL centrifuge tubes into a single centrifuge tube and repeating the centrifugation step. The supernatant was removed with a pipet, ∼2 g of the postextraction soil was removed for determination of moisture content, and the remaining soil pellet was shipped in the centrifuge tube to Alta for analysis of PCDDs/Fs in the postextraction soil.

Five samples (C27, C32, N08, C27-01, C34-01) were subjected to mass balance testing to establish whether the mass of PCDDs/Fs in the pre-extraction soil could be quantitatively recovered in the extraction fluid and post-extraction soil. Three other soils (C01, C24, N08-01) were subjected to the in vitro test without the mass balance component. Samples C02, C29, and C34 were not used for the extraction-testing portion of this study, because other samples with similar PCDD/F concentrations were selected for in vitro testing (Table 3).

Quality assurance samples included the following: (1) an extraction blank, (2) duplicate extraction spikes (spiked at 81.4 pg/L TCDD), and (3) one soil (C34-01) tested in triplicate in vitro extractions.

Results

Concentrations of PCDDs/Fs found in the 11 soil samples ranged from 5.8 pg/g (ppt) (C01) to 338 ppt TEQ (C27-01) in the <250-μm size fraction, with similar values for the 250−2000-μm size fraction (range of 10.5−514 ppt TEQ; Table 4). All TEQ values were calculated using the toxic equivalency factors (TEFs) of the World Health Organization (*33*). Comparison of the TEQ values for the two size fractions of each sample indicates that they are often nearly the same and always within a factor of 2 of each other. However,

TABLE 5. Bioaccessibility of PCDD/F Congeners in Test Soils

analyte	N08 (%)	C32 (%)	N08-01 (%)	C27 (%)	C34-01[a] (%)	C27-01 (%)	congener mean (%)	std dev (%)
2,3,7,8-TCDD	48	39	20	15	24	17	27	13.3
1,2,3,7,8,-PeCDD	14	35	19	16	25	16	21	7.6
1,2,3,4,7,8-HxCDD	27	35	16	16	27	18	23	7.6
1,2,3,6,7,8-HxCDD	29	34	16	21	27	18	24	7.0
1,2,3,7,8,9-HxCDD	19	31	16	20	28	18	22	6.2
1,2,3,4,6,7,8-HpCDD	47	33	22	23	37	26	32	9.8
OCDD	51	28	17	20	28	20	27	12.7
2,3,7,8-TCDF	20	33	14	17	22	16	20	6.9
1,2,3,7,8-PeCDF	25	32	19	19	23	17	22	5.7
2,3,4,7,8-PeCDF	24	37	14	19	21	18	22	8.2
1,2,3,4,7,8-HxCDF	37	38	19	20	21	19	26	9.2
1,2,3,6,7,8-HxCDF	35	36	17	31	15	16	25	10.0
2,3,4,6,7,8-HxCDF	24	37	18	18	24	17	23	7.3
1,2,3,7,8,9-HxCDF	29	34	22	19	22	16	24	6.6
1,2,3,4,6,7,8-HpCDF	33	40	26	24	33	23	30	6.5
1,2,3,4,7,8,9-HpCDF	44	29	33	17	23	19	27	9.8
OCDF	56	29	43	12	30	32	34	14.6
av bioaccessibility (%)	33	34	21	19	25	19		
total TEQ concn (ppt)	48.5	64.3	66.4	101.0	126.8	337.6		
mass recovery (%)	157	102	na[b]	76	92	68		

[a] Sample analyzed in triplicate. Results averaged prior to presentation in this table. [b] na, not analyzed.

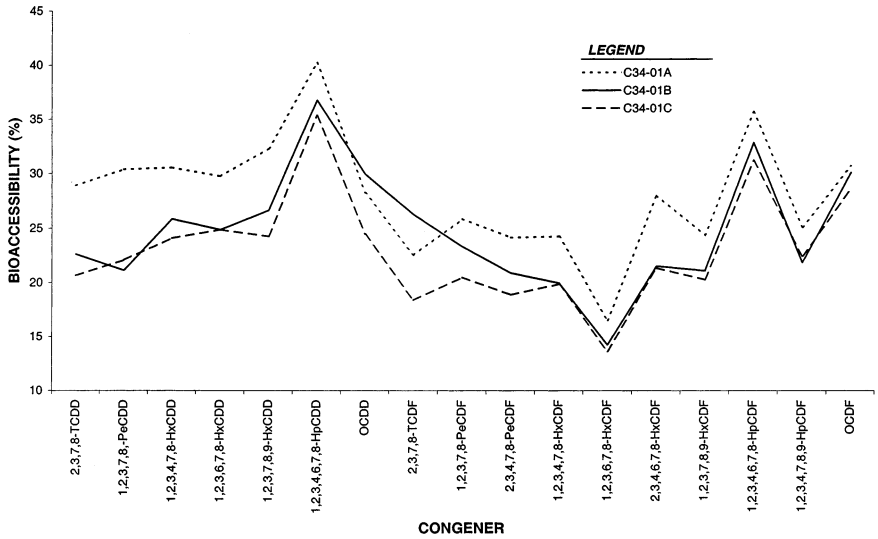

FIGURE 1. Bioaccessibility of PCDDs/Fs from triplicate analysis of sample C34-01.

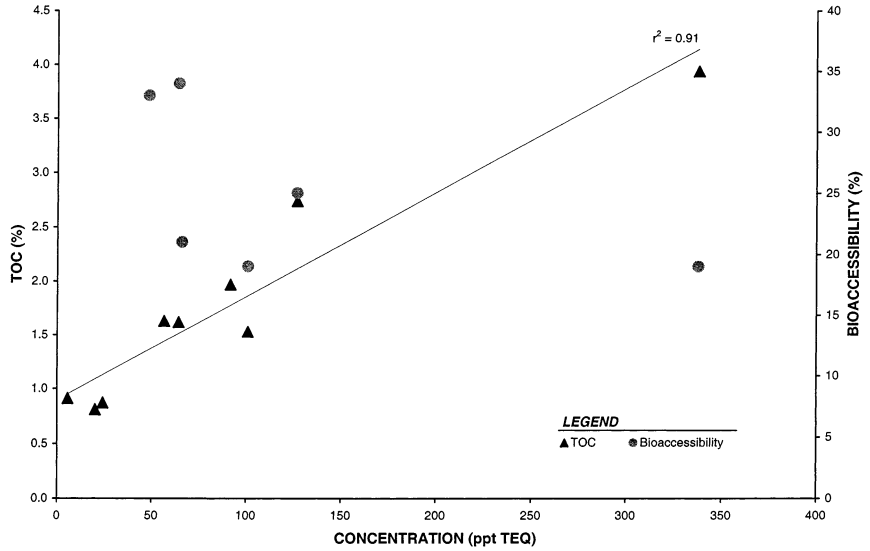

FIGURE 2. Relation between TEQ concentration (ppt) and TOC (%) and average bioaccessibility (%).

comparison of the mass of PCDDs/Fs in the fine versus the coarse fractions (Table 4) indicates that the mass of PCDDs/Fs is predominantly in the fine soil fraction (with the exception of samples N08 and N08-01, which were composed primarily of coarse material).

It is noteworthy that the three collocated sample pairs all demonstrated greater concentrations of PCDDs/Fs in the 0−1-in. samples than in the 0−3-in. samples, for both size fractions (Table 4). The higher concentrations of PCDDs/Fs in the surficial soil samples are consistent with an aerial deposition mechanism and a lack of transport down the soil column; the hydrophobic nature of PCDDs/Fs and their sorption to soil limit their mobility. This general lack of vertical transport in soil was also observed for TCDD in soil at Seveso, Italy (*34*), and at Times Beach (*35*).

The test soils had pH values in the range of 7.6−8.1, with the exception of two soils with lower pH values: 5.8 and 5.9 for samples C27 and C27-01, respectively (Table 3). The more acidic pH values of these two samples are most likely due to their location within the perimeter of some woods. TOC values ranged from 0.81 to 3.94%. The distribution of soil particle sizes was relatively consistent across the nine samples analyzed (data not shown) and indicates that, on average, these soils would classify as a loamy sand (77% sand, 22% silt, 1% clay).

The in vitro quality assurance samples demonstrated that the extraction blank was below detection limits for all compounds except 1,2,3,4,6,7,8-HpCDD (present at 113 pg/L) and that the two extraction spikes (spiked at 81.4 pg/L TCDD) were recovered at 72 and 127%, respectively. The detection limit for TCDD in the in vitro extraction fluid was 2.88 pg/L.

Although samples C01 and C24 were subjected to the in vitro extraction, it was concluded that the concentrations of PCDDs/Fs in these two samples (both below 30 ppt TEQ) were too low to produce reliable results. In the resulting data, the recovery of various individual dioxin/furan congeners ranged from 30 to 300% of that in the test soils (this was not observed with any of the other samples). It was therefore concluded that 30 ppt TEQ was a practical lower limit on the concentration of PCDDs/Fs in soil for this type of testing.

The five samples that were evaluated for mass balance of PCDDs/Fs resulted in mass recoveries ranging from 68 to 157% (average of 99%; Table 5), indicating that PCDDs/Fs in soil can be quantitatively recovered from the in vitro test system. The triplicate analysis of sample C34-01 produced average bioaccessibility values of 23, 25, and 28%, indicating good reproducibility of the in vitro test system, for both the overall sample and individual congeners (Figure 1).

Bioaccessibility results (averaged across all 17 congeners) for the 6 samples tested (excluding the two samples below the lower limit of the assay) ranged from 19 to 34% (Table 5), with an overall average of 25%. The bioaccessibility values for TCDD ranged from 15 to 48%, with an average of 27%. The data generally indicate minimal variability in the congener-specific bioaccessibility values for a given sample. A possible trend of increasing bioaccessibility values as a function of increasing degree of chlorination is apparent for dioxins in samples C27, C34-01, and C27-01 (Table 5). This latter observation is consistent with the results of Wittsiepe et al. (*14*), who observed a trend of increasing bioaccessibility with increasing degree of chlorination (i.e., the more hydrophobic PCDDs/Fs had a greater tendency to enter the fluid phase).

Discussion

The *Soil Survey of Midland County, Michigan* (*36*) indicates that the soils within the City of Midland are primarily of the Wixon-Belleville and the Pipestone-Oakville series. These soils are loamy sand and sandy, respectively, and have pH values ranging from 4.5 to 6.5 and TOC values ranging from 3.4 to 6.9%. Comparison of these soil parameters to those of the soils used in this study indicates that the typical Midland soil is somewhat more acidic than those used in this study, and also contains a greater amount of organic carbon.

Comparison of the soil chemistry data with the PCDD/F concentrations indicates a correlation between TOC and the dioxin/furan concentration (ppt TEQ) in the fine soil fraction ($r^2 = 0.91$, Figure 2) (r^2 decreases to 0.84 if the data point at 338 ppt TEQ is disregarded). This is consistent with the understanding that soil organic matter provides the primary sink for hydrophobic organic contaminants in soil (*1*, *2*). In addition, an inverse relation between dioxin/furan concentration and bioaccessibility may also be present (Figure 2), suggesting that bioaccessibility may decrease as TEQ concentration increases. This could be due to some unknown mechanism by which greater concentrations of dioxins/furans are sequestered more effectively, or it could be due to the greater TOC concentrations that are present in the soils with the higher dioxin/furan concentrations. This latter hypothesis is the more plausible, given that the soil organic matter is known to play an important role in sequestering organic compounds (*2*) and reducing their bioavailability to soil invertebrates (*1*).

This study yielded a mean bioaccessibility of 25% for the 17 2,3,7,8-substituted dioxin/furan congeners in the soils tested, which contained total PCDD/F concentrations spanning almost 1 order of magnitude (48.5−337.6 ppt TEQ). Because TCDD dosed to a human in corn oil was almost completely absorbed (*17*), it is reasonable to assume that TCDD liberated in the lipophilic environment of the in vitro extraction would also be nearly completely absorbed in humans. Thus, bioaccessibility, as determined by the in vitro assay, would be equivalent to absolute bioavailability in humans. This implies that, on average, 25% of dioxins/furans in Midland soils that were ingested would be absorbed.

Although the in vitro assay was designed for similarity to humans, rather than rodents or lagomorphs, it is informative to compare the bioaccessibility values for TCDD in Midland soils to the relative bioavailability estimates derived in various animal models. The bioaccessibility of TCDD in Midland soils ranged from 15 to 48%, with an average of 27% (Table 5), while the in vivo estimates of TCDD bioavailability from soil ranged from 16 to 63% (excluding the outlying value of 0.5%), with an average of 35% (Table 1). This similarity suggests that the in vitro test used in this study produces values in the same range as historical animal studies. This is particularly surprising given that the Midland soils were ~3 orders of magnitude lower in TCDD concentration than those used in the animal studies.

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