



Bioavailability of PCDD/F from contaminated soil in young Goettingen minipigs

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Abstract

For the general population the intake of food of animal origin is the main route of human exposure to polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F). Besides this the ingestion of contaminated soil might be an important exposure path for small children. For risk assessment the knowledge of the bioavailable fraction of soil bound contaminants is important.

In a balance study with young Goettingen minipigs the oral bioavailability of PCDD/F from contaminated soil was estimated by determination of the retention of PCDD/F from soil in different organs and tissues. Relative bioavailability was estimated by comparing the retention from soil to the retention of PCDD/F in organs and tissues after oral administration of a PCDD/F mixture extracted from the same soil by solvent. The soil had a PCDD/F-contamination of 5.3 µg I-TEq/kg and originated from a former arable land that had been treated with sludge from the port of Hamburg some years ago. Two groups of each four animals were exposed daily for 28 days via their diet either to 0.5 g soil per kg body weight and day (2.63 ng I-TEq/(kg_{bw} · d)) or to a daily dose of 1.58 ng I-TEq/(kg_{bw} · d) given to the diet by solvent. Five unexposed animals were used as a control group.

Liver, adipose tissue, muscle, brain and blood were analyzed for their PCDD/F content. Accumulation of PCDD/F from soil or solvent in comparison to control animals was only observed for congeners with 2378-chlorosubstitution and predominantly took place in the liver. Bioavailability of 2378-chlorosubstituted congeners was in the range of 0.64%–21.9% (mean: 10.1%) from soil and 2.8%–59.8% (mean: 31.5%) when administered by solvent. The soil matrix reduced the bioavailability by about 70%. Expressed as I-TEq only 13.8% of the PCDD/F contamination were bioavailable from soil. The relative bioavailability of 2378-chlorosubstituted congeners from soil in relation to administration by solvent was in the range of 2%–42.2% (mean: 28.4%).

When not considering the bioavailability, the risk by oral uptake of PCDD/F contaminated soil might be overestimated.

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1. Introduction

Generally humans are exposed to polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) mainly via intake of food of animal origin. In contrast to the oral pathway inhalation or dermal uptake of PCDD/F is of minor relevance. For young children however, the oral ingestion of contaminated soil can be a major route of PCDD/F exposure. Soil ingestion estimates for children in the range of 50–200 mg per day have

been discussed by several authors (Binder et al., 1986; Clausen et al., 1987; Calabrese et al., 1989; Calabrese et al., 1990; Calabrese et al., 1991). Children showing pica-behaviour can ingest up to several gram soil per day.

Since PCDD/F are able to bind to certain soil constituents they become progressively less available over time for uptake by organisms and exerting toxic effects. These factors are currently not reflected by most methods for determination of risk from contaminated soil and it is assumed that the risk is overestimated in most cases (Alexander, 2000).

In several animal studies the uptake of orally administered PCDD/F from different exposure media was

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investigated. When PCDD/F are administered to rats, guinea pigs, mice or monkeys by using a readily available dosing vehicle like oil or solvent a decreasing bioavailability with the grade of chlorination was observed. The values dropped from 70% to 90% for 2378-TetraCDD to 2–15% for OctaCDD (Birnbaum and Couture, 1988; van den Berg et al., 1994; Diliberto et al., 1996). Liver and adipose tissue were the major storage compartments for PCDD/F and in particular 2378-substituted congeners are accumulated (Abraham et al., 1989; van den Berg et al., 1994; Diliberto et al., 1996; Körner et al., 2002).

In relation to PCDD/F given by solvent or oil the absorption of PCDD/F administered by soil is lower. For rats, rabbits and guinea pigs bioavailability of 2378-TetraCDD from naturally contaminated soil is between 16% and 50% (Bonaccorsi et al., 1984; McConnell et al., 1984; Lucier et al., 1986; Umbreit et al., 1986; Shu et al., 1988; Umbreit et al., 1988). Poiger and Schlatter (1980) found bioavailability values of 16–24% for 2378-TetraCDD in rats when the substance was artificially added to the soil for feeding purposes in the laboratory.

The relative bioavailability of 2378-TCDD in soil, calculated as the ratio of the oral absorption of 2378-TetraCDD from soil to the absorption of 2378-TetraCDD from a readily available dosing vehicle – each based on 2378-TetraCDD-concentrations in liver of test animals –, could vary by 2 orders of magnitude (from 0.5 to 60%) and was generally in the range of 20 to 60% (Ruby et al., 2002).

Only a few studies investigated the bioavailability of PCDD/F from soil naturally contaminated with complex mixtures of PCDD/F. These studies concentrated on foraging animals and the risk for humans resulting from the intake of animal products like meat, eggs or milk.

Stephens et al. (1995) examined the uptake and accumulation in chicken which were exposed to naturally contaminated soil, caused by aerial deposition in the vicinity of a pentachlorophenol facility, at doses of 0.3–2.5 ng I-TEq/(kg_{bw}·d) through their diet. A decrease of the bioavailability with the grade of chlorination from 80% for TetraCDD to <10% for OctaCDD and a tissue-specific distribution was observed. Considering all 17 congeners with 2378-chlorosubstitution pattern 5%–30% of the intake was transferred into the eggs, 7%–54% was accumulated in the adipose tissue and less than 1% in the liver.

The *in vivo* studies on bioavailability of PCDD/F from soil summarized above were limited to 2378-TetraCDD and were performed in rodents, lagomorphs or birds in most cases. The fact that these animals have significant anatomic and physiologic differences from humans limits their applicability for human risk assessment. Moreover the distribution in chicken is quite different from that in mammals, especially because of egg-laying as a unique mechanism for excreting fat.

Besides, studies on cows fed with grass silage from a field, which had a history of repeated sewage sludge applications, showed in general similar results regarding the congener-specific bioavailability (McLachlan et al., 1990;

Richter and McLachlan, 2001). For nonlactating cows the authors observed, that the PCDD/F after dietary absorption are first sequestered primarily in the liver and then redistributed into other tissues in dependence of the perfusion rates of the different tissues and the molecule size. Redistribution is more rapid for lower chlorinated congeners, higher chlorinated congeners retained in the liver for longer periods of time.

One of the most important factors influencing the bioavailability of a chemical from soil is its mobilization from the matrix. Studies of Umbreit et al. (1986) on 2378-TetraCDD contaminated soil indicate a correlation between the extractability by organic solvents and the bioavailability. In recent time approaches for human risk assessment have been made to use physiologically based extraction tests (PBETs) to measure the fraction of PCDD/F that would be soluble in the human gastrointestinal tract and might be bioaccessible (Rotard et al., 1992; Rotard et al., 1995; Wittsiepe et al., 2001; Ruby et al., 2002). Our working group (Wittsiepe et al., 2001) compared different artificial digestive tract models to estimate the bioaccessibility of PCDD/F from the technogene slag material 'Kieselrot'. Within all tested digestive juices the rate of mobilization increased more or less with the grade of chlorination and this was observed for PCDD as well as for PCDF. The degree of mobilization depends considerably on the composition of the digestive juices, especially on bile and supplementary food material added to the test system. The great influence of bile has also been observed for other contaminants (Oomen et al., 2004). Development work for PBETs is still ongoing (Ruby, 2004).

The objective of the present study was to examine the oral uptake and accumulation of PCDD/F from a naturally contaminated soil particularly with regards to absolute and relative bioavailability with the final aim to extrapolate bioavailability data to human risk assessment.

Minipigs are supposed to be an adequate animal model because of wide physiological and biochemical similarities to humans regarding the gastrointestinal tract (Swindle and Smith, 1998). We used young pigs at the age of about 1–3 months to simulate childrens physiological age and body weight. The animals were orally exposed to known amounts of PCDD/F either soil-bound or as an extract of the same soil to determine the influence of the soil matrix on bioavailability.

2. Methods and materials

2.1. Soil preparation

The soil (30.6% sand, 36.5% silt, 32.9% clay, 6.83% organic carbon) originated from the upper layer of a former arable land which is located near the city of Hamburg in Northern Germany. The soil had been treated with sludge from the port of Hamburg some years ago. For experimental use and analysis the material was air-dried at 20 °C, only larger aggregates were carefully crushed by

hand. Soil particles >1 mm were removed by sieving. For the exposure experiments soil of the particle size fraction <1 mm was used.

PCDD/F contamination of the soil is 5.3 µg I-TEq/kg_{dry weight}, which is far above the limit values for PCDD/F in contaminated soil with respect to direct uptake given by German regulations (BMU, 1998; BMU, 1999) which is 100 ng I-TEq/kg_{dry weight} for playgrounds and 1000 ng I-TEq/kg_{dry weight} for residential areas. The congener pattern shows increasing concentrations with the grade of chlorination and is dominated by PCDF (see Table 1 and Fig. 1), which is rather unusual in comparison to patterns found in industrial or residential areas (Rotard et al., 1994).

2.2. Preparation of PCDD/F exposure solution

The PCDD/F mixture for the solvent exposure experiment was gained by extraction of the soil with hexane/acetone (50 + 50 v%, 3 times for each 2 h, then 12 h). The combined extracts were evaporated under vacuum and a clean up of the extract was performed by extraction with concentrated sulphuric acid and 10% sodium sulphate solution, followed by column chromatography on alumina oxide. The PCDD/F-concentrations of the exposure solution are shown in Table 1.

2.3. Animal treatment

Young Goettingen minipigs (Ellegaard Goettingen Minipigs ApS, Dalmose Denmark) aged 56–78 days at

the beginning of the experiment were divided into two exposure groups (“soil” and “solvent” with each 4 animals) and one control group (5 animals). Detailed data on the exposure groups are given in Table 2. The animals were housed separately in metabolic cages and were fed with a SDS standard diet (SDS Special Diet Services, Witham, Essex, England) adjusted to 3% of their body weight (bw) per day, according to the recommendations of the breeder. The feeding took place twice a day, half of the ration at 08.00 a.m. and the other half at 3.30 p.m. The animals had unlimited access to water.

On 28 consecutive days soil was administered at a dose of 0.5 g/kg_{bw} per day at 13.30 p.m. resulting in a daily uptake of 2.63 ng I-TEq/(kg_{bw} · d). For the solvent experiment PCDD/F were applied at a daily dose of 1.58 ng I-TEq/(kg_{bw} · d) at 11.00 a.m. Soil or solvent were incorporated into pellets consisting of small amounts of feed, milk powder and water to make it palatable. These pellets were fed by hand to the minipigs to ensure the complete intake. Soil and solvent doses were adjusted to the individual pig's body weight every three days.

On day 29, between 19.5 and 28.5 hours after the last administration of soil or solvent, the animals were sacrificed. Organs with assumed accumulation and contribution to the bioavailability of PCDD/F or toxicological relevance, as liver, adipose tissue, muscle, brain and blood, were taken and stored at –18 °C until analysis.

The experiments were conducted according to the German Animal Protection Law (permission 23.8720 No. 20.35, district authority Arnsberg, Germany).

Table 1
PCDD/F-concentrations of the exposure media and lipid-adjusted concentrations in liver and adipose tissue of both exposure groups

	Exposure media		Mean concentrations (±standard deviation) in tissues of minipigs			
	Soil (µg/kg _{d.w.})	Solvent (µg/l)	Soil exposure (N = 4)		Solvent exposure (N = 4)	
			Liver (pg/g fat)	Adipose tissue (pg/g fat)	Liver (pg/g fat)	Adipose tissue (pg/g fat)
2378-TetraCDD	0.051	0.079	3.7 ± 1.9	n.d. ± –	15 ^a ± 5.2	0.76 ^b ± 0.32
12378-PentaCDD	0.22	0.62	50 ± 31	1.6 ± 0.45	116 ± 39	3.2 ± 1.4
123478-HexaCDD	0.31	0.87	213 ± 81	3.7 ± 0.79	443 ± 82	11 ± 0.75
123678-HexaCDD	0.64	1.7	180 ± 73	5.2 ± 0.78	338 ± 82	15 ± 2.3
123789-HexaCDD	0.54	1.5	89 ± 45	1.4 ^b ± 0.21	208 ± 65	3.4 ± 0.66
1234678-HeptaCDD	3.6	9.9	2023 ± 1008	14 ± 2.9	4375 ± 1396	39 ± 4.9
OctaCDD	4.3	12	4875 ± 1916	17 ± 6.5	7075 ± 1981	26 _s ± 2.0
2378-TetraCDF	2.0	4.0	60 ^a ± 27	2.2 ± 0.95	115 ± 71	3.2 ± 1.6
12378-PentaCDF	5.1	14	73 ± 45	4.2 ± 1.3	162 ± 66	12 ± 1.6
23478-PentaCDF	2.5	6.5	2725 ± 2604	9.5 ± 3.2	3150 ± 465	30 ± 6.6
123478-HexaCDF	12	43	20000 ± 14445	109 ± 23	30000 ± 4397	293 ± 5.7
123678-HexaCDF	9.1	30	11975 ± 3688	60 ± 17	25500 ± 6720	160 ± 17
234678-HexaCDF	1.8	5.5	2175 ± 768	6.2 ± 1.2	3425 ± 512	13 ± 1.5
123789-HexaCDF	1.8	5.1	266 ± 171	2.8 ^a ± 0.79	508 ± 232	4.8 ± 1.5
1234678-HeptaCDF	44	130	44250 ± 11266	136 ± 48	88750 ± 14032	477 ± 72
1234789-HeptaCDF	17	49	16500 ± 4796	36 ± 10	34750 ± 6291	127 ± 5.8
OctaCDF	120	430	54750 ± 17134	108 ± 45	89250 ± 24540	387 ± 90

n.d. = not detectable.

^a n = 3, one value below detection limit or in the range of blank sample.

^b n = 2, two values below detection limit or in the range of blank sample.

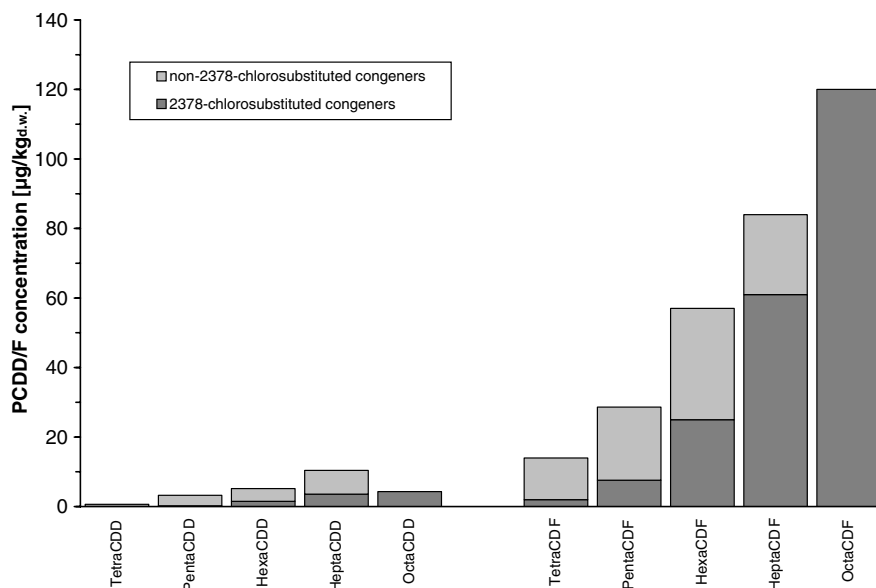


Fig. 1. Concentrations of PCDD/F in the administered soil (particle size fraction <1 mm).

Table 2
Exposure groups and basic data of the minipigs

Exposure group	Sex	At beginning of exposure		At time of death						
		Age (days)	Animal weight (g)	Age (days)	Animal weight (g)	Adipose tissue (g) ^a	Blood (g) ^b	Liver (g) ^c	Brain (g) ^c	Muscle tissue (g) ^d
Soil	<i>f</i>	76	5500	104	6900	690	269	174	43.4	n.d.
	<i>f</i>	75	4850	103	6500	650	254	173	42.7	n.d.
	<i>m</i>	78	5550	106	7050	705	550	171	41.9	n.d.
	<i>m</i>	75	5650	103	7450	745	581	178	42.1	n.d.
	Mean	76	5388	104	6975	698	414	174	42.5	–
Solvent	<i>f</i>	69	4650	97	5950	595	226	193	37.0	2677
	<i>f</i>	64	4950	92	6450	645	245	163	37.3	n.d.
	<i>m</i>	74	5050	102	6300	630	491	193	33.6	n.d.
	<i>m</i>	63	4900	91	6700	670	523	171	35.4	3015
	Mean	67.5	4888	95.5	6350	635	371	180	35.8	2846
Control	<i>f</i>	–	–	84	5400	540	211	137	n.d.	2430
	<i>f</i>	–	–	87	5450	545	213	143	n.d.	n.d.
	<i>m</i>	–	–	84	5350	535	417	166	n.d.	2407
	<i>m</i>	–	–	87	5650	565	441	111	n.d.	n.d.
	<i>m</i>	–	–	103	6150	615	480	136	n.d.	2767
	Mean	–	–	89	5600	560	352	139	–	2535

n.d. = not determined.

^a 10% of body weight based on literature data for minipigs (Holtz and Kallweit, 1981).

^b 7.8% of body weight for male and 3.9% of body weight for female animals, based on literature data for minipigs (Holtz and Kallweit, 1981).

^c weight after removal.

^d 45% of body weight, value was determined by subtracting the weight of organs, blood and excreta from the total body weight of 4 minipigs.

2.4. PCDD/F analysis

2.4.1. Extraction

- **Soil:** 30 g of soil were spiked with 17 ¹³C₁₂-labelled PCDD/F-congeners (2.5 or 5.0 ng) and Soxhlet extracted with toluene/2-methoxyethanol (90 + 10 v%) for 24 h.
- **Tissue samples:** Representative aliquots of the tissues were cut into small pieces and in most cases freeze-dried

before further preparation. The material was weighed and mixed with sea sand/sodium sulphate (1:1) until a dry and homogeneous mixture resulted. An internal standard solution containing 17 ¹³C₁₂-labelled PCDD/F-congeners (25 or 50 pg) was added and the samples were extracted with hexane/acetone (50 + 50 v%) for 24 h using a Soxhlet apparatus. The extract was dried with anhydrous sodium sulphate and the solvent evaporated at 40 °C under vacuum to constant weight. The residue, which represented the fat content,

was weighed and redissolved in hexane for sample clean up.

- **Blood:** The extractions of whole blood samples were performed as described by us previously (Wittsiepe et al., 2000).

2.4.2. Clean up

The clean up was performed by standard methods using modified silicagels, alumina and activated charcoal. After adding 2 µl of dodecane as a keeper the final sample extract was evaporated in a nitrogen stream to the keeper volume and reconstituted by adding 10 µl of toluene, containing ¹³C₁₂-1234-TCDD as an external standard.

2.4.3. GC/MS-analysis

The analytical instrument system was a VG AutoSpec high-resolution mass spectrometer and a Hewlett Packard 5890 series II gas chromatograph equipped with a Gerstel KAS 2 vaporization system [GC-parameters: column: J&W Scientific, DB-5, 60 m, 0.1 µm film thickness; temperature program: 200 °C (3 min), 5 °C/min, 220 °C (16 min), 5 °C/min, 235 °C (7 min), 5 °C/min, 330 °C (9 min); injector program: 70 °C (60 s), 12 °C/s, 330 °C (10 min), split off (1 min); split on (2 min); injection volume: 2 µl; MS-parameters: single ion recording mode; resolution 8000–10000 at 10%; electron impact ionization at 40 eV; perfluorokerosene lock mass check; observation of two ions each for native and labelled isomers; setting of five time windows]. The detection limit in tissue and blood samples was about 1 pg/g fat. Soil samples were additionally analyzed on a polar GC-column.

2.5. Mass balance calculations

For mass balance calculations the total masses of the congeners in the various tissues were calculated from the concentrations of the congeners and the total masses of the respective tissues. For liver and brain the fresh weight of the whole organ was determined after removal from the fresh dead body. The total weight of blood and adipose tissue was calculated using literature data that determined their percentage in total body weight of minipigs (Holtz and Kallweit, 1981). This practice is acceptable if a homogeneous distribution of the PCDD/F in all kinds of body fats is assumed. Literature data indicate, that an uniform PCDD/F distribution among different adipose tissues related to their lipid content is found when the animals were close to a contaminant steady state (Feil et al., 2000; Richter and McLachlan, 2001). The share of muscle tissue was determined by subtracting the weight of organs, skin, bones, blood and excreta from the total body weight of the minipigs.

2.6. Bioavailability

Estimation of bioavailability in selected tissues was calculated as the ratio of the mass of a PCDD/F-congener in

the tissue to the administered mass of the same congener from soil or solvent multiplied by 100%:

$$b_{i,j} = \frac{m_{i,j}}{M_i} * 100\%$$

$b_{i,j}$	bioavailability of congener <i>i</i> in the tissue <i>j</i> (%)
$m_{i,j}$	mass of congener <i>i</i> in tissue <i>j</i> (pg)
M_i	mass of congener <i>i</i> administered to the pig by soil or solvent (pg)

To estimate the total bioavailability in the animal we added the masses found in relevant tissues:

$$B_i = \frac{\sum_j m_{i,j}}{M_i} * 100\%$$

B_i	total bioavailability of congener <i>i</i> in the pig (%)
$m_{i,j}$	mass of congener <i>i</i> in tissue <i>j</i> (pg)
M_i	mass of congener <i>i</i> administered to the pig by soil or solvent (pg)

To compare the bioavailability from the two exposure media (soil and solvent), the relative bioavailability in a selected tissue or in the total animal was calculated as the ratio of the bioavailability in soil to the bioavailability in solvent multiplied by 100%:

$$b_{i,j,rel} = \frac{b_{i,j,soil}}{b_{i,j,solvent}} * 100\% \quad \text{or} \quad B_{i,rel} = \frac{B_{i,soil}}{B_{i,solvent}} * 100\%$$

$b_{i,j,rel}$	relative bioavailability of the congener <i>i</i> in the tissue <i>j</i> (%)
$b_{i,j,soil}$	bioavailability of the congener <i>i</i> in the tissue <i>j</i> administered by soil (%)
$b_{i,j,solvent}$	bioavailability of the congener <i>i</i> in the tissue <i>j</i> administered by solvent (%)
$B_{i,rel}$	relative total bioavailability of the congener <i>i</i> in the pig (%)
$B_{i,soil}$	total bioavailability of congener <i>i</i> in the pig administered by soil (%)
$B_{i,solvent}$	total bioavailability of congener <i>i</i> in the pig administered by solvent (%)

3. Results and discussion

In the tissue samples of the animals of the control group most PCDD/F congeners were not detectable and only a few higher chlorinated congeners were found in trace amounts. These findings ensure, that PCDD/F in the tissues of the exposed minipigs originated exclusively from the administered soil or solvent. Low levels of PCDD/F in juvenile swine have also been reported by Ruby et al. (2004).

3.1. Concentrations and accumulation of PCDD/F in tissues

PCDD/F concentrations in liver, blood, brain, muscle and different adipose tissues were calculated on fat and on

fresh weight basis. As expected, in samples of the exposed animals only congeners with 2378-chlorosubstitution pattern were found in the various tissues in different concentrations, both on fat and on fresh weight basis. Liver and adipose tissue contained the highest concentrations of PCDD/F of all tested tissues. These data are shown in Table 1. Concentrations in blood and brain are significantly smaller (<1% of the lipid-adjusted concentrations in liver) and the muscle tissue samples from the solvent exposed animal group, which were analyzed exemplarily, also show significantly smaller concentrations in comparison with liver and adipose tissue. When considering the same tissues, significantly higher concentrations were found in the solvent exposed animals. The same tissues show similar homologue patterns in the two exposure groups. As in the exposure media concentrations of PCDF are higher than those of PCDD. Within the PCDD homologue group an increase in concentrations from TetraCDD to OctaCDD for both exposure groups can be observed. Within the PCDF the

concentrations increase with the grade of chlorination up to the hepta-chlorinated congeners. 1234678-HeptaCDF shows the highest concentrations all in all.

The liver-to-adipose concentration ratio indicates an about 10 fold higher affinity of higher chlorinated congeners to the liver. In other studies similar results were observed in chicken, rats, marmoset monkeys, calves and humans (Abraham et al., 1989, 1990; Thoma et al., 1989; Thoma et al., 1990; Feil et al., 2000; Richter and McLachlan, 2001; Körner et al., 2002). Richter and McLachlan (2001) also observed a higher accumulation in liver for higher chlorinated congeners (50–75% of administered dose of Hepta-CDF and OctaCDD) while penta- and hexachlorinated congeners were mainly found in adipose tissue. The authors suggested a primary sequestration of all congeners in the liver followed by a redistribution which is more rapid for lower chlorinated congeners. Finally a steady state is reached in which the PCDD/F are homogeneously distributed in all body lipids. Since in the present study the condi-

Table 3
Means and standard deviations of bioavailability of PCDD/F from soil or solvent in liver, adipose tissue and the sum of all examined tissues and means of relative bioavailability of PCDD/F from soil in Goettingen minipigs

N = 4 minipigs exposed in each group	Bioavailability from soil (%)			Bioavailability from solvent (%)			Relative bioavailability from soil (%)		
	Liver	Adipose tissue	Total	Liver	Adipose tissue	Total	Liver	Adipose tissue	Total
2378-TetraCDD	0.75	n.d.	0.75 ± 0.34	9.3 ^a	31.2	38.2 ± 9.4	8.1	–	2.0
12378-PentaCDD	2.3	4.3	6.6 ± 2.1	9.3	11.5	20.8 ± 3.8	24.5	37.5	31.7
123478-HexaCDD	7.2	6.9	14.1 ± 2.2	24.9	34.9	59.8 ± 6.5	28.8	19.9	23.6
123678-HexaCDD	3.0	4.8	7.8 ± 1.6	10.0	27.0	37.0 ± 4.5	29.6	17.9	21.1
123789-HexaCDD	1.7	1.7 ^b	2.5 ± 1.4	6.8	6.0	12.8 ± 3.0	24.8	28.1	19.7
1234678-HeptaCDD	5.9	2.2	8.1 ± 1.8	21.5	11.8	33.4 ± 8.4	27.6	18.2	24.3
OctaCDD	11.9	2.9 ^a	14.0 ± 3.0	28.4	6.8	35.3 ± 7.9	41.8	42.0	39.8
2378-TetraCDF	0.30	0.64	0.86 ± 0.44	1.5	2.1	3.6 ± 1.4	20.5	30.1	24.1
12378-PentaCDF	0.14	0.49	0.64 ± 0.26	0.56	2.2	2.8 ± 0.66	25.8	22.1	22.8
23478-PentaCDF	10.4	2.3	12.8 ± 6.7	23.6	13.5	37.1 ± 2.6	44.2	17.3	34.4
123478-HexaCDF	16.5	5.4	21.9 ± 6.5	33.6	20.0	53.6 ± 5.9	49.1	27.1	40.9
123678-HexaCDF	13.8	4.0	17.9 ± 4.2	41.4	15.3	56.8 ± 10.5	33.4	26.2	31.5
234678-HexaCDF	12.6	2.0	14.6 ± 2.9	30.5	6.5	36.9 ± 4.0	41.2	31.1	39.4
123789-HexaCDF	1.5	0.92 ^a	2.2 ± 1.4	4.9	2.8	7.7 ± 3.1	31.0	32.6	28.6
1234678-HeptaCDF	10.7	1.9	12.7 ± 1.7	33.0	11.3	44.4 ± 5.7	32.5	17.0	28.5
1234789-HeptaCDF	10.5	1.3	11.8 ± 2.0	34.4	7.7	42.1 ± 6.0	30.5	17.0	28.0
OctaCDF	4.9	0.54	5.4 ± 1.6	9.9	3.0	12.9 ± 2.9	49.2	18.4	42.2
Minimum P(4–8)CDD	0.75	1.7	0.75	6.8	6.0	12.8	8.1	17.9	2.0
Maximum P(4–8)CDD	11.9	6.9	14.1	28.4	34.9	59.8	41.8	42.0	39.8
Mean P(4–8)CDD	4.7	3.8	7.7	15.7	18.5	33.9	26.4	27.3	23.2
Standard deviation	3.9	2.0	5.1	8.9	12.2	14.8	10.0	10.4	11.7
Minimum P(4–8)CDF	0.14	0.49	0.64	0.56	2.1	2.8	20.5	17.0	22.8
Maximum P(4–8)CDF	16.5	5.4	21.9	41.4	20.0	56.8	49.2	32.6	42.2
Mean P(4–8)CDF	8.1	2.0	10.1	21.3	8.4	29.8	35.7	23.9	32.0
Standard deviation	6.0	1.6	7.4	15.6	6.3	21.0	9.8	6.3	6.9
Minimum P(4–8)CDD/F	0.14	0.49	0.64	0.56	2.1	2.8	8.1	17.0	2.0
Maximum P(4–8)CDD/F	16.5	6.9	21.9	41.4	34.9	59.8	49.2	42.0	42.2
Mean P(4–8)CDD/F	6.7	2.7	9.1	19.0	12.6	31.5	31.9	25.2	28.4
Standard deviation	5.4	1.9	6.5	13.2	10.2	18.3	10.6	7.9	9.9

n.d. = not detectable.

^a n = 3, one value below detection limit or in the range of blank sample.

^b n = 2, two values below detection limit or in the range of blank sample.

tions of exposure were quite similar in both exposure groups and the liver-to-adipose concentration ratio is higher for the soil-exposure group it can be assumed that the absorption from soil occurs slower than absorption from solvent.

Considering the tissue weights and the PCDD/F-concentrations found in these compartments of the exposed animals, the main burden of PCDD/F is found in liver and adipose tissue. Liver shows the highest accumulation. Total masses of PCDD/F found in muscle tissue, blood and brain are negligible, as observed in previous studies on animals (Lakshmanan et al., 1986; Shu et al., 1988; van den Berg

et al., 1994; Stephens et al., 1995; Diliberto et al., 1996; Richter and McLachlan, 2001; Körner et al., 2002).

As part of this study different adipose tissue samples of the solvent exposed animals were examined (skin, back of the neck, back fat, shoulder, abdomen and kidney). The lipid-normalized concentrations were similar in skin, kidney and shoulder while concentrations in abdominal fat were significantly higher and the fat from the back or from the nape of the neck showed lower concentrations. This might be due to the fact that the animals were not in a steady state at the end of the experiment and shows that

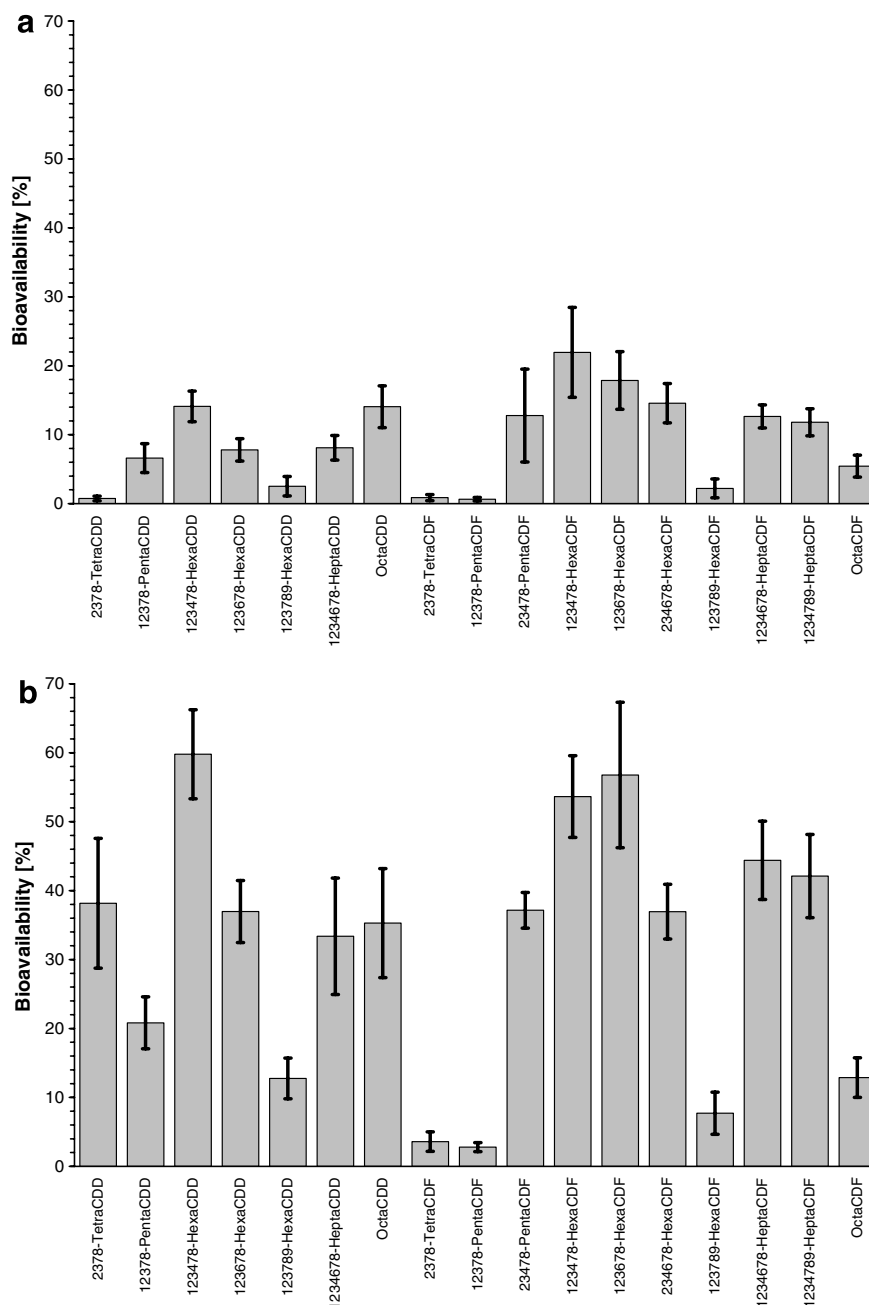


Fig. 2. Bioavailability of PCDD/F in Goettingen minipigs ($n = 4$) based on accumulation in liver, adipose tissue, brain and blood (arithmetic means and standard deviations): (a) from orally administered soil and (b) from orally administered solvent.

the estimation of the PCDD/F content in adipose tissue should be viewed critically.

3.2. Bioavailability

In view of the fact, that predominantly 2378-chlorosubstituted congeners accumulated, only these congeners are discussed below. The data presented are mean values for each PCDD/F-congener calculated from all animals of the specific exposure group. The concentrations of some PCDD/F-congeners, especially 2378-TetraCDD, in the soil were extremely low (see Table 1). As a consequence the

amount accumulated in the tissues was in some cases below the limit of detection. Values which were either below the detection limit or in the range of blank samples were not considered with respect to the calculations for the mean values.

3.2.1. Bioavailability from soil

A congener- and tissue-specific distribution of the bioavailability of PCDD/F from soil was found. The accumulation occurs mainly in liver and adipose tissue whereas in blood and brain it is considerably lower (<0.5% with respect to total bioavailability). The calculated values for

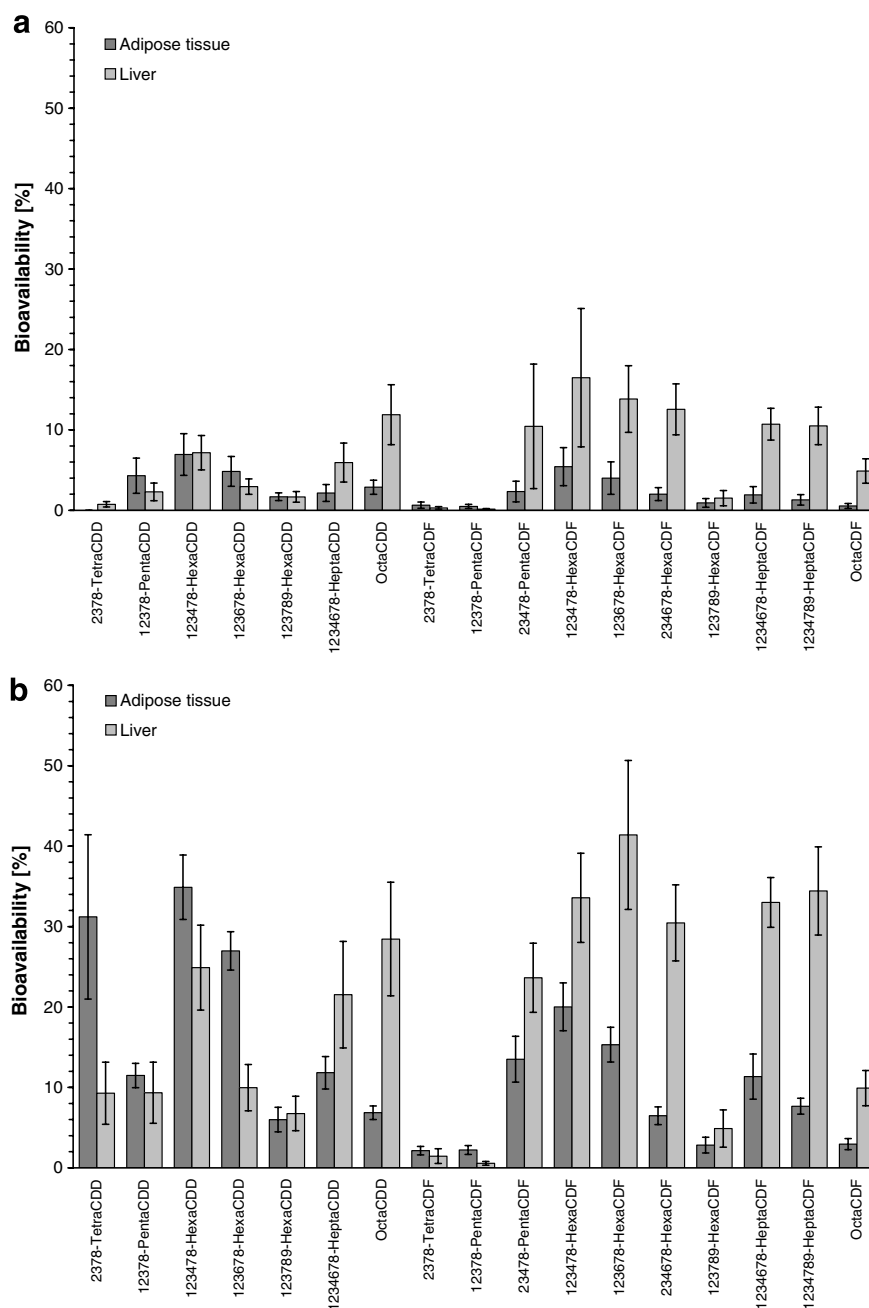


Fig. 3. Comparison of arithmetic means and standard deviations of bioavailability of PCDD/F in liver and adipose tissue of Goettingen minipigs ($n = 4$): (a) from orally administered soil ($n = 3$ for 2378-TetraCDF, OctaCDD and 123789-HexaCDF; $n = 2$ for 123789-HexaCDD) and (b) from orally administered solvent ($n = 3$ for 2378-TetraCDD).

liver and adipose tissue are shown in Table 3. Looking at the total bioavailability $B_{i, \text{soil}}$, 123478-HexaCDD and OctaCDD are the best bioavailable PCDD congeners (14.1% and 14.0%). The bioavailability of most PCDF congeners is slightly higher than those of the corresponding PCDD congeners. 123478-HexaCDF, the best bioavailable PCDF congener, is bioavailable at rates of 5.4% and 16.5% in adipose tissue and liver and to 21.9% totally (Table 3, Figs. 2a and 3a).

Averaged across all 17 2378-chlorosubstituted PCDD/F congeners the mean bioavailability from soil is 9.2% (range: 0.6% (12378-PentaCDF) to 21.9% (123478-HexaCDF)). The standard deviation varies between 0.3% and 6.7%. With respect to I-TEq values bioavailability from soil can be calculated to 13.8%. It should be mentioned, that other soils might result to other values.

3.2.2. Bioavailability from solvent

Bioavailability of PCDD/F in the solvent exposed group showed a similar congener- and tissue-specific pattern, but higher levels compared to the soil exposure group (see Fig. 2b). The highest total bioavailability was found for 123478-HexaCDD (59.8%), followed by 123678- (56.8%) and 123478-HexaCDF (53.6%).

For the higher chlorinated congeners the bioavailability is generally higher in liver than in adipose tissue (Fig. 3). A possible explanation are the parameters influencing the redistribution. After absorption from the gastro-intestinal tract and sequestration in the liver, the redistribution of the congener to outer compartments – like adipose tissues – is influenced by the perfusion rates of the tissues and by physico-chemical parameters like lipophilicity and molecule size.

3.2.3. Relative bioavailability from soil

The relative bioavailability expresses the influence of the soil matrix on the bioavailability (Table 3). Except for 2378-TetraCDD (see note above) the congener-specific values for the total relative bioavailability were in the range of 19.7–42% and thus emphasize the great influence of the soil matrix.

4. Conclusion

- Accumulation of PCDD/F from soil or solvent is only observed for congeners with 2378-chlorosubstitution.
- Bioavailability of PCDD/F is congener- and tissue-specific. Accumulation takes place predominantly in liver, which is the primary compartment, and in adipose tissue as a secondary compartment. All other tissues examined are of minor importance for calculation of bioavailability.
- The soil matrix has a significant influence on oral bioavailability. Under the chosen experimental conditions and in relation to PCDD/F orally administered by solvent, soil reduces the bioavailability of about 70%.
- Expressed as I-TEq-values the bioavailability of PCDD/F from the examined soil is 13.8%. This indicates that neglecting the bioavailability might lead to an overestimation of the risk by oral uptake of PCDD/F contaminated soil.

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