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

Jürgen Wittsiepe[1], Bibiane Erlenkämper[1], Peter Welge[1], Alfons Hack[1], Michael Wilhelm[1]

[1]Ruhr-Universtität Bochum, Abteilung für Hygiene, Sozial- und Umweltmedizin, Universitätsstraße 150, D-44801 Bochum

Introduction

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.
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 [1].

In several animal studies the uptake of oral administered PCDD/F from different exposure media was investigated, but 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 [2-4]. The applied animal models are limited in their applicability for human risk assessment because of their significant anatomic and physiologic differences from humans and because of egg-laying and continuous lactation as unique mechanisms for excreting fat.

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 finally 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 [5]. 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.

Methods and Materials

Soil preparation

The soil (30.6 % sand, 36.5 % silt, 32.9 % clay, 6.83 % organic carbon) originates 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 $_{\text{dry weight}}$, which is far above the limit values for PCDD/F in contaminated soil with respect to direct uptake given by German regulations [6, 7] which is 100 ng I-TEq/kg $_{\text{dry weight}}$ for playgrounds and 1000 ng I-TEq/kg $_{\text{dry weight}}$ for residential areas. The congener pattern shows increasing concentrations with the grade of chlorination and is dominated by PCDF (see Table 1), which is rather unusual in comparison to patterns found in industrial or residential areas [8].

Preparation of PCDD/F exposure solution

The PCDD/F mixture for the solvent exposure experiment was gained by extraction with hexane/acetone (50 + 50 v %, 3 times for each 2 h, then 12 h) of the soil. 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 final PCDD/F-concentrations of the exposure solution are shown in Table 1.

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). 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. 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 pallatible. 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 known 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 Laws.

PCDD/F analysis

Extraction

- *Soil:* 30 g of soil were spiked with 17 $^{13}C_{12}$-labelled PCDD/F-congeners (2.5 or 5.0 ng) and Soxhlet extracted with toluene/2-methoxyethanol (90 + 10 v %) for 24 hours.
- *Tissue samples:* Representative aliquotes 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 $^{13}C_{12}$-labelled PCDD/F-congeners (25 or 50 pg) was added and the samples were extracted with hexane/acetone (50 + 50 v %) for 24 hours 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.

Extraction of blood samples, *clean up* and *GC/MS-analysis* were performed as described by us previously [9]. Soil samples were additionally analyzed on a polar GC-column.

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 [10]. 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 [4, 11]. 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.

Bioavailability

Bioavailability in selected tissues is here defined 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 %. To estimate the total bioavailabilty in the animal we added the masses found in relevant selected tissues. 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 %.

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.

Concentrations and accumulation of PCDD/F in tissues

As expected, only congeners with 2378-chlorosubstitution pattern were found in the various tissues with highest concentrations in liver and adipose tissue. Concentrations in blood and brain were significantly smaller (< 1 % of the lipid-adjusted concentrations in liver). When considering the same tissues in both exposure groups, significantly higher concentrations were found in the solvent exposed animals, whereas the homologue patterns were similar. As in the exposure media concentrations of PCDF are higher than those of PCDD. Within the PCDD homologue groups an increase in concentrations from TetraCDD to OctaCDD for both exposure groups was observed and within the PCDF the concentrations increased with the grade of chlorination up to the hepta-chlorinated congeners. 1234678-HeptaCDF showed the highest concentrations all in all.

Liver and adipose tissue contained the main burden of PCDD/F. Total masses of PCDD/F found in muscle tissue, blood and brain are negligible low. These observations on minipigs correspond to results published on other animal species [2, 4, 12-16].

Bioavailability

In view of the fact, that 2378-chlorosubstituted congeners accumulated predominantly, 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.

Bioavailability from soil

A congener- and tissue-specific distribution of the bioavailability of PCDD/F from soil was found. Looking at the total bioavailability based on accumulation in liver, adipose tissue, brain and blood, 123478-HexaCDD and OctaCDD were the best bioavailable PCDD congeners (14.1 and 14.0 %). The bioavailability of most PCDF congeners was slightly higher than those of the corresponding PCDD congeners. 123478-HexaCDF, the best bioavailable PCDF congener, was bioavailable at rates of 5.4 % or 16.5 % in adipose tissue or liver and to 21.9 % in total (see Figure 1 a). Averaged across all 17 2378-chlorosubstituted PCDD/F congeners the mean bioavailability from soil was 9.2 %, with respect to I-TEq values bioavailability from soil can be calculated to 13.8 %.

Bioavailability from solvent

Bioavailablity of PCDD/F in the solvent exposed group showed a similar congener- and tissue-specific pattern, but higher levels compared to the soil exposed group (see Figure 1 b). 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 bioavailability in both exposure groups is generally higher in liver than in adipose tissue. This can be explained, because after the first absorption from the gastro-intestinal tract and sequestration in the liver, the redistribution of the congener to outer compartiments - like adipose tissues - is influenced by the perfusion rates of the tissues and by physico-chemical parameters like lipophilicity and molecule size.

Relative bioavailability from soil

The relative bioavailability expresses the influence of the soil matrix on the bioavailability (Table 1). Except for 2378-TetraCDD (see note above) the congener-specific values for the total relative bioavailability were in the range of 19.7 to 42 % and describe the great influence of the soil matrix.

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

a)

b)

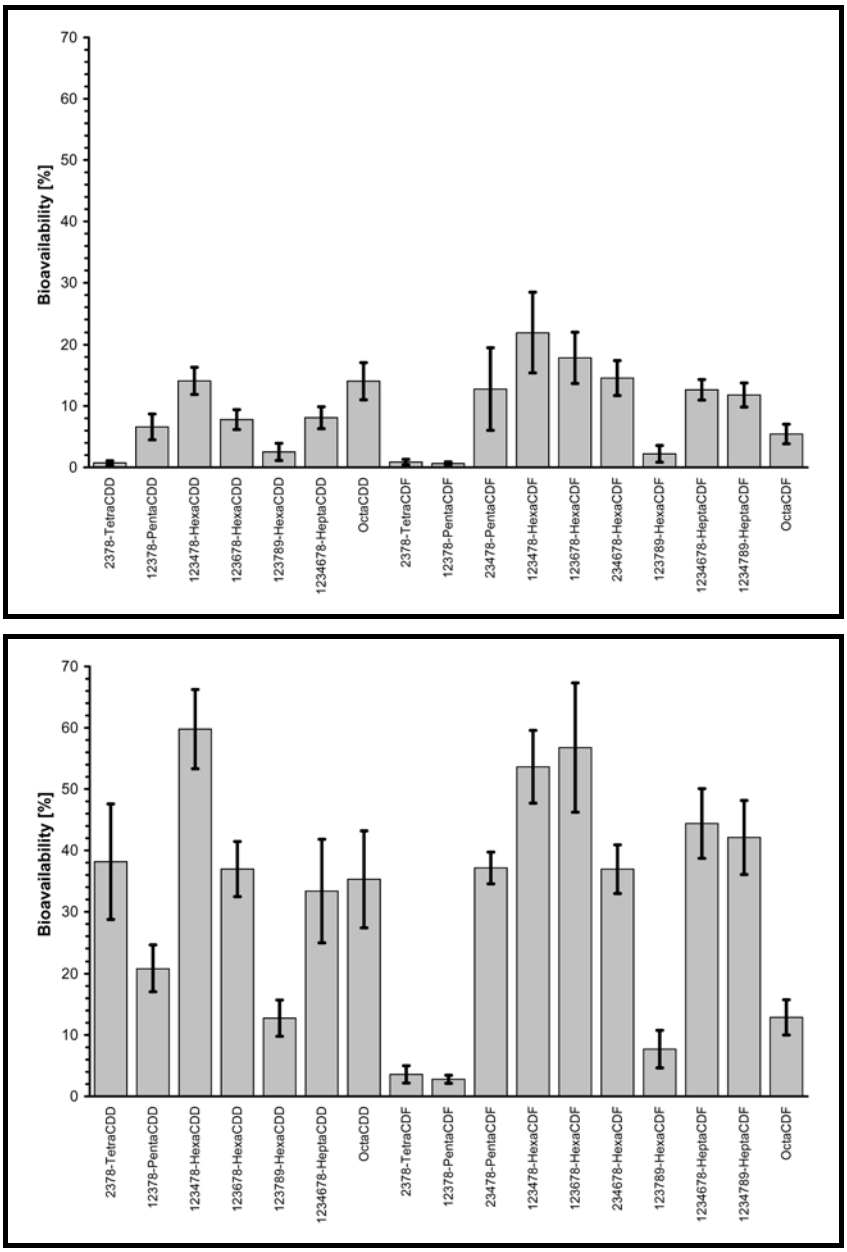

Figure 1: Bioavailability of PCDD/F in Goettingen minipigs (n = 4) based on accumulation in liver, adipose tissue, brain and blood (arithmetic means and standard deviations)
a) from oral administered soil
b) from oral administered solvent

Table 1: PCDD/F-concentrations of the exposure media and means of relative bioavailability of PCDD/F from soil in Göttingen minipigs

	PCDD/F-concentration of exposure media		Relative bioavailability from soil
	Soil [µg/kg $_{d.w.}$]	Solvent [µg/l]	[%]
2378-TetraCDD	0.051	0.079	2.0
12378-PentaCDD	0.22	0.62	31.7
123478-HexaCDD	0.31	0.87	23.6
123678-HexaCDD	0.64	1.7	21.1
123789-HexaCDD	0.54	1.5	19.7
1234678-HeptaCDD	3.6	9.9	24.3
OctaCDD	4.3	12	39.8
2378-TetraCDF	2.0	4.0	24.1
12378-PentaCDF	5.1	14	22.8
23478-PentaCDF	2.5	6.5	34.4
123478-HexaCDF	12	43	40.9
123678-HexaCDF	9.1	30	31.5
234678-HexaCDF	1.8	5.5	39.4
123789-HexaCDF	1.8	5.1	28.6
1234678-HeptaCDF	44	130	28.5
1234789-HeptaCDF	17	49	28.0
OctaCDF	120	430	42.2

Acknowledgements

Parts of the investigations were supported by the Umweltbundesamt, Germany (FKZ 29873227/ 01). The technical assistance of Michaela Fey, Heidemarie Joppich and Sonja Schaub is greatfully acknowledged.

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