INVESTIGATIONS ON THE *IN VIVO* FORMATION OF PCDD/F USING RADIOACTIVE LABELLED PENTACHLOROPHENOL AS PRECURSOR

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Introduction:

Main objective of this study was the examination of the potential formation of PCDD/F in biological systems. There have been an increasing number of studies about PCDD/F which reported anomalous distribution patterns of PCDD/Fs with increased portions of OCDD and little to no PCDF. [1,2,3] These are usually present when PCDD/F originate from anthropogenic sources.
Before this background the hypothesis is postulated, that there must be another source for such dioxins and especially OCDD, possibly a natural one. Other studies with experiments trying to prove and explain biological formation, were published recently. However, those studies relied more on *in vitro* and simulated conditions. [4,5,6,7] In this study living animals (Göttingen Minipigs) were used in an *in vivo* experiment, and forest soil litter (from different regions in Bavaria and an indication of formation by exhibiting relative high EOX values) as a natural matrix.
A radiotracer method was applied to increase traceability and for better quantification and differentiation from native OCDD. As precursor Pentachlorophenol was employed because it is potentially abundant and can also be formed biologically from hexachlorobenzene via oxidative dehalogenation. The PCP was synthesized from hexachlorobenzene with a single substitution of the beta radiator carbon 14 in advance. The different samples were analysed using scintillation counting and isotope dilution methodology with GC-MS.

Materials and Methods:

In advance the radioactive labelled PCP was dissolved in aqueous 2 N NaOH and purified by extraction with n-hexane in a ultrasonic bath 30 times to remove all lipophile by-products such as PCDD/Fs. The progress of the purification was monitored every four steps with scintillation counting and every eight steps with GC-MS, resulting in a highly concentrated and purified stock solution. For experimental use this solution was diluted to a non toxic level but sufficiently concentrated radioactivity for later detection assuming formation rates below 1 ppm.
The two experiments were performed as follows:
Two male, six month old Göttingen Minipigs were housed in separate metabolism cages. The PCP was added to the food prior feeding. The exposure lasted for two month. After the first month the dose of PCP was doubled. Urine and faeces of the animals were sampled daily. Finally the Minipigs were sacrificed and samples taken of all organs and body tissues.
Forest soil samples from two different areas were crushed, mixed with labelled PCP separately and distributed into Erlenmeyer flasks under equal conditions. The total incubation time was three month. Every two weeks three flasks each were frozen at –40°C.

For analysis all samples had to be homogenized and dried. This was achieved using freeze drying and a shredder mill. Part of the samples was then burned with a Tri Carb Sample Oxidizer and analysed with a scintillation counter. The other part was Soxhlet-extracted with Toluene for 24 hours. Prior to high resolution GC-MS analysis the extracts were purified by column chromatography executing three steps [8]. After each different step small sub-samples were taken and analysed by scintillation counting, to monitor the decrease in radiation along the clean up.

Results and Discussion

Minipig experiment:
The mass-balance results in a recovery rate of 88.3% of the amount of radioactivity employed. The missing 11.7% were lost via respiration, loss of some urine samples, and incompletely investigated body tissues such as muscle flesh. Only a very small quantity of labelled OCDD could be found in the animal experiment. The concentrations vary between 17 and 60 pg/g dry mass (figure 1). Calculation of a conversion rate is not possible because only the daily dose of PCP is known.

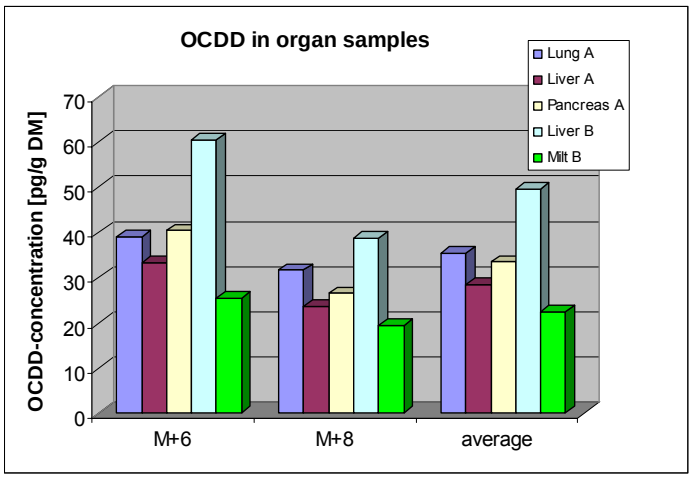

Figure 1: Labelled OCDD in organs of the minipigs

The relevance of the findings is difficult to estimate due to the high amount of precursor. However, formation of OCDD from PCP in a living organism is indicated. Unfortunately the phenomenon of the anomalous distribution patterns can not be clarified conclusively.
Forest soil experiment:
Recording of time series yielded no obvious trend over the period of three months. Concentrations of radioactive OCDD were slightly higher than in the animal experiment, but still in the range of picogram per gram (figure 2).

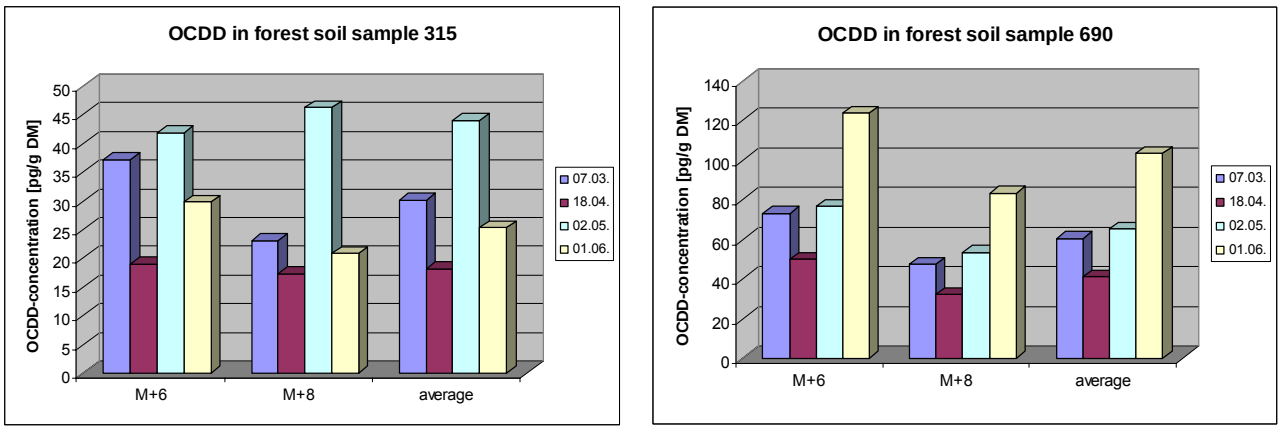

Figure 2: Labelled OCDD in forest soil samples

Conversion rates relating to the amount of PCP used per gram of forest soil resulted in values close below 1 ppm (figure 3). In one special case, only shown in figure 3 because of scaling reasons, a concentration of OCDD thirty times higher than in the other forest soil samples was detected. Following the same calculation, this leads to a conversion rate of 30.2 ppm (cut of in figure 3).

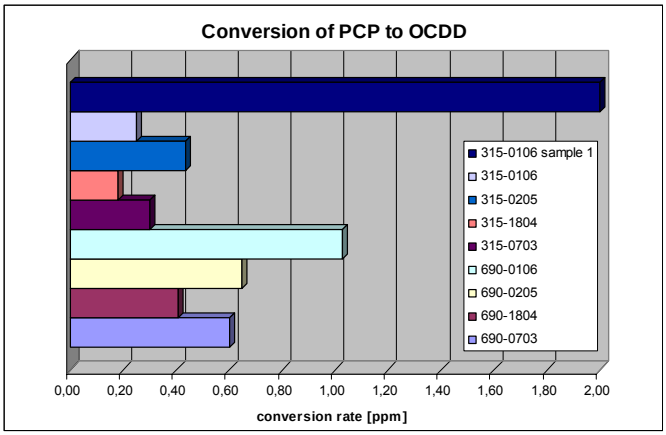

Figure 3: Formation rates of OCDD in forest soil

Formation is therefore proven as shown in table 1. The conversion rate of about 1 ppm in combination with accumulation processes of persitent OCDD formed, e.g. in sediments, could be the reason for the dominance of OCDD in some studies. The single unusual high value and conversion rate of the special sample points to unique possibly aerobic/anaerobic intermediate conditions. A corresponding high radioactivity of the lipophilic part of the sample makes an instrumental error or artefacts during clean-up very unlikely. However due to the preparation method in terms of subdividing samples from a homogenized lot a subsample with higher than average mass of precursor could not completely be excluded.

Table 1: Data Summary

	Starting Concentration	*Concentration (pg/g dm)*	*Conversion rate (ppm)*
stock solution	n.d.		
Lung A		34	n.a.
Liver A		28	n.a.
Pancreas A		33	n.a.
Liver B		50	n.a.
Milt B		22	n.a.
315 (07.03.)		30	0.30
315 (18.04.)		18	0.18
315 (02.05.)		44	0.43
315 (01.06.)		25	0.25
315 (increased)		3043	30.2
690 (07.03.)		60	0.60
690 (18.04.)		41	0.41
690 (02.05.)		65	0.65
690 (01.06.)		103	1.03

Outlook

This study demonstrates the possibility for the biological formation of PCDD/Fs, especially OCDD. Results in the minipig experiment hint at a potential but low hazard level directly through the human digestion system. But a real human experiment would be needed for conclusive proof. Formation of OCDD was also found in the experiment with forest soil litter. But special conditions, probably at interfaces, seem to be required for increased, resp. pronounced formation rates.

In addition further experiments with different precursors and different matrices and condidtions would be reasonable to quantify the extent of formation more securely. Finally detailed investigations to identify formation paths and reaction mechanisms should follow.

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