ENANTIOMER FRACTIONS OF *o,p'*-DDD IN PLASMA AND TISSUES FROM GÖTTINGEN MINIPIGS

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Abstract
Five minipigs were given a single oral dose of *o,p'*-DDD (30 mg/kg, a racemic mixture with EF = 0.49). Following administration plasma and adipose tissue samples were collected during a period of 180 days. At the end of the experiment kidney, brain and liver samples were collected. The enantiomer fractions (EF) of *o,p'*-DDD were determined in plasma, adipose tissue and kidney using GC/ECD equipped with a chiral column (BGB-172). The EFs of individual minipigs showed large variability, ranging from 0.21-0.64 after 24 hours in plasma and from 0.20-0.66 after 90 days in adipose tissue. Hence in some of the minipigs one of the *o,p'*-DDD enantiomer dominated while the other enantiomer dominated in other minpigs. The fact that different enantiomers are dominating after some time but in different minipigs possibly implies polymorphism among the minipigs in this study.

Introduction
Technical DDT contains about 70% *p,p'*-DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane), 25% *o,p'*-DDT (1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane) and other impurities[1]. In the environment *o,p'*-DDT is degraded to *o,p'*-DDE (1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethene) and *o,p'*-DDD (1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane). *o,p'*-DDT and its metabolite *o,p'*-DDD are both chiral. Chiral chemicals used for technical purposes and as pesticides are usually released into the environment as racemic mixtures. The relative abundances of enantiomers can however change in the environment due to microbiological degradation or after being taken up in biota due to e.g. enantioselective uptake, metabolism and/or bioaccumulation. Enantiomeric composition in biota has been used as a tracer for exposure and biotransformation but also for atmospheric long range transport.

There has been few studies evaluating the enantiomeric composition of *o,p'*-DDD in biota. The enantiomeric ratio (ER) of *o,p'*-DDD in human placenta deviated significantly from ER = 1 showing a faster degradation of the first eluting enantiomer[2]. However, usually the concentrations of *o,p'*-DDD found in biota are too low to make it possible to determine enantiomeric ratios. *o,p'*-DDD has proved to be a tissue selective toxicant in the adrenal cortex in several species including human [3], dog[4], birds[5] and mink[6]. Enantiomers could have different biological activities and effects, e.g. (-) *o,p'*-DDT has been shown to have estrogenic activity in humans[7].

o,p'-DDD, with the commercial names Lysodren or Mitotane, has for 40 years been used as an adrenocorticolytic drug for treatment of adrenocortical carcinoma (ACC)[8] and Cushing's syndrome[9] due to its toxicity to the adrenal gland. In the medical literature there is a controversy about its efficacy and *o,p'*-DDD is often associated with severe side effects[10].

The aim of the present study is to identify the enantiomer pattern of *o,p'*-DDD in plasma and tissues taken from the Göttingen minipig given *o,p'*-DDD considering the fact that to our knowledge Lysodren given to ACC patients is racemic.

Material and methods
Animals and samples: Five female minipigs, 6-7 months old, were obtained from Ellegaard, Dalmose, Denmark. The pigs were administrated a single oral dose (30 mg *o,p'*-DDD/kg body weight dissolved in corn oil). The given dose corresponds to approximately half a daily dose *o,p'*-DDD given to adrenocortical carcinoma patients. Blood samples were taken from *vena jugularis* at 0.5, 1, 3, 8, 24, 48 hours and 4, 10, 30, 60, 90, 120 days after administration. 30, 60, 90, 120 days after administration the pigs were weighed and subcutaneous fat samples

from the chin were collected with a biopsy punch after a local anaesthesia. After 180 days the pigs were put to death and liver, kidney, and brain samples were taken.

Chemicals and instruments: o,p'-DDD (purity 99%) was obtained from Aldrich Chemical Company Inc, Milwaukee, USA. All solvents used were of analytical grade. Analysis and quantification of *o,p'*-DDD was performed on a Varian 3400 gas chromatograph with electron capture detection (GC-ECD). A non-polar column containing CP-SIL 8CB (25m x 0.15mm x 0.12µm); Chrompack, (EA Middelburg, The Netherlands) was used for the quantification. For the enantioselective analysis a BGB-172 column (30m x 0.25mm x 0.25µm film) from BGB Analytik, Switzerland was used.

Analysis: The extraction and the clean up of the blood samples was carried out as described earlier[11], but due to the small amount of plasma the method had to be slightly modified. Plasma (0.2 g) was transferred to a screw cap tube and the internal standard (CB-189) was added. The samples were denatured with hydrochloric acid and 2-propanol. The denatured plasma was extracted twice by adding hexane/methyl-*tert*-butyl ether (1:1) and inverting them for 5 min. The organic phase was partitioned into a potassium chloride solution by gentle mixing. After centrifugation the organic phase was transferred to a pre-weighed test tube and the solvent was evaporated. The lipid content was determined gravimetrically. To separate the phenolic compounds from the neutrals the extract was dissolved in hexane and was partitioned with an aqueous solution of potassium hydroxide. The lipids were removed by a multilayer column containing 0.1 g activated silica gel, 0.4 g 0.1 M potassium hydroxide/silica gel (1:2) and 0.8 g 90 % (w/w) sulfuric acid/silica gel (1:2) as described elsewhere[12]. The analytes were eluted with hexane/dichloromethane (1:1) to obtain the *o,p'*-DDD fraction.

The method used for extraction of the fat samples has been described by Jensen *et al*, 1983[13], but due to the small sample amount (100-200 mg) the method has been scaled down. The samples were mixed with hexane:acetone (2:5) and extracted twice with hexane:methyl-*tert*-butyl ether (9:1). The lipid amount was determined gravimetrically. The samples were dissolved with hexane and spiked with CB-189 before lipid removal. The phenolic compounds were separated as described above. A first lipid reduction from the neutral fraction was performed by sulfuric acid treatment. Further lipid removal was performed with a column of silica gel/sulfuric acid (2:1, w/w, 1 g) and the analytes were eluted with hexane.

Liver, kidney and brain samples were homogenized according to the method described by Jensen *et al,* 2003[14]. The samples were spiked with CB-189 after extraction. The extracted lipids were removed as mentioned above. All the samples were analyzed and quantified by GC-ECD with a non-polar column (CP-SIL 8CB).

The enantiomer fractions (EF) of *o,p'*-DDD were determined by GC-ECD equipped with a chiral column (BGB-172). EF was calculated as suggested by Harner *et a*l[15] where $EF=E_1/(E_1+E_2)$. E_1 and E_2 representing the area of the first and second eluted enantiomer when analysed on a chiral column when the absolute structure is not known.

Results and discussion

o,p'-DDD was given to the minipigs in a racemic mixture (EF= 0.49) as a single oral dose. The EFs of individual minipigs showed large variability in plasma and adipose tissue. At the initial time the EFs were below 0.49 suggesting the E_2 enantiomer being more abundant, but after 3 hours the mean EF value was nearly racemic (0.46, ranging from 0.4-0.52). This could perhaps indicate a slower uptake for the E_1 enantiomer. However after 24 hours the EFs changed. Two minpigs showed an excess of the first eluting enantiomer E_1 in plasma after 2 days, while the other three minipigs showed an excess of the second eluting enantiomer E_2 at the same time (see figure 1). This clearly shows a different retention pattern of the enantiomers indicating perhaps different enantioselective biotransformation rates. In adipose tissue the same pattern was found between day 30 and day 120 (see figure 2); two minpigs showed an excess of the E_2 enantiomer and the other three showed an excess of the E_1 enantiomer. It is notable that different enantiomers are dominating in different pigs after some time possibly implying polymorphism among the minipigs.

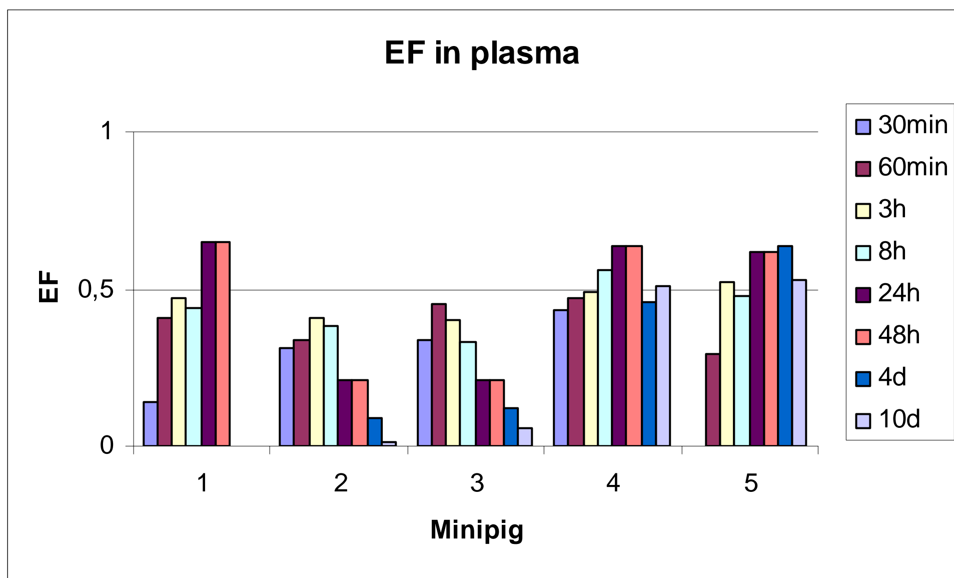

Figure 1. EFs of *o,p'*-DDD in plasma collected at different times after a single oral dose of a racemic mixture of *o,p*'-DDD with an EF=0.49.

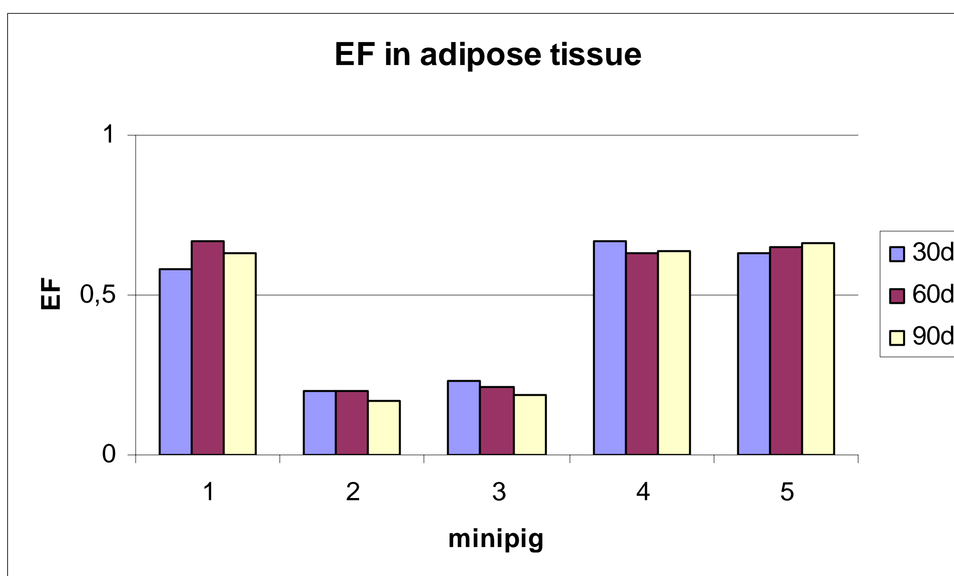

Figure 2. EFs of *o,p'*-DDD in adipose tissue collected at different times after a single oral dose of a racemic mixture of *o,p'*-DDD with an EF=0.49.

In kidney, samples taken 180 days after exposure the EFs were nearly racemic (0.49) for all five minipigs with a mean EF value of 0.46 ranging from 0.40-0.51. The concentrations of *o,p'*-DDD in the liver samples (mean 0.48 ng/g f. w.) and brain samples (below LOQ) were too low to make it possible to analyse them on a chiral column.

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