ANALYSIS OF CHIRAL *o,p'*-DDD IN PLASMA AND ADIPOSE TISSUE FROM GÖTTINGEN MINIPIGS

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Introduction
Technical DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) contains about 70% *p,p'*-DDT, 25% *o,p'*-DDT and other impurities [1]. In the environment *o,p'*-DDT is slowly degradated 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). Both DDE and DDD are lipophilic and are found in humans, wild mammals, birds and fish. *o,p'*-DDD has proved to be a tissue selective toxicant in the adrenal cortex in several species including human, dog, domestic fowl and mink.

o,p'-DDD (Lysodren, Mitotane) has for 40 years also been used as an adrenocorticolytic drug for treatment of adrenocortical carcinoma (ACC) and Cushing's syndrome due to its tissue-selective activity [2]. In the medical literature there is a controversy about its efficacy and *o,p'*-DDD is often associated with severe side effects [3].

o,p'-DDT and its metabolite *o,p'*-DDD are both chiral. Chiral chemicals are usually released into the environment in a racemic mixture. The relative abundances of enantiomers can however change after enantioselective uptake, metabolism and/or bioaccumulation. Enantiomeric composition in biota has been used as a tracer for exposure, atmospheric long range transport and biotransformation. There has been few studies evaluating the enantiomeric composition of *o,p'*-DDD in biota. Enantiomers could have different biological activities and effects, e.g. (-)*o,p'*-DDT has been associated with estrogenic activity in humans [4].

The aim of the present study is to describe the kinetics of *o,p'*-DDD and to compare it to the kinetics for 3-$MeSO_2$-DDE which has been suggested as a possible alternative to *o,p'*-DDD in treatment of ACC [5] (preliminary data for 3-$MeSO_2$-DDE is presented by Hermansson et al [6] at this symposium). Since patients with adrenocortical cancer and Cushing's syndrome often show characteristic accumulations of fat, the Göttingen minipig was chosen as a model because of their body fat storages.

We have also identified the enantiomer pattern of *o,p'*-DDD in plasma and adipose tissue taken from the Göttingen minipigs considering the fact that to our knowledge the active chemical in Lysodren is racemic.

Material and methods
Animals and samples: Five female minipigs, 6-7 months old, were obtained from Ellegaard, Delmose, Denmark. All animals were housed in the same pen with free access to water and they were fed twice a day according to the guidelines from the supplier. The pigs were administrated with a single dose, 30 mg *o,p'*-DDD/kg body weight dissolved in corn oil. The given dose was chosen to correspond to the daily dose *o,p'*-DDD given to adrenocortical carcinoma patients. Blood samples were taken from *vena jugularis* before administration and at 0.5, 1, 3, 8, 24, 48 hours and at 4, 10, 30, 60, 90, 120 days after administration. The 180 days blood samples were taken from the heart after anaesthesia. 30, 60, 90, 120 and 180 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 with an injected overdose of Pentorbital.

Chemicals and instruments: *o,p'*-DDD was obtained from Fluka Chemie, Switzerland. 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 chiral analyses a heptakis (6-Ot-butyldimethylsilyl-2,3-di-o-methyl)-β-cyklodextrin, 50% in OV 1701 w/w, (15m x 0.25mm i.d.), Supelco, USA, column was used.

Analysis: The extraction and the clean up of the blood samples was carried out as described earlier [7], 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 with hexane/methyl-*tert*-butyl ether and 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 a potassium hydroxide solution. The organic phase was transferred to another test tube and represents the neutral fraction. The lipids were removed by a multilayer column containing activated silica gel (0.1 g), potassium hydroxide(1 M)/silica gel (1:2, 0.4 g) and 0.8 g 90 % (w/w) sulfuric acid/silica gel (1:2) as described elsewhere [8]. The analytes were eluted with hexane/dichloromethane 1:1 (8 ml) to obtain the *o,p'*-DDD fraction. The compounds were analyzed and quantified by GC-ECD.

The method used for extraction of the fat samples has been described by Jensen et al, 1983 [9], 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 CB189 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 (15 ml). The compounds were analyzed and quantified by GC-ECD.

The enantiomer fractions (EF) of *o,p'*-DDD were determined by GC-ECD equipped with the cyklodextrin column. EF was calculated using the formula suggested by Harner et al [10] where $EF=E_1/(E_1+E_2)$. E_1 and E_2 representing the area of the first and second enantiomer when analysed on a chiral column. In this case we used the peak height instead of the area due to insufficient resolution of the two peaks.

Results and discussion

Exposure to *o,p'*-DDD did not appear to influence the health of the minipigs. Plasma concentrations of *o,p'*-DDD are shown in figure 1. Each data point represents a mean value from the five pigs. The plasma concentrations (ng/g plasma) increased rapidly during the first hours after administration and have the maximum uptake at 8 hours in all individuals, the mean value being 780 ng/g plasma. After 8 hours the concentration of *o,p'*-DDD was rapidly decreasing having the highest depuration rate between 8 and 48 hours (780-58 ng/g plasma) reaching a constant level after 30 days (2-0.5 ng/g plasma).

The concentrations of *o,p'*-DDD in the adipose tissue were similar for day 30 and day 60 (2960 ng/g l.w. and 2740 ng/g l.w respectively) and the concentrations were 3 times higher than in plasma in lipid weight basis at the same sampling time. After day 60 the concentration decreases rapidly and more slowly after 120 days. Although the concentrations in both the plasma and adipose samples decreased over time, a detectable amount still remains after 180 days, ranging from 0.15-0.95 ng/g plasma and 11-135 ng/g l.w for adipose.

o,p'-DDD was given to the minipigs as the racemic mixture (EF= 0.5). The enantiomer fraction changed after 3 hours in plasma. The enantiomer composition, EF (calculated for one pig, figure 2), increased from 0.5 to 0.8 during the first 4 days, the E_1 enantiomer being the more prominent. This could perhaps indicate a greater

enantioselective biotransformation rate of the E_2 enantiomer. Between day 4 and day 30 the EF was constant 0.8. A steady state situation may have developed after 4 days. In adipose tissue the EF was ranging from 0.73-0.74 during the sampling time 30-90 days.

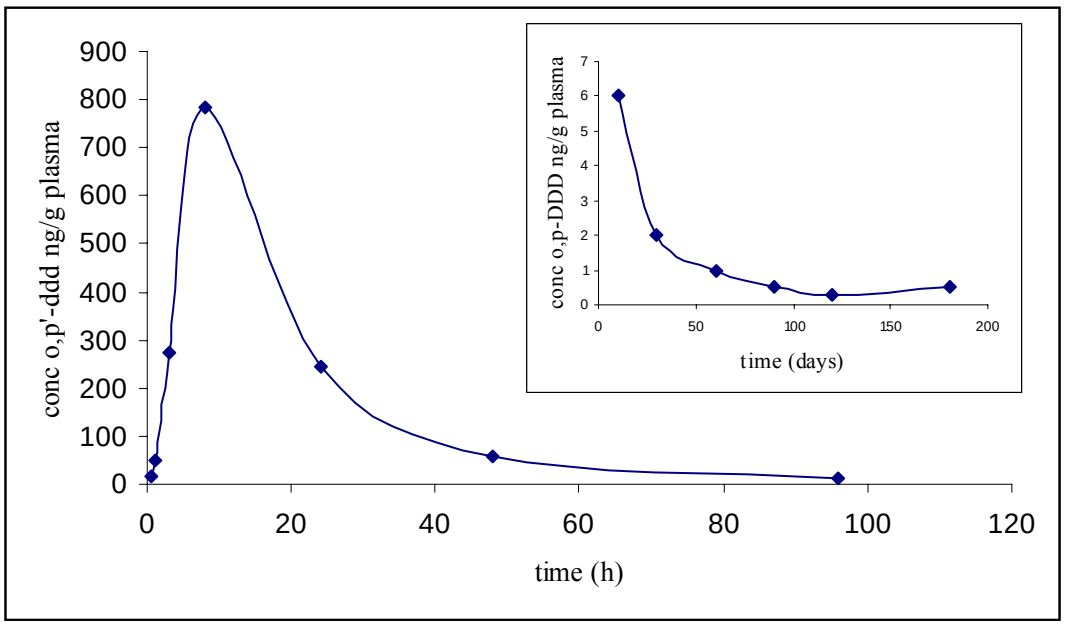

Figure 1.Plasma concentrations of *o,p'*-DDD (ng/g plasma) plotted versus time, during the first 4 days. The smaller graph shows plasma concentrations of *o,p'*-DDD for the remaining days (10-180 days).

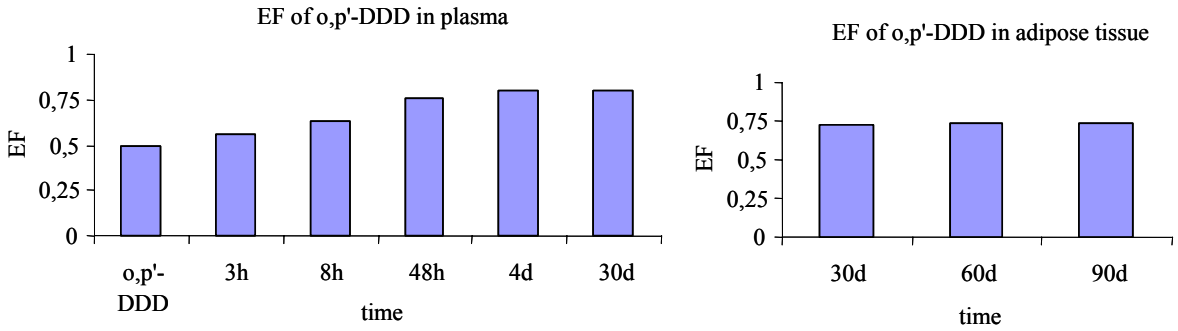

Figure 2.Enantiomer fraction (EF) of *o,p'*-DDD as a racemic form (EF=0,5), in plasma and in adipose tissue from one individual minipig versus time.

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