Kinetic properties of three polyfluorinated dibenzo-p-dioxins in rats

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Introduction

The polychlorinated and polybrominated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDF and PBDD/PBDF) are formed as unwanted byproducts in a variety of chemical and thermal processes. These compounds are widespread in the environment and their lipophilic nature leads to a transport into the foodchain with pronounced accumulation in human tissues. Among this group, the 2,3,7,8-substituted congeners are the most potent compounds with a broad spectrum of biological and toxicological effects. Results from previous studies indicate comparable kinetic properties and biological activity of the chlorinated and the corresponding brominated congeners.[1-3].

To obtain basic data and reference substances of corresponding fluorinated compounds for environmental and toxicological investigations a number of polyfluorinated dibenzo-p-dioxins (PFDD) were synthesized for the first time [4-6].
The presence of polyfluorinated dibenzo-p-dioxins and dibenzofurans (PFDD/PFDF) in environmental samples could so far not be demonstrated [7]. However, in samples of fly ash from aluminum-producing industries and from the pyrolysis of Freons some monofluoropolychloro-benzenes, -biphenyls and -furans were detected [8]. In contrast to these results, in electrofilter ash from aluminum-producing industries and from the thermolysis of teflon, penta- and hexafluorobenzene PFDD/F were detected in the range of µg/kg up to mg/kg [9].

Up to now, there are no relevant data available on the kinetics of 2,3,7,8-tetrafluorodibenzo-p-dioxin (TFDD) and other PFDD/PFDF in rat tissues and only little information exists on the relative toxicity of these compounds (e.g. induction of ethoxyresorufin O-deethylase activity, EROD; [4, 6]). Therefore, we investigated the tissue distribution of 2,3,7,8-tetrafluorodibenzo-p-dioxin in comparison with two hexasubstituted congeners: 1,2,3,4,7,8-hexafluorodibenzo-p-dioxin (1,2,3,4,7,8-HFDD) and 1,2,4,6,7,9-hexafluorodibenzo-p-dioxin (1,2,4,6,7,9-HFDD) following a single intravenous application in rats. In addition, we investigated the inductive potency of these compounds by measurement of the EROD activity in liver microsomes.

Material and Methods

Animal treatment

The congeners were dissolved in toluene and applied as a suspension in peanut oil/0.9% NaCl (1+9, v/v), the suspension contained less than 5% toluene, the applied volume was 0.5 ml/kg body wt.
A group of female Wistar rats (n=15) weighing 190 - 290 g was treated intravenously (through the tail vein) with a mixture containing 50 µg/kg body wt of each congener. Tissue concentrations (liver, adipose tissue and thymus) were determined 30 minutes and 3, 6, 24, and 96 hours following treatment (n=3). In addition, hepatic EROD activity was measured 24 hours after injection.

Preparation of liver microsomes and determination of EROD activity.

After sacrifice the livers were removed, weighed, kept on ice and aliquots homogenized with 4 volume of 0.25 M sucrose using a Potter-Elvehjem glass/Teflon homogenizer. Following the centrifugation of the homogenate (at 9.500 x g for 20 min) the supernatant was filtered through gauze and centrifuged at 100.000 x g for 60 min. The pellet was washed in 100 mM Tris HCl buffer pH=7.4, containing 150 mM KCl and centrifuged again at 100.000 x g for 60 min. The microsomal pellet was resuspended in 100 mM potassium phosphate buffer, pH=7.4, containing 20% glycerol and 1 mM EDTA. The EROD activity was measured in the liver homogenate according to the method of Burke et al., (7) modified by using a NADPH-regeneration system. Final concentrations were: 2.5 mM $MgCl_2$, 0.25 mM NADP, 5 mM glucose-6-phosphate and 0.5 units glucose-6-phosphate dehydrogenase per ml. The substrate concentration was 0.5 µM ethoxyresorufin dissolved in DMSO. The measurement of resorufin formation was performed with a spectrofluorometer (type RF 540, Shimadzu, Kyoto, Japan) at 37°C at pH 7.8 (0.1 M potassium phosphate buffer). The fluorometer settings were: excitation and emission wave lengths 550 nm and 585 nm, respectively; excitation and emission slits: 5 nm. The calibration was performed with purified resorufin.

Analytical procedure

The PFDD were synthesized via an intermolecular nucleophilic aromatic substitution of polyfluorinated phenols followed by an intramolecular one.

The pooled frozen tissue samples were ground with sand/ Na_2SO_4 and extracted with n-hexane for 24 hours. The extract was evaporated with a Kuderna Danish apparatus to 5 ml, then chromatographed over an alumina super B1 column (id.25 mm, 25 g alumina) with 1.: 50 ml benzene; 2.: 20 ml n-hexane/ dichloromethane (98:2); 3.: 250 ml n-hexane/ dichloromethane (9:1). After evaporation of the third fraction the concentrate was chromatographed over a layered silica column (id. 25 mm, 10 g Na_2SO_4, 2 g SiO_2, 5 g NaOH/SiO_2, 2 g SiO_2, 10 g H_2SO_4/SiO_2, 2 g SiO_2, 2 g Na_2SO_4) with 300 ml n-hexane. Subsequently the eluate was evaporated to 1 ml and concentrated in an argon stream to 20 µl in micro vessels.

The GC/MS-analysis for PFDD was carried out on a MD 800 (FISONS Instruments). A DB5 fused silica capillary column (30 m, 0,25 mm id, 0,25 µm df) was used. An aliquot of 1µl sample was injected splitless (septum split also closed for 1 min) at an injector temperature of 250 °C and the following oven temperature program: 2 min 60°C; 50°/min, 140°C; 3°/min, 180°C; 50°/min, 250°C; 2 min; transfer line temperature 280°C. Carrier gas was helium with a head pressure of 100 kPa. The mass spectra were obtained in EI-mode with an ionisation energy of 70 eV. For identification and quantification the GC/MS was run in SIM-mode. The identification was carried out by the registering of the retention time and the M^+-signal. The quantitative determination was made by an external calibration with the observed three compounds as standards and 1,2,6,7-TFDD as an internal standard.

Recoveries varied between 50 and 90%; in some cases it was lower. The specified concentrations relate to the moist weight of the tissues.

Results

Tissue concentrations

Considerable differences were observed in the kinetics of the three applied congeners in the investigated tissues. 0.5 hour after treatment **2,3,7,8-TFDD** reached similar concentrations in liver, thymus and adipose tissue (**Figure 1**). Concentrations of 2,3,7,8-TFDD in the liver revealed a rapid decrease from 5.8 ng/g (30 minutes) to 0.1 ng/g (3 hours after injection). In adipose tissue, however, more than 4-fold increase could be observed during this time and highest concentrations were reached 3 hours after treatment (21.6 ng/g). The subsequent decrease of the concentrations pointed at a biphasic elimination of 2,3,7,8-TFDD from adipose tissue. Using a semi-logarithmic plot, an elimination half-life of approx. 100 hours was calculated for the slow phase between 6 hours and 96 hours. Concentrations of 2,3,7,8-TFDD in the thymus were more than 10-fold compared to levels in the liver and did not reveal any time dependent changes.

In contrast to the rapid elimination of 2,3,7,8-TFDD from liver tissue, concentrations of **1,2,3,4,7,8-HFDD** in the liver increased to a maximum of 15 ng/g (3 hours after treatment, **Figure 2**). These values were approximately 7-times higher compared to corresponding concentrations in the adipose tissue. In the following time values in the liver decreased, but in the adipose tissue remained at the same level. Thus, 4 days after treatment similar concentrations were measured in both tissues.
2,3,7,8-subst.-HFDD content in the thymus increased after treatment and reached highest values of 13.1 ng/g 6 hours after treatment (**Figure 2**). At the 4th day after treatment concentrations in the thymus decreased to similar levels as observed in liver and adipose tissue.

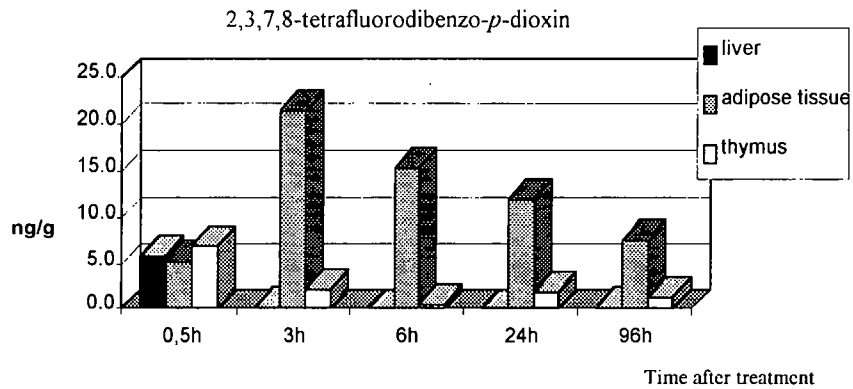

Figure 1: Time course of concentrations of 2,3,7,8-TFDD in rat tissues after i.v. injection of a mixture containing 50 µg/kg body wt of 2,3,7,8-TFDD, 1,2,3,4,7,8-HFDD and 1,2,4,6,7,9-HFDD.

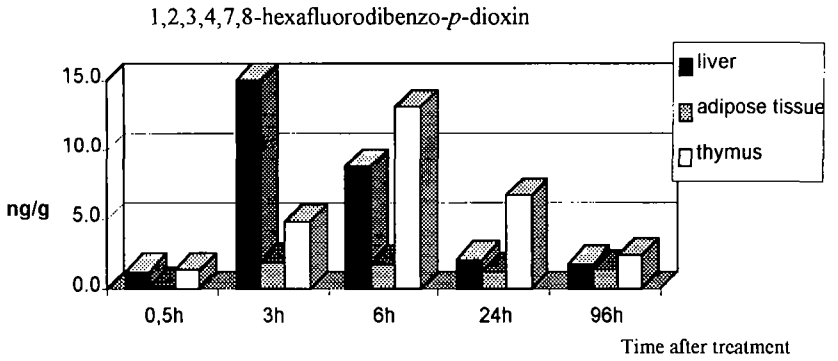

Figure 2: Time course of concentrations of 1,2,3,4,7,8-HFDD in rat tissues after i.v. injection of a mixture containing 50 µg/kg body wt of 2,3,7,8-TFDD, 1,2,3,4,7,8-HFDD and 1,2,4,6,7,9-HFDD.

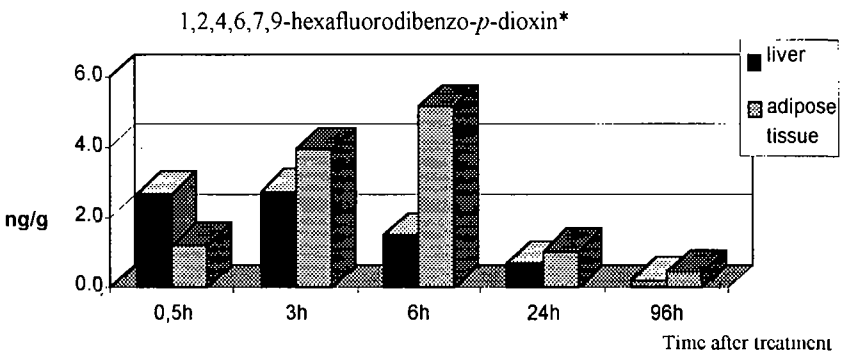

Figure 3: Time course of concentrations of 1,2,4,6,7,9-HFDD in rat tissues after i.v. injection of a mixture containing 50 µg/kg body wt of 2,3,7,8-TFDD, 1,2,3,4,7,8-HFDD and 1,2,4,6,7,9-HFDD.

*) The results of the thymus study are not shown here because of unexpected results, which have to be further investigated.

Concentrations of **1,2,4,6,7,9-HFDD** in the liver measured 0.5 hour after injection were 2fold higher compared to levels of the 1,2,3,4,7,8-HFDD (2.7 ng/g and 1.3 ng/g, respectively; **Figure 3**). However, 3 hours after injection values of 1,2,4,6,7,9-HFDD remained at the same level, while concentrations of 1,2,3,4,7,8-HFDD increased more than 10fold during this time period. Subsequent decrease of concentrations in the liver was similar for both congeners.

In the adipose tissue, however, a contrary concentrations-relation for both congeners was found when comparing with the liver. The levels of 1,2,4,6,7,9-HFDD increased more than 4fold during the first 6 hours after treatment (from 1.2 ng/g to 5.1 ng/g, 0.5hour and 6 hours after treatment, respectively). In

contrast, levels of 1,2,3,4,7,8-HFDD did not reveal any time dependent changes and ranged between 1.3 ng/g and 2.1 ng/g (3 hours and 96 hours after treatment, respectively).

EROD induction

To investigate the possible effects of the fluorinated congeners on the hepatic monooxygenases, the activity of Ethoxyresorufin O-deethylase (EROD) in the liver microsomes was measured 24 hours after treatment (n=3). In contrast to previous reports [10], no induction of the EROD activity (when compared to control values) could be observed in treated rats.

Discussion

Previous studies *in vivo* demonstrated a very rapid elimination of 2,3,7,8-tetrafluorodibenzo-p-dioxin from mice after a single i.p. injection of 100 µg/kg body wt [7, 10]. The authors described a biphasic kinetics of elimination from blood and liver tissue and calculated an elimination half-life of 5 and 7.5 hours (liver and blood, respectively) in the slow phase. Studies *in vitro* [12] indicated a considerable metabolic degradation of polyfluorinated and mixed polyfluorinated/ polychlorinated congeners in mouse liver homogenates, whereas for 2,3,7,8-TCDD and 2,3,7,8-TCDF no metabolic degradation was observed over 3 h of incubation.

Considering the rapid elimination of 2,3,7,8-TFDD from liver tissue, these data are in good agreement with our findings after i.v. injection of this compounds in rats. However, considerable differences arise, when we compare peak liver concentrations measured 30 minutes after treatment in both experiments: approx. 90 ng/g were measured after i.p. application of 100 µg TFDD/kg body wt in mice liver [7, 10], but only 5.8 ng/g after i.v. injection of 50 µg/kg body wt in liver tissue of rats (this study).

Furthermore, our preliminary results indicate considerably differences in the kinetics between the two hexafluorinated dibenzo-p-dioxins: 2,3,7,8-subst.-HFDD and non-2,3,7,8-subst.-HFDD. Considering the peak values in the liver, concentrations of 2,3,7,8-subst.-HFDD were more than 5 times higher then levels of the non-2,3,7,8-substituted congener. In the adipose tissue, however, the peak values of non-2,3,7,8-subst.-HFDD were 2.5 times higher compared to concentrations of 2,3,7,8-subst.-HFDD. Further investigations are necessary to elucidate the different behaviour of 2,3,7,8-substituted and non-2,3,7,8-substituted polyfluorinated dibenzo-p-dioxins.

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