Metabolic Degradation in Mouse Liver Homogenate and Inducing Potency in Hepa-1 cells of Polyfluorinated and mixed Polyfluorinated/polychlorinated Dibenzo-p-dioxins and Dibenzofurans

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Introduction

While the presence of fluorinated dibenzodioxins (PFDDs) and dibenzofurans (PFDFs) in environmental samples has so far not been demonstrated, we have found perhalogenated monofluoropolychloro-benzenes, -biphenyls, and -furans in samples from aluminum-producing industry and from the pyrolysis of Freons (See analytical section).
In order to assess their toxicological relevance, we synthesized various mixed chlorinated/ fluorinated dibenzofurans (PCFDF) and dibenzodioxins (PCFDD). It was found in previous studies with PCDDs and PCDFs that there exists a good correlation between the binding to the Ah-receptor, the induction of Cytochrom (CYP) P4501A1 and the toxic potential [1,2]. In the very sensitive EROD test the induction of 7-ethoxyresorufin O-deethylase (EROD) activity can be determined. It is a measure for the P4501A1 induction [3,4]. The dioxin receptor-mediated EROD activity in Hepa-1 cells was tested for 2,8 difluoro-3,7 dichlorodibenzofuran in comparison to 2,3,7,8 TCDD/F and 2,3,7,8 TFDD/F. In a previous study 2,3,7,8 TFDD was shown to be a potent inducer of cytochrome P450 1A1-catalyzed EROD activity [5].
In the present study, we further investigated the metabolic degradation of various PCFDD/F and PFDD/F in mouse liver homogenate fortified with an NADPH-regenerating system. The results provide an explanation for the rapid elimination of 2,3,7,8 TFDD from blood and liver found in a previous study [5] in male NMRI mice.

Materials and Methods

Liver homogenates were obtained from male NMRI mice weighing 20-23 g. The animals were pretreated with corn oil or with a total amount of 900 mg ß-naphthoflavone (i.p.; dissolved in corn oil) at three consecutive days prior to sacrifice. Livers were perfused via the portal vein with sterile saline, removed, and minced in 4 ml Tris/sucrose buffer (5 mM Tris, 250 mM sucrose, pH 7.4). The tissue was then homogenized in an ice-cooled Dounce homogenizer, centrifuged at 500 x g, and stored at -70°C. Incubations were performed at 37°C by mixing 200 µl 1M Tris-HCl (pH 7.4), 200 µl 1 mM $MgCl_2$, 200 µl 5 mM NADP, 200 µl 50 mM sodium isocitrate, and 40 µl isocitrate dehydrogenase/H_2O (1:10) (Boehringer, Mannheim) with liver homogenate containing 1.2 mg protein, and with a substrate mixture dissolved in 10 µl DMSO. After various times, the incubations were stopped by the addition of 1 ml toluene containing internal standards. Polyhalogenated dibenzo-p-dioxins and dibenzofurans were extracted twice for 15 min with 1 ml

toluene under sonification. The organic phase was collected, the solvent was evaporated to a final volume of 40 ml, and the samples were analyzed by HRGC/LRMS.
For analysis of CYP1A1 induction, Hepa-1 cells were incubated for 48 h with 2,3,7,8-TCDD, 2,3,7,8-TCDF, or 2,8-difluoro-3,7-dichlorodibenzofuran, and for 9 h with 2,3,7,8-TFDD or 2,3,7,8-TFDF, and EROD activity was determined as described (3).
Mixed polyfluorinated/polychlorinated dibenzofurans/dioxins were synthesized by heating chlorinated/fluorinated phenols in a glass ampoule under N_2 at 380°C for 2 hours. The resulting congeners were separated on an alumina column (50g Alumina B-Super1, ICN) with 180 ml heptane/dichloromethane (87,5:12,5) followed by 240 ml heptane/dichloromethane (80:20). From the second fraction 24 separate samples were collected. These 24 samples were analyzed via GC/MS. Fractions containing the same congener were combined..

Results

In mouse liver homogenate, no metabolic degradation of 2,3,7,8-TCDD and 2,3,7,8-TCDF was detectable over an incubation period of 3 h (Fig. 1). In contrast, stepwise fluorination of the lateral positions of the molecules led to a considerable rate of degradation in particular in liver homogenate from ß-naphthoflavone-treated mice. A number of non-2,3,7,8-substituted PFDDs [Fig.2] and 1,7 dichloro-2,8 difluorodibenzofuran [Fig.1] were exceptionally labile towards metabolic degradation and in most instances were completely metabolized after 3 h incubation with homogenate from pretreated animals. Inactivation of the homogenate by heating almost completely abrogated metabolic degradation (not shown).
The octafluorinated congeners OFDD and OFDF were however significantly more stable and did not show higher instability in liver homogenates from pretreated animals [Fig.2].
Log-probit calculations from EROD induction data in Hepa-1 cells are shown in Fig. 3. The curves allow the calculation of EC_{50} values as parameters for the relative inducing potency of each congener. 2,3,7,8-TFDD was about ten-fold less potent than 2,3,7,8-TCDD and 2,3,7,8-TCDF. For dibenzofurans substitution of two chlorine atoms by fluorine led to a dramatic decrease in potency while the completely 2,3,7,8-fluorinated derivative (2,3,7,8-TFDF) was inactive. The finding of a complete lack of EROD inducing potency of 2,3,7,8-TFDF and of a lower potency of 2,8-difluoro-3,7-dichlorodibenzofuran compared to 2,3,7,8-TCDF may result both from metabolic instability in Hepa-1 cells and from alterations in receptor binding/activation. Both aspects are currently under investigation in our laboratories. In conclusion, our findings suggest that NADPH-consuming enzymes, presumably ß-naphthoflavone-inducible hepatic CYP1A1/1A2 play a major role in the degradation of most PFDD/PFDF and mixed fluorinated/chlorinated congeners. In general, 2,3,7,8-substituted congeners were more resistant towards degradation than non-2,3,7,8 substituted congeners. The octa-fluorinated congeners OFDD and OFDF, however, warrant further toxicological investigation because of their higher metabolic stability.

References

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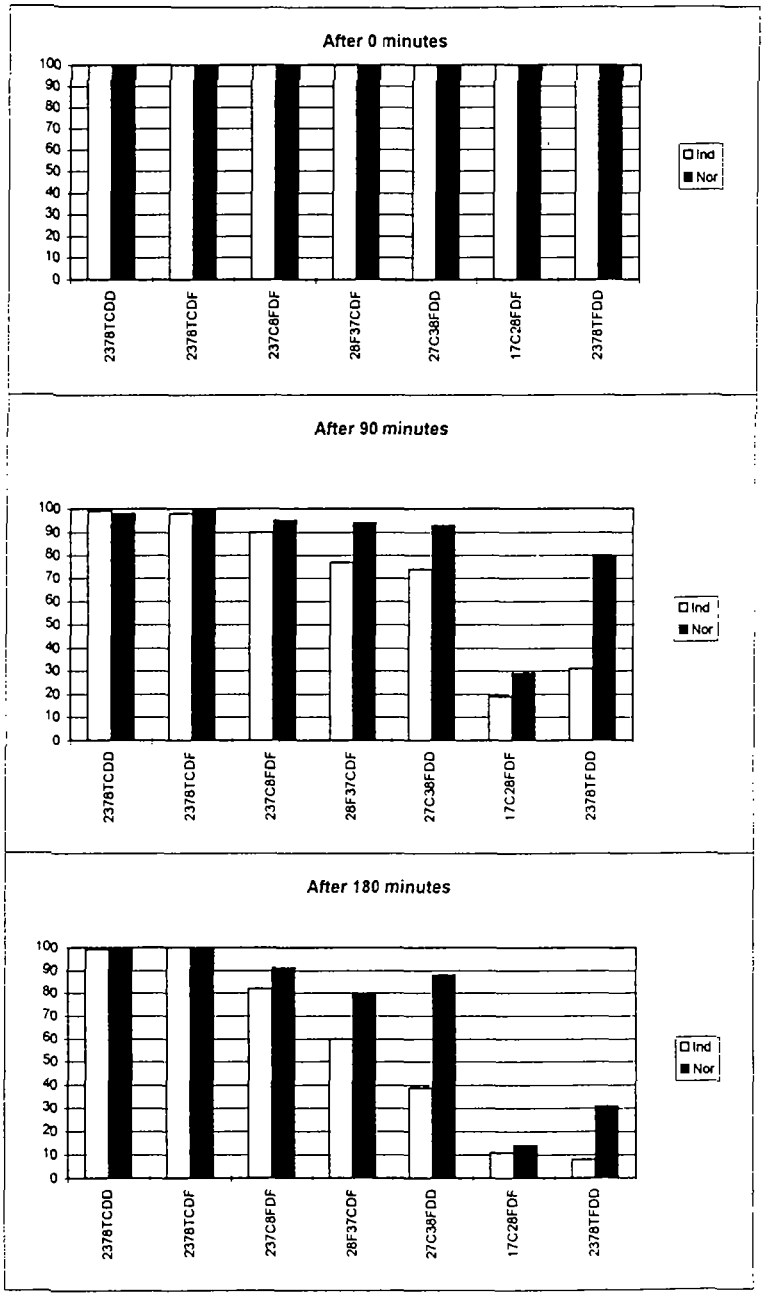

Figure 1: Metabolic degredation of fluorinated dibenzodioxins and dibenzofurans in mouse liver homogenate (0 min.=100%; 5 nanomol Σdioxin/furan in 1,2 mg protein)
ind.= β-naphthoflavone induced homogenate nor.= not induced homogenate

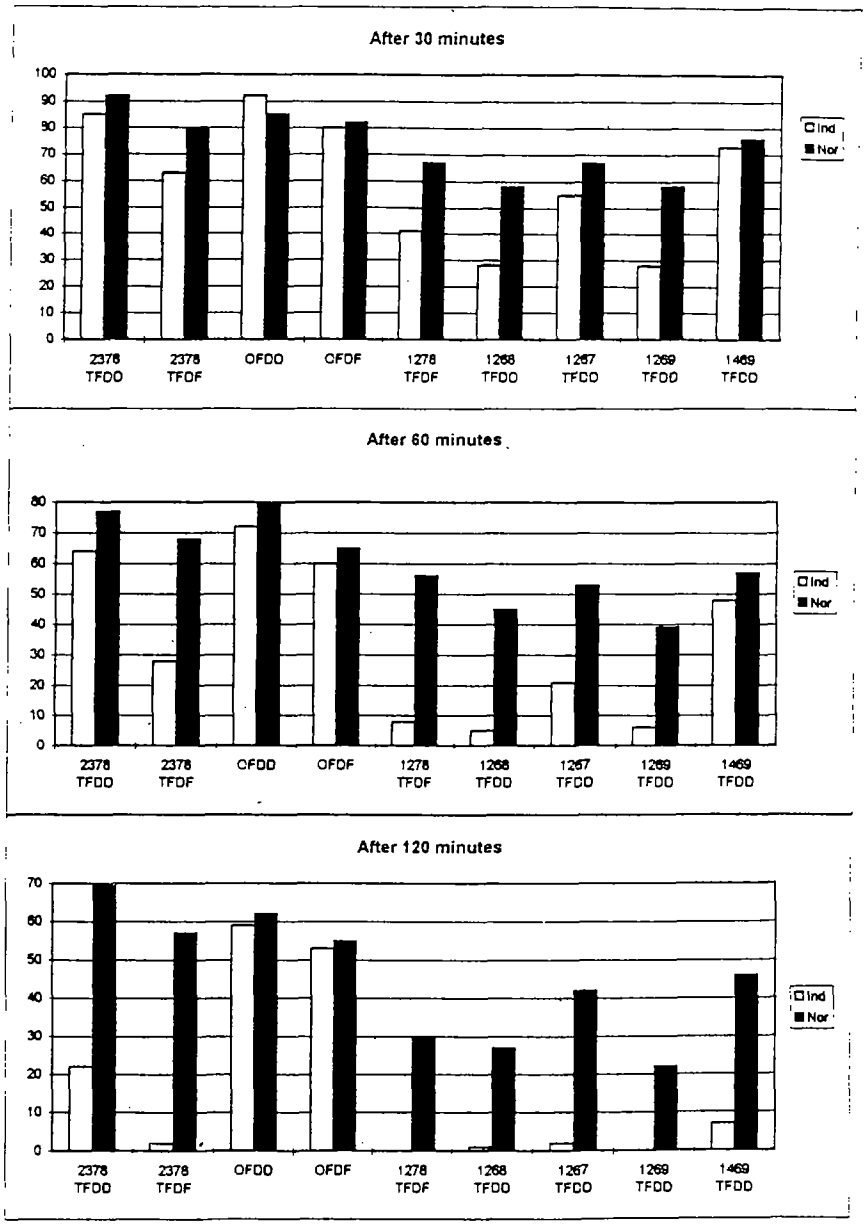

Figure 2: Metabolic degredation of fluorinated dibenzodioxins and dibenzofurans in mouse liver homogenate (0 min.=100%; 5 nanomol ∑dioxin/furan in 1,2 mg protein)

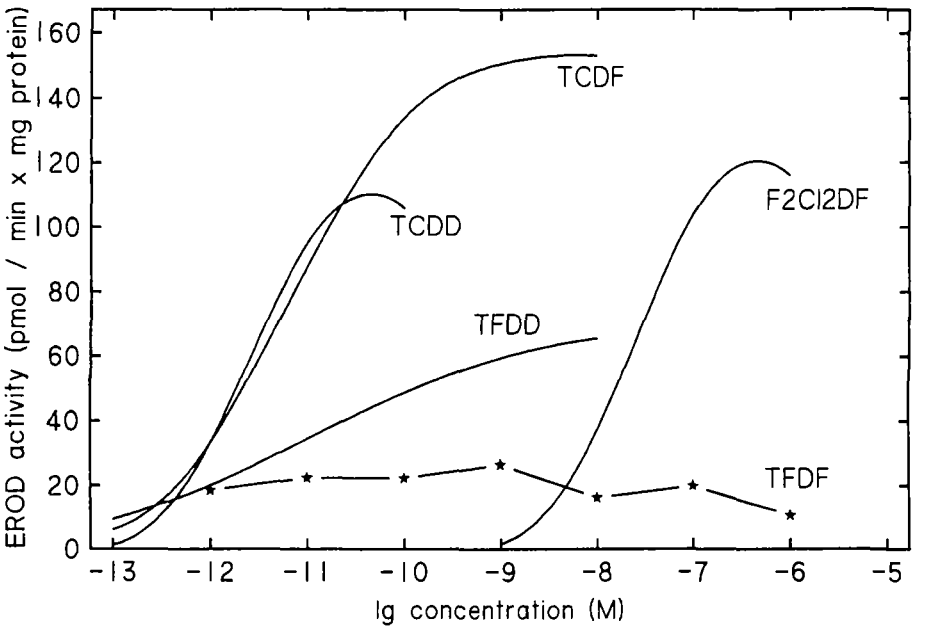

Figure 3: EROD activity of 2,3,7,8 TFDD/F, TCDD/F, and 2,8-difluoro-3,7-dichlorodibenzofuran