THE RELATIVE POTENCIES OF PCDD AND PCDF CONGENERS TO REDUCE HEPATIC VITAMIN A LEVELS FOLLOWING SUBCHRONIC DIETARY EXPOSURE

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Introduction

The toxic equivalency (TEQ) concept has been developed as a tool to estimate the toxicological risk due to environmental exposure of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) sharing a common mechanism of action with the 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (TCDD) (1). This approach is based on the assumption that binding to the *Ah*-receptor is the first step for all important biological effects of these compounds and they act in an additive manner. In fact, a good correlation between *Ah*-receptor mediated biochemical effects, such as induction of aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-O-deethylase (EROD) activity, and different toxic effects has been proved (2,3). Although CYP1A1-related enzyme induction and body and organ weight effects constitute a majority of the experimental data in the data base from which the present harmonised World Health Organisation/Toxicity Equivalency Factors (WHO/TEF) are derived (4), there is no obvious relationship between these end-points and more complex effects such as cancer, reproductive disturbances or developmental effects. During recent years much attention has been paid to the fact that persistent organohalogen compounds interfere with endocrine systems (5). The retinoid system has been addressed as one of the endocrine targets for PCDDs and PCDFs (5-7). The present study was undertaken to further investigate the suitability of using hepatic vitamin A reduction as a biomarker for dioxin exposure. The specific aims were: 1) to investigate the dose-response relationship for hepatic vitamin A reduction, 2) to estimate the individual relative potency (REP) values for this effect and to investigate if they were in accordance with the observed potency to produce subchronic toxicity and enzyme induction and 3) to investigate if the effect on hepatic vitamin A was additive with respect to the set REP values for the individual congeners.

Materials and Methods

Liver tissue was obtained from four separate studies, which investigated the subchronic toxicity of PCDDs/PCDFs in the rat. Detailed descriptions of the experimental designs have been published previously (8-11). Briefly, in experiments I to III, groups of 6, approximately 7 weeks old, male or female Iva: SIV 50 (SD) rats were maintained for 13 weeks on diets (NAFAG No 890 cereal based diet, containing 3 mg vitamin A/kg diet) containing different PCDD/PCDF congeners and water *ad libitum*. The congeners investigated and the administered doses were the following:
experiment I: TCDD (0.2, 2, 20 μg/kg diet); 2,3,4,7,8-PeCDF (2, 20, 200 μg/kg diet).
experiment II: TCDD (2 μg/kg diet); 1,2,3,4,8-PeCDF (600, 6000 μg/kg diet); 1,2,3,7,8-PeCDF (2, 20, 200 μg/kg diet); 1,2,3,6,7,8-HeCDF (2, 20, 200 μg/kg diet); low dose mixture: TCDD, 2,3,4,7,8-PeCDF, 1,2,3,6,7,8-HeCDF and 1,2,3,7,8-PeCDD (0.2, 2, 1 and 1 μg/kg diet, respectively); high dose mixture: TCDD, 2,3,4,7,8-PeCDF, 1,2,3,6,7,8-HeCDF, 1,2,3,7,8-PeCDD (2, 20, 10, and 10 μg/kg diet, respectively).
experiment III: TCDD (2, 10 μg/kg diet); 1,2,3,7,8-PeCDD (20, 100 μg/kg diet);
experiment IV: TCDD (2 μg/kg diet); OCDF (80, 800 μg/kg diet); OCDD (80, 800 μg/kg diet). experiment IV was done with female rats only, and the groups of animals were maintained for 26 and 39 weeks in addition to the 13-week feeding period.
Tissue extraction and vitamin A analysis in triplicate were made as previously described (12), i.e. tissues were completely hydrolyzed before extraction of vitamin A, which was analyzed as retinol by high pressure liquid chromatography. REP values for hepatic vitamin A reduction were estimated for males and females separately. The equation utilized to approximate the dose-response relationship was the following:

1. $Y = 100 \cdot X^n/(b^n+X^n)$

Where *Y* is the hepatic vitamin A reduction expressed as percentage of the amount of vitamin A in the corresponding control group, *X* is the administered dose (μg/kg diet), *b* is the estimated EC50 value (μg/kg diet).
For TCDD, all individual data of all experiments, were used to fit into one single dose- response curve for males and for females, respectively (Fig. 1). Individual data for 2,3,4,7,8-PeCDF (experiment I), 1,2,3,7,8-PeCDF and 1,2,3,6,7,8-HeCDF (experiment II) were also fitted in a dose response curve using equation 1 and the REP values were calculated as the ratio between the EC50 for TCDD and the EC50 for the congener. In experiments III and IV the REP values were estimated by the equivalent TCDD dose predicted by the dose-response curve for TCDD. This curve was also utilized to predict the equivalent TCDD dose for vitamin A reduction induced from the mixtures.

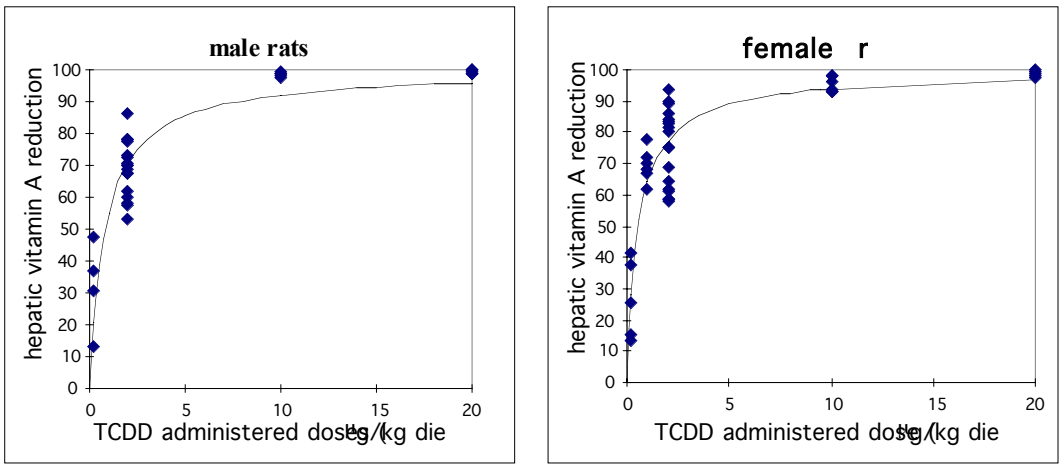

Fig. 1. Dose-response curves for hepatic vitamin A reduction (expressed as % of the corresponding control value) in male and female rats following 13 weeks of dietary exposure to TCDD.

Results and Discussion

The estimated feed intake and thus the dietary intake of vitamin A was similar in exposed groups and corresponding controls, except for groups receiving 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDD which showed a decrease intake of vitamin A ranging from 16 to 35%.

All the congeners under investigation affected in a significant way the amount of hepatic vitamin A at the end of the study, with the exception of 1,2,3,4,8-PeCDF. The most dramatic reductions were observed for TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF and 1,2,3,6,7,8-HeCDF which induced, at the highest doses, a reduction of hepatic vitamin A close to 100% in comparison to the corresponding controls. The REP values for hepatic vitamin A reduction were estimated for male and female rats separately and they did not show major differences (Table 1). However, females were generally found to be slightly more sensitive to the hepatic vitamin A reduction, since the EC50 value for TCDD was lower for female rats (EC50 = 0.5 μg/kg diet) in comparison to the males (EC50 = 0.8 μg/kg diet). OCDD showed a non-significant decrease of hepatic vitamin A after 13 weeks of exposure at the highest dose. Nevertheless, a significant vitamin A decrease was observed after 39 weeks of exposure to 800 μg/kg. The TEQ content in the low and high dose mixtures were calculated by applying the REP values derived in this study multiplied by the corresponding concentrations in the mixture. The equivalent TCDD doses inducing the same effect of the low dose mixture was also predicted by the dose-response curves of Fig. 1. Slightly lower than expected TEQ values were found for both male and female rats.

Data from the present study clearly show that reduction of hepatic vitamin A in rats occurs after dietary subchronic exposure to individual PCDD/F congeners and not as a consequence of decreased dietary intake due to reduced food. Previously estimated REP values (8-11) based on the subchronic toxicity symptoms and REP values based on hepatic vitamin A reduction were found to be fairly similar for all the congeners under investigation (Table 1). Results from the present study

suggest that OCDF can have different toxicokinetic proprieties in comparison to OCDD since significant effect appeared already after 13 weeks of exposure of OCDF, instead of 39 weeks as for OCDD. Data from the present study also indicate that the individual congeners act in an additive manner with respect to hepatic vitamin A reduction. The ability of the low and high dose mixtures to decrease hepatic vitamin A levels was stronger than the effect of each congener alone. Nevertheless for male rats the TEQ content of the low dose mixture calculated by the REP values derived in this study is somewhat higher than the equivalent TCDD dose predicted by the TCDD dose response curves of Fig. 1, while for female rats these values were not significantly different. Thus these results suggest that for female rats the effect on hepatic vitamin A of the individual congeners in the mixture is close to pure additivity, while for male rats the effect tended to be somewhat lower.

In conclusion these structure-activity data strongly suggest a role for the *Ah*-receptor on hepatic vitamin reduction and retinoid disruption in dioxin toxicity.

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TABLE 1
Relative potencies values derived from four different 13-weeks subchronic toxicity studies and harmonised World Health Organization/Toxic Equivalents Factors (WHO/TEFs) for human health risk assessment.

CONGENER	**Vitamin A**		**Subchronic toxicity** [b]	***WHO/TEFs***
	males [a]	females [a]		
TCDD	1	1	1	1
1,2,3,7,8-PeCDD	0.68	0.7	0.39	1
OCDD	not tested	<0.001		0.0001
1,2,3,4,8-PeCDF	<0.0003	<0.0003		
1,2,3,7,8-PeCDF	0.01	0.01	0.02	0.05
2,3,4,7,8-PeCDF	0.14	0.17	0.4	0.5
1,2,3,6,7,8-HeCDF	0.03	0.03		0.1
OCDF	not tested	0.0007	0.0016	0.0001

[a] Data derived in this study. [b] Derived from different subchronic toxicological effects (8-11).

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