COMPARISON OF THE CAPACITY OF 2,3,7,8-TCDD AND TWO DEFINED PCDD- AND PCDF-MIXTURES TO INDUCE HEPATIC EROD ACTIVITY IN RATS DURING REPEATED ADMINISTRATION

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ABSTRACT

Male Wistar rats were treated 16 times (every third day) with 75 ng TCDD/kg body wt. This dosing schedule leads to a steady state level of about 80%. Another two groups of rats received the PCDD- or PCDF-mixture. The applied dose of the mixtures were calculated to contain 82 and 85 ng TE/kg body wt using modified UBA/BGA "toxic-equivalency" factors (the factor for 1,2,3,7,8-P5CDD and 2,3,4,7,8 P5CDF was increased from 0.1 to 0.5). The corresponding I-TE values (NATO 1988) were 57 (PCDD-mix) and 39 (PCDF-mix) ng/kg body wt.

EROD induction in liver microsomes was determined one day after the 3rd, 8th and 16th treatment. In addition, EROD activity was measured 13 and 34 days after the last dosing. The following results were obtained:

1. Hepatic EROD activity steadily increased during the study and was apparently close to reaching a plateau after the 16th injection. The maximum EROD activity reached after a six week treatment period (16 doses) with TCDD was: 4.400 ± 718 pmoles resorufin formed/mg protein x min.
2. Subsequent to the treatment with the dibenzodioxin- or the dibenzofuran-mixtures the maximum EROD activities (after 16 doses) were about 2600 and 2300 pmoles resorufin/mg protein x min, respectively.
3. Hepatic EROD activity decreased after the treatment with TCDD with an apparent half-life of about 15 days.
4. The decline in EROD activity after termination of the treatment with the two mixtures was clearly slower than that found for TCDD. The limited data available suggest that the apparent half-life of the decline in the case of the PCDD- or PCDF-mixtures may have been in the range of 45 days.
5. No differences in EROD activity were observed between the vehicle-treated group and untreated animals.

The results indicate that the EROD inducing potency of the PCDD- and PCDF-mixtures used here is well-predicted when using the I-TE factors, but is overestimated with the modified TE factors of the UBA/BGA. Since the difference in the equivalency calculations is due to the fact that the I-TEF procedure neglects the presence of the *non-2,3,7,8-substituted* congeners, our results support this approach.

KEYWORDS

Wistar rats; 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin = TCDD; PCDDs/PCDFs; Ethoxyresorufin O-deethylase; TCDD-toxic-equivalency factors

ABBREVIATIONS

PCDDs	= Polychlorinated dibenzo-*p*-dioxins;
PCDFs	= Polychlorinated dibenzofurans;
TCDD	= 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin;
OCDD	= Octachlorodibenzo-*p*-dioxin;
OCDF	= Octachlorodibenzofuran;
EROD	= Ethoxyresorufin O-deethylase;
DMSO	= Dimethylsulfoxide;
TE	= TCDD-toxic-equivalency factors;
I-TEF	= International TCDD-toxic-equivalency factors

INTRODUCTION

Studies on the capacity of different PCDDs/PCDFs to induce biological effects have so far almost exclusively been performed with single compounds (POIGER et al., 1989). Here we report on studies with two defined mixtures of PCDDs or PCDFs containing no 2,3,7,8-TCDD. The biological effect evaluated was the induction of hepatic EROD activity, the most susceptible biological (not necessarily toxic) reaction known to be caused by TCDD. One aim of this study was to contribute to the assessment of the significance and predictive value of "TCDD-toxic-equivalency" factors, as suggested by UBA/BGA (1985) and NATO (1988). The data are also relevant for answering the question of the biological and toxicological significance of *non-2,3,7,8-substituted* congeners.

MATERIAL AND METHODS

Animal maintenance and treatment
The maintenance of the animals is described elsewhere (SCHULZ-SCHALGE et al., this issue). ^{14}C-TCDD was supplied by Cambridge Isotope Laboratories (Woburn, USA). The mixture of PCDDs and PCDFs was obtained by catalytic dechlorination/hydrogenation of OCDD or OCDF (HAGENMAIER et al., 1987; WIESMUELLER, 1990) and kindly provided by Prof. Hagenmaier, Tübingen. The composition of the mixture is given in Table 1, calculated as administered dose per kilogram body weight. The original TEFs (UBA/BGA, 1985) for 1,2,3,7,8-P5CDD and 2,3,4,7,8 P5CDF were changed from 0.1 to 0.5.

The substances were dissolved in a toluene/DMSO mixture (1+2; vol/vol). The solutions were injected subcutaneously into the back of the animal at a volume of 0.2 ml/kg body wt using a 100 µl-Hamilton^{R}-syringe (Bonaduz, Switzerland).

Male Wistar rats received 16 separate doses of 75 ng TCDD/kg body wt, or TCDD equivalencies/kg body wt of the PCDD- or PCDF-mixture as indicated (Table 1). The treatment was performed every third day. EROD induction in liver microsomes was determined one day after the 3rd, 8th and 16th treatment. In addition, EROD activity was measured 13 and 34 days subsequent to the last application. *Vehicle-treated* rats received the vehicle according to the above mentioned scheme and *control* rats received no treatment.

Preparation of microsomes and measurement of EROD activity
Details of the methods used have been described elsewhere (SCHULZ-SCHALGE et al., this issue).

RESULTS

The increase of the EROD activity during the TCDD treatment period is shown in Table 1 and Figure 1. Between the 8th and the 16th TCDD treatment the increase of EROD activity was slower than in the first part of the study, indicating that the TCDD concentration was close to reaching a plateau. Kinetic calculations indicate that about 80% of the steady state level is reached after the 16th injection. A maximum value of 4400 ± 718 pmole resorufin formed/mg protein x min was measured one day after the 16th injection.

After the treatment was stopped a clear-cut decrease of the EROD activity was observed, the apparent half-life of the decrease being about 15 days. At the end of the investigation period (34 days following the last application) EROD activity was found to be 1090 ± 207 pmole resorufin formed/mg protein x min.

EROD induction following application of the PCDD- or PCDF-mixture was considerably slower when compared with induction after TCDD treatment. The EROD activities during the entire study period remained much lower than those measured in the TCDD treated rats (Fig. 1). The highest values measured one day after the last application of the PCDD-mixture were 2470 and 3290 pmole resorufin/mg protein x min. Similar results (2320 and 2720 pmole resorufin/mg protein x min) were obtained following the PCDF administration.

After the treatment period the decrease in EROD activity observed in PCDD or PCDF treated rats may have been lower than that following TCDD treatment. At the end of the study period similar levels of EROD activity were evaluated in the TCDD treated, as well as in the PCDD or PCDF treated rats.

No differences were observed in the EROD activities between the *vehicle-treated group* (58 ± 34 pmole resorufin/mg protein x min) and the *control group* (65 ± 34 pmole resorufin/mg protein x min).

DISCUSSION

Previous investigations made in our institute on the inducing activity of TCDD showed a close relationship between the administered dose or hepatic tissue concentrations and the induced EROD activity (ABRAHAM et al., 1988) in the dose range studied.

Thus, in the rat changes in EROD activity after the appropriate TCDD doses may be taken as a measure for TCDD levels and biological actions. Experimental conditions chosen for these repeated dose studies were such as to achieve almost steady state levels of TCDD (about 80% of steady state). This is confirmed by the results obtained (Fig. 1). The EROD activity after the 16th application was very similar to that observed in microsomes of male rats seven days after a single treatment with 644 ng TCDD/kg body wt (SCHULZ-SCHALGE et al., this issue). These results were in good aggreement with the calculation of HARTMANN et al. (this issue) who estimated a cumulative dose of 632 ng TCDD/kg body wt for the treatment schedule used in our studies.

When attempting to compare the biological potency induced after injection of TCDD with those after administration of the PCDD- or PCDF-mixtures some type of TCDD-toxic-equivalency factor has to be used. We decided to evaluate the factors proposed by UBA/BGA (1985) modified according to suggestions by POIGER et al. (1989) with international equivalency factors (I-TE) as proposed by NATO (1988). These two calculations differ *only* in the fact that the I-TE approach does not consider the *non-2,3,7,8-substituted* congeners, while the UBA/BGA suggestion also provides factors for these congeners. There is good reason and considerable evidence available today (cf. e.g. NEUBERT et al. 1990a,b; HAGENMAIER et al. 1990) that these *non-2,3,7,8-substituted* congeners are non-persistent in the mammalian organism and are of minor (or even no) biological significance.

2,3,7,8-TCDD had been removed from the PCDD-mixture used in this study. Thus, a 2,3,7,8-TCDD-free mixture was used in which 1,2,3,7,8-P5CDD made up (Table 1) about 60% and *2,3,7,8-substituted* H6CDDs about 30% of the toxic equivalents (NATO, 1988), and *non-2,3,7,8-substituted* congeners represented 30% of the TEs (according to UBA/BGA). In the PCDF-mixture the *2,3,7,8-substituted* H6CDF congeners made up about 50%, P5CDFs about 21% and H7CDFs about 13% (NATO, 1988), and *non-2,3,7,8-substituted* congeners represented about 50% of the TEs (according to UBA/BGA, 1985).

The results of our studies indicate that 2,3,7,8-TCDD-free mixtures of PCDDs/PCDFs induce hepatic EROD activities similar to that of TCDD, but the other congeners are less potent. Both the strategies of UBA/BGA and NATO lead to interpretations of our data which are within a range of about two. However, when applying the NATO approach, the EROD-inducing potencies of the PCDD- and PCDF-mixture studied can be well-predicted with the I-TE factors. On the other hand, with the modified UBA/BGA-TE-factors the EROD-inducing capacity of these mixtures is overestimated. This result suggests that the *non-2,3,7,8-substituted* congeners play a minor role when assessing the biological potency *in vivo*.

From the data obtained so far a slower elimination of some compounds in the mixtures when compared with TCDD cannot be excluded. Therefore, toxicokinetic data are needed for a final evaluation. Such studies are in progress in collaboration with Prof. Hagenmaier in Tübingen.

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Table 1: Composition of the administered PCDD- and PCDF-mixture

The doses injected (ng/kg body wt) and the corresponding TCDD-toxic-equivalents are given. The TCDD-toxic-equivalency factors (UBA/BGA, 1985) were modified as suggested by Poiger et al., 1989: the TE-factors of 1,2,3,7,8-P5CDD and 2,3,4,7,8-P5CDF were increased from 0.1 to 0.5. In addition, the values are indicated when using the international equivalency factors (I-TEF; NATO, 1988).

PCDDs	Dose (ng/kg)	TE mod. UBA (ng/kg)	I-TE NATO (ng/kg)	PCDFs	Dose (ng/kg)	TE mod. UBA (ng/kg)	I-TE NATO (ng/kg)
T4CDDs				T4CDFs			
2378	0	0	0	2378	22	2.2	2.2 (6%)
non-2,3,7,8-sub.	440	4.4	0	*non*-2,3,7,8-sub.	1880	18.8	0
Sum T4CDDs	*440*	*4.4*	*0*	*Sum T4CDFs*	*1902*	*21.0*	*2.2*
P5CDDs				P5CDFs			
12378	70	35.0	35.0 (61%)	12378,12348	89.0	8.9	4.4
				23478	16.6	8.3	8.3 (21%)
non-2,3,7,8-sub.	940	9.4	0	*non*-2,3,7,8-sub.	1540.0	15.4	0
Sum P5CDDs	*1010*	*44.4*	*35.0*	*Sum P5CDFs*	*1645.6*	*32.6*	*12.7*
H6CDDs				H6CDFs			
2,3,7,8-sub.	180	18.0	18.0 (32%)	2,3,7,8-sub.	189	18.9	18.9 (49%)
non-2,3,7,8-sub.	1050	10.5	0	*non*-2,3,7,8-sub.	700	7.0	0
Sum H6CDDs	*1230*	*28.5*	*18.0*	*Sum H6CDFs*	*889*	*25.9*	*18.9*
H7CDDs				H7CDFs			
2,3,7,8-sub.	370	3.7	3.7 (7%)	2,3,7,8-sub.	490	4.9	4.9 (13%)
non-2,3,7,8-sub.	600	0.6	0	*non*-2,3,7,8-sub.	90	0.09	0
Sum H7CDDs	*970*	*4.3*	*3.7*	*Sum H7CDFs*	*580*	*5.0*	*4.9*
OCDD	300	0.3 (0.4%)	0.3 (0.5%)	OCDF	94	0.09	0.09 (0.2%)
Sum PCDDs	**3,950**	**81.9**	**57.0**	**Sum PCDFs**	**5,111**	**84.6**	**38.8**
Sum 2,3,7,8-subst.	920	57.0	57.0		900	43.3	38.8
Sum excluded OCDDs	620	56.7	56.7		807	43.2	38.7
Sum non-2,3,7,8-subst.	3030	24.9	0		4210	41.3	0
% non-2,3,7,8-subst.	77%	30%	0		82%	49%	0

Figure 1 :

Induction of EROD activity in rat liver microsomes after repeated administration of TCDD and of PCDD – , PCDF – mixtures