CYTOCHROME P-450, EROD AND PROD ACTIVITY IN THE LIVER OF MALE C57Bl/6J MICE AFTER A SINGLE ORAL DOSE OF 1,2,3,7,8-PnCDD AND 2,2',4,4',5,5'-HxCB OR A MIXTURE OF BOTH COMPOUNDS *).

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ABSTRACT

The effect of a single oral dose of mixtures of 1,2,3,7,8-PnCDD and 2,2',4,4',5,5'-HxCB on cytochrome P-450 dependent enzyme activities was studied in the liver of C57Bl/6J mice and compared to the effect of the single congeners. An antagonistic effect was found on EROD activity with a mixture of 5 nmol/kg PnCDD and 500 umol/kg HxCB.

INTRODUCTION

1,2,3,7,8-PnCDD (PnCDD) and 2,2',4,4',5,5'-HxCB (HxCB) are both widespread environmental pollutants. Both compounds accumulate through the foodchain and are usually found together in relatively high concentrations in a wide variety of environmental biota samples, e.g. human milk. The knowledge about the possible mixture interactions of these classes of chemicals is very limited and is of great interest for both risk assesment and understanding of the fundamental mechanisms involved. PnCDD is known as a specific cytochrome P-450I inducer and HxCB as a specific P-450II inducer. For this reason 7-ethoxyresorufine-O-deethylation (EROD) and 7-pentoxyresorufine-O-depentylation (PROD) are good markers for the enzyme-inducing potency of the respective componds.

EXPERIMENTAL

Two groups of four C57Bl/6J mice received either a single oral dose of 5 nmol/kg PnCDD or 500 umol/kg HxCB. In addition a single oral dose of a mixture of 5 nmol/kg PnCDD and either 500, 100, 20, 5 or 1 μmol/kg HxCB was administered to five other groups of mice. One group served as controls and received vehicle only (Arachides oil, 5 ml/kg). After seven days the animals were sacrificed and a microsomal fraction was prepared from the livers.

Activities of 7-Ethoxyresorufine-O-deethylation (EROD) and 7-Pentoxyresorufine-O-depentylation (PROD) were determined according to the method of Burke et al. 1985. Total cytochrome P-450 contents was measured according to the method of Omura and Sato, 1964.

RESULTS AND DISCUSSION

As can be seen from table 1, cytochrome P-450 concentrations in all treated groups are significantly elevated above control level. The groups dosed with mixtures show an addditive response compared to groups 1 and 2, and corrected for cytochrome P-450 control values.

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PROD activity per nanomol of cytochrome P-450 is elevated in all treated groups. Non-additive interaction effects are not appearent. EROD activity is significantly above the control value in all treated groups, except for the HxCB treated group. A significant interaction between both compounds appears in group 7, where the EROD activity per nanomol cytochrome P-450 is significantly lower than in the group treated with PnCDD only.

Table 1: Total cytochrome P-450 concentration (nmol/mg protein), EROD and PROD activities (pmol /minute/nmol cytochrome P-450) in the liver of male C57Bl/6J mice after a single or mixed oral dose (μmol/kg) of PnCDD and HxCB

Group#	Dose HxCB or PnCDD		Cytochrome P-450	EROD	PROD
0	-	-	0.73 ± 0.09	67 ± 11	28 ± 5
1	500	-	1.17 ± 0.04	70 ± 7	55 ± 8
2	-	0.005	1.42 ± 0.10	1917 ± 154	60 ± 5
3	1	0.005	1.24 ± 0.04	1919 ± 178	55 ± 5
4	5	0.005	1.39 ± 0.24	1717 ± 344	49 ± 9
5	20	0.005	1.56 ± 0.12	1919 ± 172	63 ± 7
6	100	0.005	1.58 ± 0.13	1693 ± 117	58 ± 6
7	500	0.005	1.73 ± 0.10	1473 ± 53 *	89 ± 10

Values for all treated groups (#1-7) are significantly above control level (#0).
* = Significant mixture interaction in group #7 as compared with #1 and #2.

One reason for this antogonistic respons may be a toxicokinetic interaction effect in the liver. A similar study has been performed with 2,3,7,8-TCDD (TCDD) and HxCB on C57Bl/6J mice, and reported similar antagonistic effects on EROD activity (Biegel et al, 1989). In this study it was also shown, that co-administration of HxCB did not lower the TCDD concentration in the liver. However, animals were dosed via intra-peritoneal injection, while in our study dosing was done by gavage. In the same study a more mechanistic explanation for the interaction effects is given. HxCB given at high doses, displaces TCDD from the Ah receptor protein, but is not active as an inducer of EROD activity. This could also be a good explanation for the results of our study, because both PnCDD and TCDD express at least a part of their biological activities through the Ah receptor. Since it is known that cytochrome P-450II activity, for which PROD is a good marker, is not mediated through the Ah receptor, this would also explain the absence of interaction effects on PROD activity in our study.

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