Interactive Effects Between PCDDs, PCDFs and PCBs on Their Hepatic Disposition in C57BL/6J Mice

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INTRODUCTION

Information about toxicokinetic interactions between PCDDs, PCDFs and PCBs is limited, but of importance to risk assessment. In addition this might provide insight in the fundamental mechanisms behind these interactive effects. Studies on interactive effects on enzyme induction, immunologic responses and teratogenicity are scarce and are usually not supported by toxicokinetic data.

It has been shown that dosage of binary PCB mixtures can cause an enhanced liver retention as compared to dosage of the single mixture components. This accounts for some of the interactive effects observed on cytochrome P450 1A activity in the liver of rat and mouse[1,2].

The present study was designed to observe possible interactive effects on the toxicokinetics of PCDDs, PCDFs and PCBs in the liver of the Ah-responsive C57BL/6J mouse after dosage in mixtures. 1,2,3,7,8-PnCDD (PnCDD), 1,2,3,6,7,8-HxCDD (HxCDD), 2,3,4,7,8-PnCDF (PnCDF) and 2,2',4,4',5',5-HxCB (HxCB) are all present in environmental biota samples in relatively high concentrations. PnCDD, HxCDD and PnCDF are strong inducers of cytochrome P450 1A related enzyme activities. HxCB does not induce 1A activity but is known to cause interactive effects with 2,3,7,8-substituted PCDDs and some coplanar or mono-ortho substituted PCBs[1,2,3].

Table 1: Dosage of single congeners and mixtures in this study (nmol/kg).

Group:	dosage:	PnCDD	HxCDD	PnCDF	HxCB
1A	single	1	-	-	-
1B	" + HxCB	1	-	-	$300 * 10^3$
2A	single	-	10	-	-
2B	" + HxCB	-	10	-	$300 * 10^3$
3A	single	-	-	5	-
3B	" + HxCB	-	-	5	$300 * 10^3$
4A	mixture	1	10	5	-
4B	" + HxCB	1	10	5	$300 * 10^3$

These Studies are supported by the Netherlands Organization for the advancement of Pure Science (N.W.O).

EXPERIMENTAL

8 groups of 16 male C57BL/6J mice, weighing 20 ± 2 grams, age 5-7 weeks, were dosed according to the schedule in table 1. 4 animals of each dose group were sacrificed after 7, 14, 42 and 105 days. Soxhlet extraction, column chromatography and GC/MS were used to determine the liver concentrations of PnCDD, HxCDD and PnCDF as described earlier[4].

RESULTS

Liver retention of PnCDD was significantly increased after coadministration with HxCB 7 and 14 days after dosing as compared to the single dose group (Figure 1a). Administration of PnCDD in combination with HxCDD and PnCDF (group 4A) also caused an increase of its liver retention at day 7 and 14. An additional HxCB coadministration with this mixed dose group (4B) futher increased the liver retention during the whole experimental period.

HxCB coadministration caused only a slight and insignificant effect on the liver retention of HxCDD (Fig 1b). The only significant enhancement of HxCDD retention in the liver was observed in group 4B, coadministrated with PnCDD, PnCDF as well as HxCB, when compared to the single dose group at day 7. HxCDD retention in the liver of the other dose groups was not significantly altered at any timepoint (Figure 2b).

No significant effect of HxCB coadministration on the liver disposition of PnCDF was found at any timepoint in any of the dose groups (Figure 1c).

The elimination rates of the three compounds from the liver were calculated using first-order kinetics. Comparison of these data shows that there is no substantial difference between halflives in single and mixed dose groups (Table 2).

Table 2: Estimated halflives of PnCDD, HxCDD and PnCDF in days.

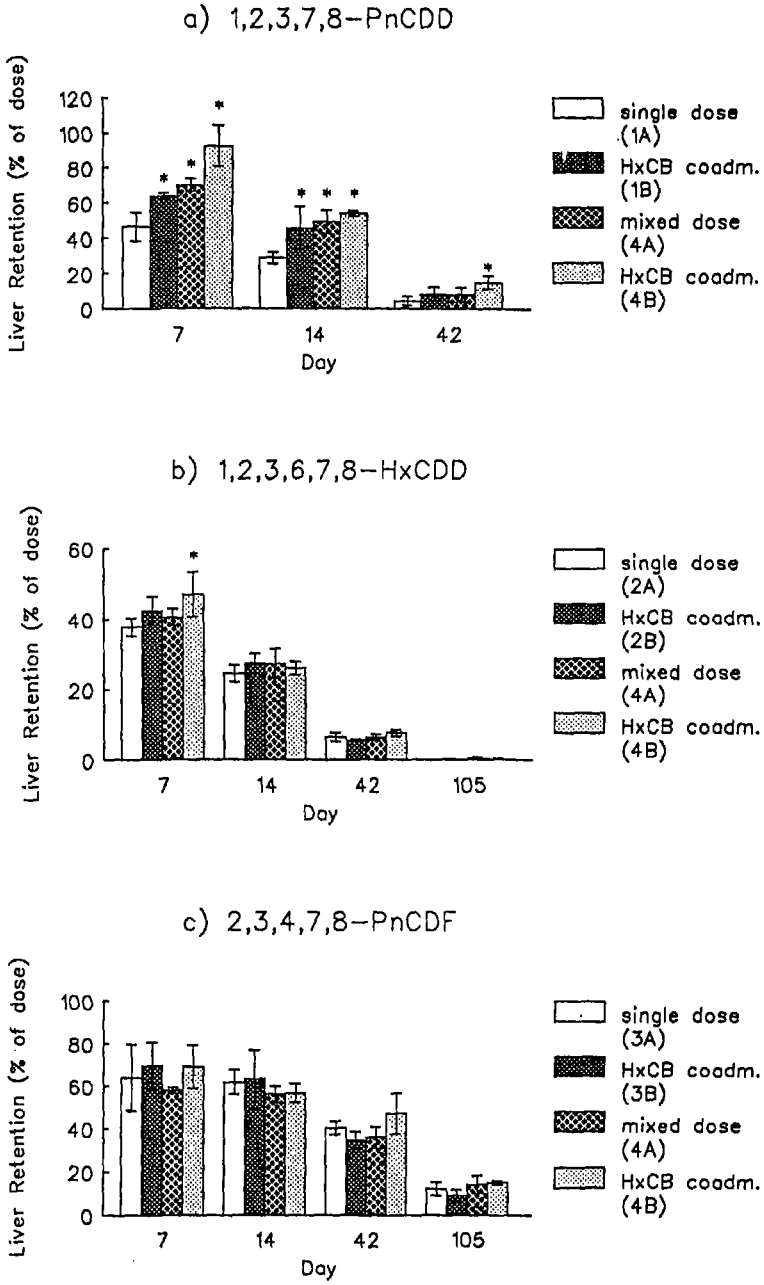
Dosage:	PnCDD	HxCDD	PnCDF
single	10	13	40
+ HxCB	11	13	32
mixture	10	15	46
+ HxCB	13	13	46

DISCUSSION AND CONCLUSIONS

It has been shown before that HxCB coadministration can modulate liver retention of some mono-ortho and coplanar PCBs[1]. The modulation of PnCDD toxicokinetics as observed in this study is therefore not unexpected, since these PCB congeners are isostereomers of 2,3,7,8-TCDD, with a similar mechanism of action.

The modulating effect on PnCDD liver retention by HxCDD and PnCDD, as seen in dose group 4A, resembles the results from a similar study with 2,3,7,8-TCDD in mice, using preadministration with the same compound[5].

From the data presented above, it can be concluded that PnCDD, HxCDD and



Retention in the liver of C57BL/6J mice after single or mixed dosage with PnCDD (a), HxCDD (b) and PnCDF (c).
(*significantly different from the corresponding single dosed group, $P<0.05$)

PnCDF showed distinct differences in their respective toxicokinetic interactions when present in mixtures. PnCDD liver kinetics showed the highest sensitivity towards toxicokinetic modulation. HxCDD toxicokinetics showed some effect but no effect was seen on PnCDF liver retention. These differences are remarkable in view of the closely related structures of the three compounds. It can be argued that the ability of a compound to show an increased liver disposition after dosage in a mixture is dependent on its distribution between the liver and other organs. Data on the liver/adipose tissue distribution ratio in rats show large differences among 2,3,7,8-substituted PCDDs and PCDFs[6]. As a result compounds with a relative high liver disposition, such as PnCDF, are much less liable to toxicokinetic modulation.

The results from this study again indicate, that the observed interactive effects are likely based on the presence of an inducible, quantitatively important binding site in the liver. The existence of such an inducible binding site has been has been proposed before[5]. Results from our study indicate that induction of this binding site could originate from more than one mechanism. This can be derived from the fact that the observed toxicokinetic modulation is caused by two types of compounds with distinctly different mechanisms of action. HxCB belongs to the group of di-ortho substituted PCBs, inducing isoenzymes of cytochrome P450 2B, whereas PnCDD, HxCDD and PnCDF are strong inducers of P450 1A isoenzymes. It can be postulated that the inducing properties of HxCB for the de novo synthesis of the Ah-receptor could play a key role in this[7].

REFERENCES

1 De Jongh J, Wondergem F, Seinen W, Van den Berg M. Toxicokinetic interactions in the liver of the C57BL/6J mouse after administration of a single oral dose of a binairy mixture of some PCBs. In: Proceedings of the 11th International Symposium on Chlorinated Dioxins and Related Compounds. Chapel Hill, NC, USA, September 23-27, 1991: p. 211.

2 Leece B, Denomme MA, Towner R, Li A, Landers J, Safe S. Nonadditive interactive effects of polychlorinated biphenyl congeners in rats: role of the 2,3,7,8-TCDD receptor. *Can J Physiol Pharmacol* 1987;65:1908-12.

3 Bannister R, Safe S. Synergistic interactions of 2,3,7,8-TCDD and 2,2',4,4',5,5'-hexachlorobiphenyl in C57BL/6J and DBA/2J mice: role of the Ah receptor. *Toxicology* 1987;44:159-69.

4 de Jongh J, Wondergem F, Seinen W, Van den Berg M. Absence of mixture interactions on hepatic disposition and EROD activity in the C57BL/6J mouse after coadministration of 1,2,3,7,8-PnCDD and 2,4,5,2',4',5'-HxCB. *Toxicology* 1992 (submitted).

5 Curtis L, Kerkvliet N, Beacher-Steppan L, Carpenter H. 2,3,7,8-Tetrachlorodibenzo-p-dioxin pretreatment of female mice altered tissue distribution but not hepatic metabolism of a subsequent dose. *Fund Appl Toxicol* 1989;14:523-31.

6 Abraham K, Wiesmuller T, Brunner H, Krowke R, Hagenmaier H, Neubert D. Absorbtion and tissue distrubution of various polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) in the rat. *Arch Toxicol* 1989;63:193-202.

7 Denomme MA, Leece B, Li A, Towner R, Safe S. Elevation of 2,3,7,8-TCDD rat hepatic receptor levels by polychlorinated biphenyls. Structure-activity relationships. *Biochem Pharmacol* 1986;35:277-82.