Tumour Promoting Activity of Different Polychlorinated Biphenyls and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

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Introduction

Polychlorinated biphenyls (PCBs) have been used in diverse applications in the industry due to their chemical stability. The persistence of PCBs has resulted in a world wide distribution in the environment. The most toxic PCB-congeners are those substituted in both *para*- and at least two *meta*-positions (fig. 1). With none or only one chlorine substituent in *ortho*-position they can assume a coplanar conformation and are thereby approximate stereoisomers of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These coplanar PCB-congeners also cause biological effects resembling those reported for TCDD, i.e. skin lesion, thymus atrophy, liver damage, a wasting syndrome and increased mortality. There seems to be a general consensus that these effects, and the carcinogenic effects caused by TCDD, are mediated via the arylhydrocarbon-receptor[1]. The biological effects caused by coplanar PCB-congeners are probably mediated via the same receptor-mechanism as TCDD. The molecular mechanism underlying the biological effects elicited by the non-planar PCB-congeners (i.e. substituted in at least two *ortho*-position) is not as clear as that for coplanar PCBs and TCDD.

The potencies of individual aromatic hydrocarbons have been determined relative to TCDD using toxic equivalency factors (TEFs)[2,3]. In the present study the relative potency of three different PCB-congeners (PCB126, PCB105, PCB153) and TCDD in promoting altered hepatic foci was studied in female Sprague-Dawley rats. The doses were chosen on the basis of the TEFs assigned the various congeners by Safe[2], which are 0.1 for PCB126, 0.001 for PCB105, 0.00002 for PCB153 and consequently 1 for TCDD. In addition, a combination of the PCB126 and TCDD were administered to rats in order to investigate possible interactions.

Experimental design

The animals were partially hepatectomized and 24 hours later initiated with an intraperitoneal injection with nitrosodiethylamine (NDEA) (30 mg/kg). Five weeks later the promotion treatment was started. The test substances were administered by subcutaneous injections once a week for 20 weeks. During the first treatment the animals were given a loading dose which was five times higher than the following doses.

The weekly doses were:

PCB126 0.316, 1, 3.16, 10, 100 and 1000 μg/kg body weight
PCB105 500, 1500 and 5000 μg/kg body weight
PCB153 1000, 5000 and 20000 μg/kg body weight
TCDD 0.1, 0.316 and 1 μg/kg body weight

In the coexposure experiment the animals were given PCB126 and TCDD in combination. The doses were 1+0.1, 3.16+0.316 and 10+1 μg PCB126+TCDD per kg body weight, respectively.

At sacrifice, samples from the liver were fixed in acetone and embedded in paraffin. Additional samples were frozen until used. Sections of acetone fixed tissue were stained for γ-glutamyltranspeptidase (GGT)[4] and the number and size of GGT-positive altered hepatic foci were evaluated as previously reported[5]. Frozen liver samples were analyzed for cytochrome P450 activity, measured as *O*-dealkylation of 7-ethoxyresorufin (EROD)[6].

Results and discussion

PCB126 administered at the highest dose proved to be lethal within four weeks. Rats administered 1 μg TCDD/kg and 100 μg PCB126/kg had a marked decrease in mean body weight gain compared to the control group. The volume fraction of GGT-positive foci were statistically significant increased in all animals treated with PCB105 and PCB153 (table 1). Only the highest dose of TCDD, 1 μg/kg, resulted in a significant increase of the volume fraction, as did the two highest doses of PCB126, 10 and 100 μg/kg. The potencies, in promoting altered hepatic foci, of the compounds tested can be ordered as follows; TCDD > PCB126 >> PCB105 > PCB153. This order is in agreement with the structure activity relationships for the TCDD-like effects indicated above.

The calculated TEFs, based on the results from the present study, for PCB126 and PCB153 were in the same order of magnitude as those based on overall toxicity assigned by Safe, whilst the TEF for PCB105 was 10 % of Safes TEF. However, comparisons between different PCB-congeners must be interpreted with caution, at least when they are assumed to act through different mechanistical pathways.

When TEFs are assigned to mixtures it has been assumed that the compounds included are acting in an additive manner. Consequently, calculating the TCDD-equivalents given to the animals in this study, the real dose was multiplied with the respective TEF as follows: TCDD 1*1 + PCB126 10*0.1 = 2. The results indicate that the foci formation in animals given TCDD and PCB126 in combination was additive (fig. 2A). By the same approach, the EROD-activity was plotted against the number of TCDD-equivalents given to the animals (fig. 2B). The lack of additive response, when measuring this parameter, could be due to the fact that the maximum, or near maximum response was reach by the individual compounds. In addition, competitive inhibition by PCB126 could reduce the TCDD binding to the receptor, which may decrease the effectiveness of the enzyme induction.

Conclusion

The present investigation demonstrate that all PCB-congeners investigated can act as tumour promoters. A risk assessment based on the TEF concept imply an additive response of the individual congeners. Such a concept will be feasible only when the individual compounds mechanistical pathways, as well as possible interactions, are elucidated. Results from the coexposure to PCB126 and TCDD suggest that these compounds acts via the same mechanistical pathway. This result support that the TEF concept can be used for TCDD-like tumour promoters.

References

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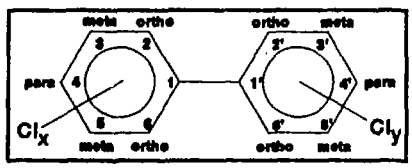
Figure 1. The structure of the PCB molecule.



PCB126 - 3,4,5,3',4'-pentachlorobiphenyl
PCB105 - 2,3,4,3',4'-pentachlorobiphenyl
PCB153 - 2,4,5,2',4',5'-hexachlorobiphenyl

Table 1.

	dose µg/kg	% liver occupied by GGT^+-foci
control[a]		0.6 (0.4–0.9)
control[b]		0.5 (0.4–0.6)
TCDD	0.1[a]	0.8 (0.6–1.0)
	0.316[a]	0.8 (0.4–1.6)
	1.0[a]	2.3*(1.4–3.6)
	1.0[b]	6.7*(3.9–11.5)
PCB126	0.316[a]	0.6 (0.3–1.1)
	1.0[a]	0.6 (0.3–1.1)
	3.16[a]	1.0 (0.7–1.5)
	10[a]	2.4*(1.2–4.9)
	10[b]	4.1*(2.6–6.5)
	100[b]	21.5*(15.6–29.7)
PCB105	500[b]	1.0*(0.6–1.4)
	1500[b]	1.3*(0.8–2.2)
	5000[b]	1.0*(0.6–1.6)
PCB153	1000[b]	1.0*(0.6–1.5)
	5000[b]	2.3*(1.8–3.1)
	20000[b]	2.2*(1.6–2.9)
PCB126 + TCDD	1 + 0.1[a]	1.1 (0.6–1.8)
	3.16 + 0.136[a]	1.2*(0.9–1.7)
	10 + 1[a]	5.3*(3.2–8.9)

* statistical significant different from the corresponding control group (a or b, respectively).

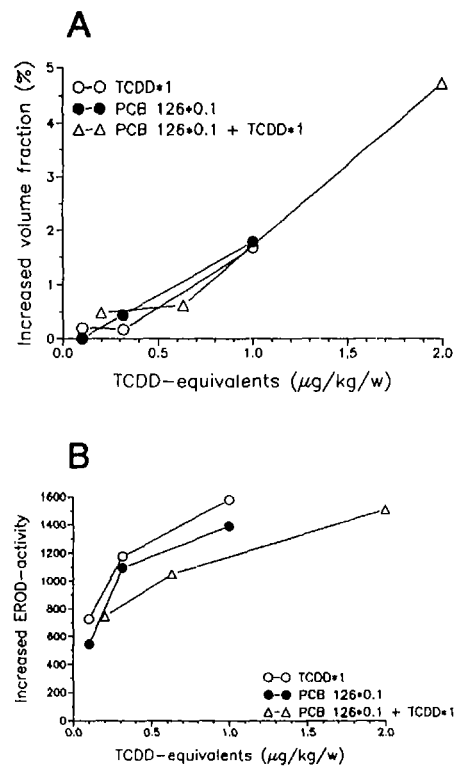


Figure 2. The increase in % liver occupied by GGT^+ foci (A) and increased EROD-activity (B) plotted against the number of TCDD-equivalents given to the animals.