TOX III

Liver Tumour Promoting Activity of 2,3,3',4,4',5-hexachlorobiphenyl in Female Sprague-Dawley Rats.

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1. Introduction

Polychlorinated biphenyls (PCBs) constitute a group of 209 different congeners and the numbers and sites of chlorine substitution determine the chemical and biological characteristics of a specific congener. The general structure of a PCB molecule is shown in Figure 1. The most toxic PCBs are those substituted in both *para*- and at least two *meta*-positions and lacking substitutions in the *ortho*-position. Such congeners have structural and biological similarities with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). If one or more chlorine substituents are introduced in an *ortho*-position, a decreased degree of co-planarity between the two phenyl rings will occur due to steric interactions. In general, the mono-*ortho*-substituted congeners have lower toxic potency than the co-planar, TCDD-like congeners[1].

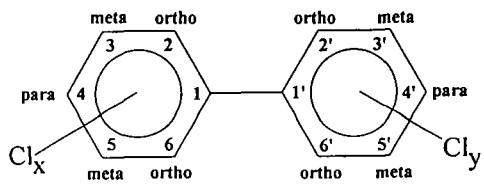

Figure 1. The general PCB structure.

Long-term exposure to mixtures of PCBs has caused cancer in rodent livers. Although some PCB-congeners exhibit a weak genotoxic potential, their primary mode of action in the carcinogenic process is believed to be through tumour promotion[2]. It is important to determine the tumour promoting activity of individual congeners in order to assess their carcinogenic potential and aid human risk assessment.

In the present study, a medium-term two-stage initiation/promotion protocol was used to investigate the ability of 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) to promote the development of glutathione *S*-transferase P (GST-P) positive altered hepatic foci in female Sprague-Dawley rats. An increase in the development and growth of such foci is generally considered to indicate a tumour promotive activity[3].

PCB 156 was chosen because of the high concentrations of this congener in human milk and adipose tissue[4,5] and its high toxicity relative to other mono-*ortho*-substituted PCBs[6]. Furthermore, there is at present no information available regarding the carcinogenic or tumour promotive properties of this PCB congener.

2. Materials and Methods

Test compounds

3,3',4,4',5-pentachlorobiphenyl (PCB 126) and PCB 156 were kindly provided by Professor Å. Bergman, Wallenberg laboratory, University of Stockholm, Sweden. Solutions of PCB 126 and PCB 156 for subcutaneous injections were prepared in corn oil. *N*-nitrosodiethylamine (NDEA) was from Fluka AG Chem Fabrik (Buchs, Switzerland). All other chemicals were obtained commercially in appropriate grades of purity.

Animal treatments

Female Sprague-Dawley rats, weighing approximately 120 g were obtained from B&K Unversal AB (Sollentuna, Sweden) and were housed in wire bottomed, plastic cages (five rats per cage) at 21-22°C under a 12-hours light-dark cycle. After one week of acclimatization, the animals were partially (2/3) hepatectomized and 24 hours later initiated with a single intraperitoneal administration of 30 mg NDEA/kg body weight. Five weeks after the initiation procedure the promotion treatment started. The test compound and PCB 126, which was used as a positive control[7], were administered by subcutaneous injections once a week for 20 weeks. The weekly doses used were 50, 300, 1500 and 7500 µg of PCB 156/kg body weight and 5µg of PCB 126/kg body weight. The first PCB treatment was given as a loading dose which was five times higher than the following 19 maintenance doses. The vehicle control groups were administered corn oil only. One week after the end of the promotion period the rats were killed. Samples taken from the liver were fixed in ice-cold acetone and embedded in paraffin. Sections of this tissue were stained for GST-P positive foci and analyzed according to a method described by Flodström et al. (1988)[8].

Statistics

Data were analyzed by One-Way ANOVA and the Duncan's multiple range test was used to determine the significance of differences between individual treatment groups. Primary data were log-transformed before statistical evaluation to achieve homogeneity of variances.

3. Results

The results from the evaluation of altered hepatic foci are shown in Table 1. The volume fraction of the liver occupied by GST-P positive foci was significantly increased to 3%, 3.5% and 12% in the groups treated with 300, 1500 and 7500 µg PCB 156/kg body weight/week respectively. PCB 126 (5 µg/kg body weight/week) also induced a significant increase in volume fraction of GST-P positive foci.

High doses of PCB 156 caused a decreased body weight gain, thymic atrophy, increased relative liver weight, induction of hepatic cytochrome P450 1A1/2 (CYP1A1/2) and CYP2B1/2 activities, and an increase in the activities of aspartate aminotransferase and γ-glutamyltransferase in plasma (data not shown).

Table 1. Volume fraction of liver occupied by glutathione-S-transferase *P* positive foci after various treatments.

Treatment		Volume fraction (%)	
Substance	Dose	Mean	Standard deviation
Corn oil	-	1.24	0.60
PCB 156	50 μg/kg/week	1.70	1.00
PCB 156	300 μg/kg/week	2.91*	1.19
PCB 156	1500 μg/kg/week	3.34*	1.92
PCB 156	7500 μg/kg/week	11.77*	8.01
PCB 126	5 μg/kg/week	5.21*	3.00

* Significantly different ($P<0.05$) from the vehicle control group.

4. Discussion

PCB 156 increased the volume fraction in a dose-dependent manner after initiation with NDEA and partial hepatectomy, which shows that this mono-*ortho*-substituted congener acts as a growth stimulus of initiated cells and is probably a liver tumour promoter.

Results from this and other studies have demonstrated that both co-planar and non-planar PCB congeners can act as tumour promoters and enhance the development of pre-neoplastic foci and liver tumours in rats and mice initiated by various genotoxic carcinogens[9,10]. Non-planar congeners are reported to be less potent than the co-planar, TCDD-like congeners[11]. Considering the administered doses in this study, the mono-*ortho*-substituted PCB 156 was a less potent enhancer of the development of altered hepatic foci as compared to the non-*ortho*-substituted PCB 126.

The potencies of individual polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls relative to TCDD have been determined using data from short- or medium-term tests for calculations of 'toxic equivalency factors' (TEFs). TCDD, the most potent of these compounds, is assigned a TEF value of one and other compounds are thus given TEF values that are a fraction of the TEF value for TCDD. A TEF for PCB 156 of 0.0005, based on a large data base of *in vitro* and *in vivo* data, has been proposed by Ahlborg et al. (1994)[12]. Safe (1994)[13] has proposed a TEF for PCB 156 of 0.0003, which is also based on *in vitro* and *in vivo* data. In the present study, the TEF for PCB 156 was calculated as volume fraction of liver occupied by foci (PCB 156)/dose (PCB 156) divided by volume fraction (PCB 126)/dose (PCB 126) for the three dose-groups of PCB 156 that were significantly

different from the vehicle control. The resulting fractions were then multiplied by 0.1 (which is a TEF for promotional activity of PCB 126 proposed by Hemming et al., 1995[14]) in order to relate to TCDD. In this way, a TEF for PCB 156 of 0.0001 - 0.001 was achieved. This TEF is in the same range as the TEFs calculated by Ahlborg et al. (1994)[12] and Safe (1994)[13].

In conclusion, the results from the present study show that the mono-*ortho*-substituted congener PCB 156 can cause enhancement of altered hepatic foci in female rats and probably act as a potent tumour promoter of hepatocarcinogenesis. A TEF for PCB 156 based on promotional activity is proposed to be 0.0001 - 0.001 and is comparable to the TEFs for PCB 156 based on studies of other endpoints.

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5. References

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