HEPATOCARCINOGENICITY OF PCB CONGENERS

I. Initiation and promotion of enzyme-altered rat liver foci by 2,2',4,5'-tetra- and 2,2',4,4',5,5'-heaxachlorobiphenyl

R.J. Laib[1], N. Rose[1] and H. Brunn[2] §

[1] Institut für Arbeitsphysiologie an der Universität Dortmund, Ardeystr. 67 D-4600 Dortmund, FRG

[2] Staatliches Medizinal-, Lebensmittel- und Veterinäruntersuchungsamt Mittelhessen, Marburger Str. 54 D-6300 Gießen, FRG

Abstract:

Since about 1980 only technical mixtures of low-chlorinated biphenyls have been used in the Federal Republic of Germany. Although these are more readily degraded in the environment than are mixtures containing high-chlorinated compounds, low-chlorinated congeners such as PCB No. 49 (2,2',4,5') have entered the food chain and are detectable in foodstuffs and in human breast milk. In order to estimate the hepatocarcinogenic properties of low-chlorinated PCB congeners, the ability to initiate and to promote focal alterations of preneoplastic enzymes was determined in rats. The low-chlorinated 2,2',4,5'-tetrachlorobiphenyl which can be metabolised, was compared to the high-chlorinated and persistent PCB No. 153 (2,2',4,4'5,5'-hexachlorbiphenyl). PCB No. 49 initiated ATPase-negative foci in rat liver. Administration of both congeners resulted in growth promotion of ATPase-deficient-foci and GGTase-positive foci initiated by diethylnitrosamine. The promoting ability of the two congeners was approximately equal.

§: For correspondence and reprint requests

Introduction:

An experimental-toxicological evaluation of all polychlorinated biphenyls present in foodstuffs and in human breast milk cannot be practically carried out. For this reason we have suggested six PCBs which, with regard to metabolic degradation and inducer type (PB or MC), could represent all PCBs [1]. For the present experiments we have chosen the two congeners, the **low-chlorinated** 2,2',4,5'-tetrachlorobiphenyl (PCB No. 49) and the **high-chlorinated** 2,2',4,4',5,5'-hexachlorobiphenyl (PCB No. 153). These compounds represent model substances of the phenobarbital type [4] which occur in foodstuffs and in human breast milk and are either subject to metabolic degradation or are persistent. The aim of this study was to quantify the tumour-initiating and tumour-promoting properties of these model substances. A very sensitive animal test system was used, based on quantitation of focal preneoplastic enzyme alteration (Nucleoside-5'-triphosphatase = ATPase, gamma-glutamyltranspeptidase = GGTase) in the liver of newborn rats [5]. These focal alterations must be considered precursors of liver tumours and can be used by morphometric methods to quantify the carcinogenic properties of a substance.

Experimental Part:

Chemicals:

PCB No. 49 was synthesised by CADOGAN coupling and PCB No. 153 was synthesised by ULLMANN condensation using argon as inert-gas protection. The preparations were purified by double-column chromatography on aluminium oxide / florisil and double recrystallisation from ethanol. The purity of the preparations was 99%. They were free of detectable chlorinated dibenzodioxins and dibenzofurans. Diethylnitrosamine (1-nitroso-diethylamine, DENA) was from Serva (Heidelberg, FRG). All other chemicals were obtained from Merck (Darmstadt, FRG) and were of p.a. quality.

Procedure:

Possible tumour-initiating properties of both PCB congeners were studied as follows:
4 groups of 10 each new-born, female Sprague-Dawley rats received orally, once daily 5 mg/kg body weight PCB No. 49 or PCB No. 153 dissolved in condensed milk, for a period of three weeks. Two of the thus-treated animal groups additionally received phenobarbital (0.05% in their drinking water for an additional 8 weeks, beginning one week after the end of PCB treatment.

To test both compounds for tumour-promoting properties, four groups of new-born animals were treated with a single i.p. injection of the liver carcinogen, diethylnitrosamine (DENA) at a dose of 15 mg/kg body weight in order to initiate preneoplastic liver lesions. After a period of four weeks, the animals received PCB No. 49 or PCB No. 153 orally once weekly at a dose of 50 mg/kg body weight for eight weeks. The corresponding control groups received either the solvent or were not further treated in any manner.

At an age of 13 weeks, the animals were sacrificed, the livers were excised, cryosections were prepared and examined for preneoplastic ATPase-negative or GGTase-positive foci. The percentage of areas with focal enzyme alterations relative to the total area of liver tissue measured was chosen as the critical parameter for the relative tumour-initiating and promoting efficiency of the respective PCB [5].

Abbreviations:

PB: Phenobarbital MC: Methylcholanthrene DENA: Diethylnitrosamine ATPase: Nucleoside-5'-triphosphatase GGTase: gamma-Glutamyltranspeptidase (EC 2.3.2.2)

Results and Discussion:

Both congeners acted as growth promoters of ATPase-negative and GGTase-positive foci that were initiated by DENA (Fig. 1, 2, Schedule B). These results corroborate other studies that attribute growth-promoting properties to PCB congeners [6]. It would appear that the most important factor in determining the degree of promotion is the inducer type (MC or PB), however, the biological half-life may also play a role [4,6]. In the test system used here, growth promotion by the low-chlorinated congener No.49 was about equivalent to that of the high-chlorinated biphenyl No. 153.

The low-chlorinated and metabolisible congener No. 49, initiated ATPase-deficient foci, but failed to initiate GGTase-positive foci (Fig. 1, 2, Schedule A). An increase in the number and area of ATPase-negative foci was not observed as a result of phenobarbital treatment. The area of ATPase-negative foci was about a factor of 20 smaller after three-weeks treatment with PCB No. 49 than it was after a single dose of DENA. The results of the present study indicate that the weak tumour-initiating properties of technical mixtures [7] are a result of the metabolisible congeners. With regard to a possible carcinogenic risk of PCBs present in foodstuffs and human breast milk, the low-chlorinated biphenyls would thus appear to be of major importance.

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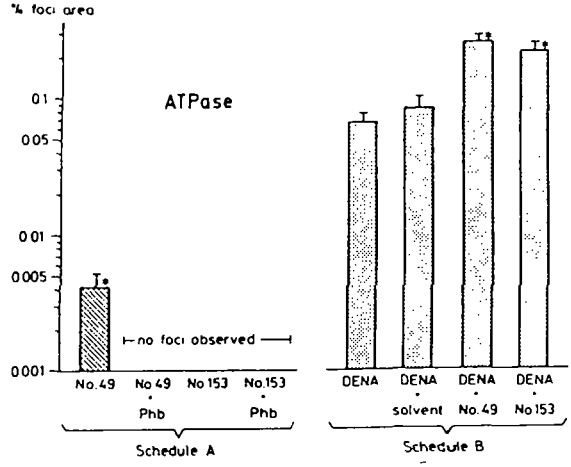

Fig. 1: Initiation and promotion of ATPase-negative foci in rat liver by 2,2',4,5'-tetra- and 2,2',4,4',5,5'-hexachlorobiphenyl.

*: $p < 0.05$ (Control: DENA + solvent)

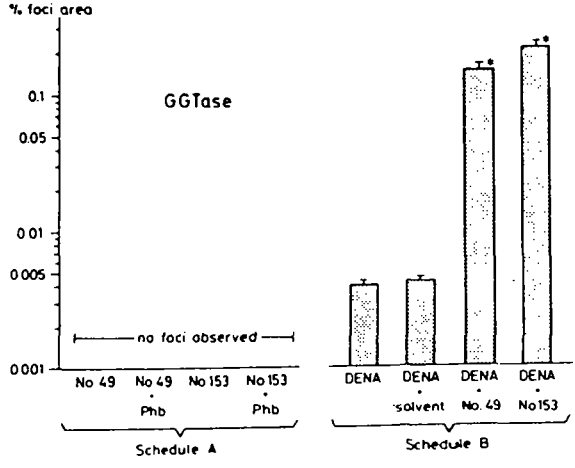

Fig. 2: Initiation and promotion of GGTase-positive foci in rat liver by 2,2',4,5'-tetra- and 2,2',4,4',5,5'-hexachlorobiphenyl.

*: $p < 0.05$ (Control: DENA + solvent)