HEPATOCARCINOGENICITY OF PCB CONGENERS

II. Covalent binding to liver macromolecules and modification of carbohydrate metabolism

A. Braun[1*], E. Eigenbrodt[2], R.J. Laib[3] and H. Brunn [1**]

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

[2] Institut für Biochemie und Endokrinologie des Fachbereiches Veterinärmedizin der Justus-Liebig-Universität Gießen, Frankfurter Str. 100 D-6300 Gießen, FRG

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

Abstract:

We have investigated the hepatocarcinogenic mechanisms of 2,2',4,5'-tetrachlorobiphenyl (PCB No. 49) and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB No. 153). PCB No. 49 was incubated with isolated hepatocytes from rats and chickens that had been stimulated with phenobarbital. We could show that this low-chlorinated biphenyl is metabolically activated in the liver cells and covalently binds to nucleic acids and proteins in vitro. Enzyme activities (for pyruvate kinase, fructose-1,6-bisphosphatase and malic enzyme), which show specific changes in tumour cells, were determined in liver homogenates of rats and chickens treated with diethylnitrosamine (DENA) and PCB. Our results support the concept that tumour promotors that affect the liver, alter hepatocellular activities of key enzymes.

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**For correspondence and reprint requests

Introduction:

Since the biotransformation of low-chlorinated biphenyls occurs more readily than does that of higher-chlorinated congeners, the former molecules are more likely to be metabolically activated to reactive epoxides (arenoxides), which may covalently interact with nucleic acids. Consequently, the question arises as to a possible gene toxicity of 2,2',4,5'-tetrachlorobiphenyl (PCB No. 49).

Tumour promotors are thought to alter carbohydrate metabolism in the liver by the activation/deactivation of key enzymes [1]. Such changes can be measured in transformed liver cells, in primary liver tumours and in tissue of the host liver [2,3]. For chlorbiphenyls, however, no data have yet been obtained which would support these hypotheses.
Studies were carried out on the mechanisms of carcinogenicity of 2,2',4,5'-tetra- (PCB No.49) and 2,2'4,4'5,5'-hexachlorobiphenyl (PCB No. 153). Radioactive labelled PCB No. 49 was incubated with isolated hepatocytes from phenobarbital-stimulated rats and chickens and covalent binding to nucleic acids and cell proteins was measured in vitro. In liver homogenates from chickens and rats which had been treated with diethylnitrosamine (DENA) and subsequently with PCB, changes in enzyme activities were measured for pyruvate kinase (PK), fructose-1,6-bisphosphatase (FBPase) and malic enzyme (ME), all of which are specifically altered in tumour cells. In addition, the relative liver weights from the experimental animals were determined.

Experimental Part:

The synthesis of ^{14}C-labelled PCB No. 49 from 2,4-dichloro^{14}C-anilin was done by CADOGAN-coupling. Isolation and incubation of hepatocytes, detection of radioactively labelled metabolites from the incubation medium, isolation and purification of hepatocellular DNA, RNA and proteins as well as the quantification of covalently bound radioactivity and determination of enzyme activities in liver homogenates was performed according to standard procedures. For the treatment of rats and chickens with DENA and PCB No. 49 and No. 153 see Methods in part 1 of this report in this volume [4]).

Abbreviations:

PK: pyruvate kinase (EC 2.7.1.40) FBPase: fructose-1,6-bisphosphatase (EC 3.1.3.11) ME: NADP Malic Enzyme (EC 1.1.40), TCBE: Equivalents of radioactive labelled 2,2',4,5'-tetrachlorobiphenyl

Results and Discussion:

Mass-spectrometric determination of a radioactively labelled 2,2',4,5'-tetrachlorbiphenylol in the supernatant of the hepatocyte fraction from chickens, showed that PCB No.49 was activated, apparently through oxidatative metabolism, by the liver cells. Consequently, PCB No. 49 was able to covalently bind to DNA and proteins from isolated rat and chicken hepatocytes after incubation in vitro (Rats: 12 pmol TCBE/mg DNA[1)], 1050 pmol TCBE/mg protein[1)]. Chickens: 3.5 pmol TCBE/mg DNA[1)], 565 pmol TCBE/mg protein[1)]). Our results indicate a connection between metabolic activation, covalent DNA binding and liver-tumour initiation for PCB No. 49. The demonstration of covalent binding in vitro support our results on weak initiating properties of PCB No. 49 seen in vivo[4].

[1)] mean values from three animals

Technical mixtures are weak tumour initiators. Metabolic epoxidation and covalent binding to DNA may thus be the general principle for tumour initiation by chlorobiphenyls with low chlorine content.

Tumour promotion is considered to be related to changes in carbohydrate metabolism [1-3]. Consequently, activities were determined for the glycolytic enzyme PK, the gluconeogenic enzyme FBPase and the glutaminolytic enzyme ME in liver homogenates from rats (Fig. 1) and chickens (Fig. 2) that had been treated with DENA alone or in combination with PCB No. 49 and No. 153: The application of the tumour initiator, DENA practically did not change these enzymatic activities in the livers of rats and chickens. Consequently, the reduction in PK activity observed in both species is interpreted as specific for the tumour-promoting effect of the two PCB congeners examined. A statistically significant reduction in FBPase activity could only be found after treatment with the high-chlorinated congener. This can be explained by a presumed greater promoting capability of this congener compared to that of PCB No. 49. A significant reduction in FBPase activity seen in chickens treated with DENA alone cannot be explained at this time. Only rats showed an increase in activity of ME, which had been treated with DENA or with DENA and PCB. In addition to an early rise in ME activity in preneoplastic cells [5], xenobiotics, which are biotransformed by a detoxification reaction, thus consuming NADPH, result in a rise in activity of this enzyme in the rat liver [6]. Accordingly, these results may be explained by a stimulation of the metabolism of xenobiotics and/or conversion of carbohydrate metabolism in the form of compensation of the pyruvate deficit.

Our results are corroborated by the measurements of relative mass of the livers of DENA as well as DENA plus PCB treated animals (Fig. 3). Both PCB congeners led to a significant increase in the relative liver mass of both rats and chickens when compared to the controls (DENA alone).

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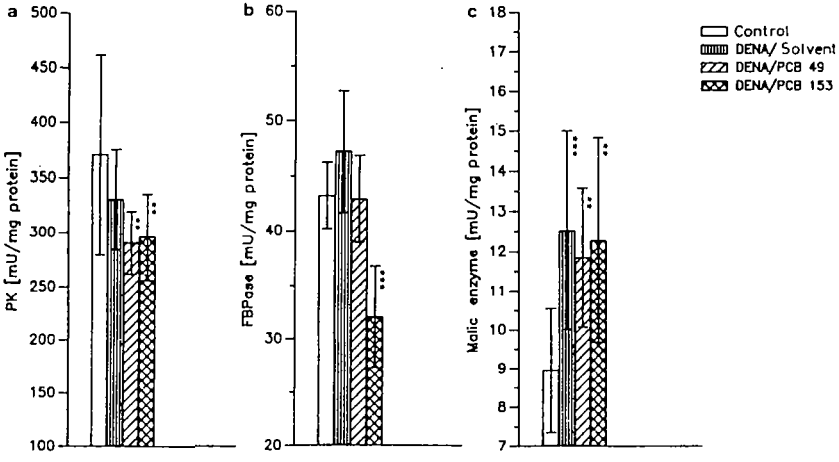

Fig.1: Enzymatic activities of PK, FBPase and ME in liver homogenates of DENA and DENA + PCB-congener-treated rats.

*: $p < 0.05$ **: $p < 0.01$ ***: $p < 0.001$

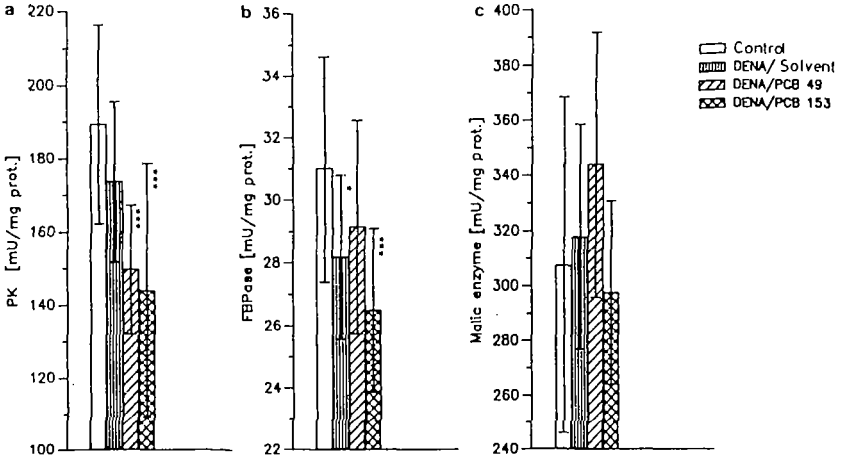

Fig.2: Enzymatic activities of PK, FBPase and ME in liver homogenates of DENA and DENA + PCB-congener-treated chickens.

*: $p < 0.05$ **: $p < 0.01$ ***: $p < 0.001$

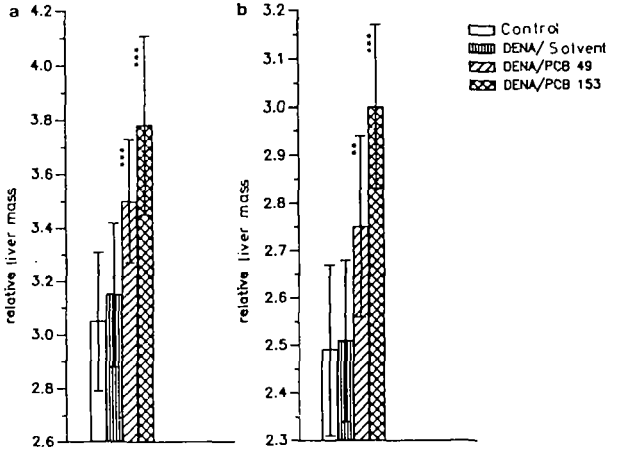

Fig. 3: Relative liver mass (g/100g BW) of DENA and DENA + PCB-congener-treated rats (a) and chickens (b).

*: $p < 0.05$ **: $p < 0.01$ ***: $p < 0.001$