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# Effects of Polychlorinated Biphenyls (PCBs) on *in vitro* Biosynthesis of Testosterone and Cell Viability in Mouse Leydig Cells

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Polychlorinated biphenyls (PCBs) were used for many years in capacitors, transformers, hydraulic fluids, paints etc. However, since the discovery that they are accumulated in the environment (Jensen 1966) and have detrimental effects on organisms in the ecosystem, especially with respect to reproduction, their use has been severly restricted. PCBs are inducers of the hepatic microsomal monooxygenase system (Yoshimura et al. 1979), for which cytochrome P-450 is the terminal oxidase. Besides being responsible for metabolism of toxic substances the cytochrome P-450 system is involved in the metabolism of endogenous substances, such as steroid hormones (Conney and Kuntzman 1971), and it has been shown that PCBs can increase the metabolism of steroids via this system (Nowicki and Norman 1972; Conney et al. 1973). The increased metabolism of the steroid hormones is considered responsible for the effects on reproduction observed in many species of animals after exposure to PCBs (Örberg and Kihlström 1973; Allen et al. 1979; Biessmann 1982; Sager 1983; a.o.). However, it has also been shown that the decreased levels of steroid hormones seem to be soon compensated for by increased synthesis, owing to feedback mechanisms (Örberg and Lundberg 1974). Induction of steroid metabolism is probably not the only way that PCBs can affect steroid hormone levels: there are also indications that PCBs can directly affect synthesis of steroid hormones in the adrenals and gonads (Freeman and Sangalang 1977; Fuller et al. 1980). It has furthermore been shown that the related substance 2,3,7,8,-tetrachlorodibenzo-p-dioxin (TCDD), which causes androgenic deficiency (Moore et al. 1985), induces certain subspecies of the cytochrome P-450 system but not the ones responsible for steroid metabolism (Hook et al. 1975). Tofilon and Piper (1982) attributed decreased androgen synthesis after TCDD exposure to a decrease in the amount of microsomal cytochrome P-450, which is an essential enzyme for androgen biosynthesis in the testis.

Some PCBs show a tendency to accumulate in steroid-producing organs such a the adrenals, testes (Brandt 1977) and ovaries (Bi-

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essmann 1981). Moreover, some hexachlorobiphenyls are accumulated in the interstitial part of the testis, where the steroid-producing cells are located (Brandt 1977).

In an earlier study (Johansson 1987) we investigated the in vivo effects of PCBs on mice. We could not find any evidence for effects of Clophen A50 and 2,2',4,4',5,5'-hexachlorobiphenyl on plasma testosterone levels or on the ability of the Leydig cells to respond to luteinizing hormone (LH). Despite these results we wanted to determine whether PCBs have any effect on testosterone synthesis when administered to Leydig cells in vitro, since it has been shown earlier that a substance having no effects on testosterone synthesis when given in vivo can have drastic effects when administered in vitro (Pont et al. 1982).

## MATERIALS AND METHODS

Twenty 10 to 12 wk-old male mice (NMRI) were purchased from ALAB, Sollentuna, Sweden. They were housed 10 per cage at room temperature under a 12 h light/12 h dark cycle and fed standardized pellet food (Ewos, Sweden) and tap water ad lib.

1,2,6,7-$^{3}$H-Testosterone (specific activity: 107 Ci/mmol) was purchased from the Radiochemical Center, Amersham, England. Antiserum to testosterone was generously provided by Dr. Sten Cekan, Reproductive Endocrinology Research Unit, Karolinska Hospital, Stockholm, Sweden. The 2nd international reference preparation of pituitary FSH and LH (ICSH) human for bioassay (code 78/549) was kindly provided by the National Institute for Biological Standards and Control, London, England. Clophen A40 (average M.W. 295) and Clophen A50 (average M.W. 329) were purchased from Bayer, Leverkusen, BRD. 3,3',4,4'-Tetrachlorobiphenyl (purity >98%, M.W. 292.0) was provided by Dr. Å. Bergman, Wallenberg Laboratory, University of Stockholm, Sweden, and 2,2',4,4',5,5'-hexachlorobiphenyl (purity >99%, M.W. 360.9) was supplied by Drs. C.A. Wachmeister and G. Sundström at the same laboratory. For details about all other chemicals, see Johansson (1987).

Testes from four mice were used for each in vitro incubation. After the testes had been decapsulated, a cell suspension was prepared by mechanical dissociation, as described by Van Damme et al. (1974).

To test tubes containing 100 µL of phosphate buffer with 0.1% bovine serum albumin or 100 µL of LH (400 µIU) and 200 µL of the interstitial cell suspension (approx. $3 \times 10^{4}$ cells), 50 µL of each of the various substances studied was added. Five replicates were used for each treatment. Before addition, the substances were first dissolved in 99.5% ethanol and diluted (1:10) in cell culture medium (Medium 199 with Earle's salts supplemented with 2% newborn bovine serum and 0.68 mM L-glutamine obtained from Flowlab, Stockholm, Sweden). Ethanol diluted in medium was used as a control. The doses administered were 0.5, 5.0 and 50.0 µg, with the exception of 3,3',4,4'-tetrachlorobiphenyl which was given at 0.05, 0.5 and 5.0 µg doses. These mixtures were incubated for 3 hr

at 34°C, 6.5% $CO_2$ in air and 80% humidity with continous shaking of the tubes (350 rpm). Two tubes from each treatment were used to check cell viability. After centrifugation at 780 rpm (130xg) the supernatants were discarded, 200 µL of a trypan blue solution (0.1% in phosphate buffer) added and the cells counted in a Bürker chamber. To the tubes (in triplicates) to be used for testosterone assay 500 µL of phosphate buffer with 0.1% BSA was added, after which the tubes were placed in a refrigerator overnight.

Aliquots (200µL) of the contents in the tubes were transferred to new tubes, and the amount of testosterone formed was measured by radioimmunoassay without extraction or chromatography. One hundred microliters of a mixture of $^3$H-testosterone (21 nCi) and antiserum to testosterone (1/1500) was added to all tubes, except the ones used for non-specific binding (no antiserum). The samples were incubated at 60°C for 10 min and at 30°C for 20 min, after which they were put on ice for 5 min. To separate antibody-bound from free hormone charcoal was used. After the charcoal suspension had been added the tubes were gently stirred and incubated at 4°C for 30 min and then centrifuged (4°C, 1700xg) for 5 min. The supernatant containing the antibody-bound hormone was decanted into plastic scintillation vials and mixed with 4 ml of liquid scintillator (Miniria 20, Koch-Light, England). The level of radioactivity was then determined with a liquid scintillation counter (Ultrobeta 1210, LKB-Wallac, Finland).

Student's t-test, with correction of the t-values for small samples according to de V. Weir (1960), was used for the statistical analyses. The level of significance was set at p-values less than 0.05.

## RESULTS AND DISCUSSION

Testosterone synthesis was stimulated when cells were exposed to 2,2',4,4',5,5'-hexachlorobiphenyl in the absence of LH (Fig. 1). This effect was somewhat unexpected, since the viability of the cells was very low at the end of the incubation. It could perhaps be explained by a time lag between the addition of the substance and the response. The increased level of testosterone found after 3 hr incubation could have resulted from an increased rate of synthesis during the first hr, when most of the cells were still viable. Even for LH, the natural regulator of testosterone synthesis, there is a time lag between addition and response. One possible explanation for this stimulation could be that 2,2',4,4',5,5'-hexachlorobiphenyl was able to bind to LH-receptors and possessed a hormone-like effect. Various low-chlorinated PCBs and the structurally related DDT have been shown to bind to estrogen receptors and exert estrogen-like effects (Bitman and Cecil 1970). The other substances tested had no effect on the basal synthesis of testosterone.

The two commercial mixtures Clophen A40 and A50, which seemed to inhibit LH-stimulated testosterone synthesis in a dose-dependent way (Fig. 1), also caused a dose-dependent decrease in cell viability. Calculations of testosterone levels per viable cell did not

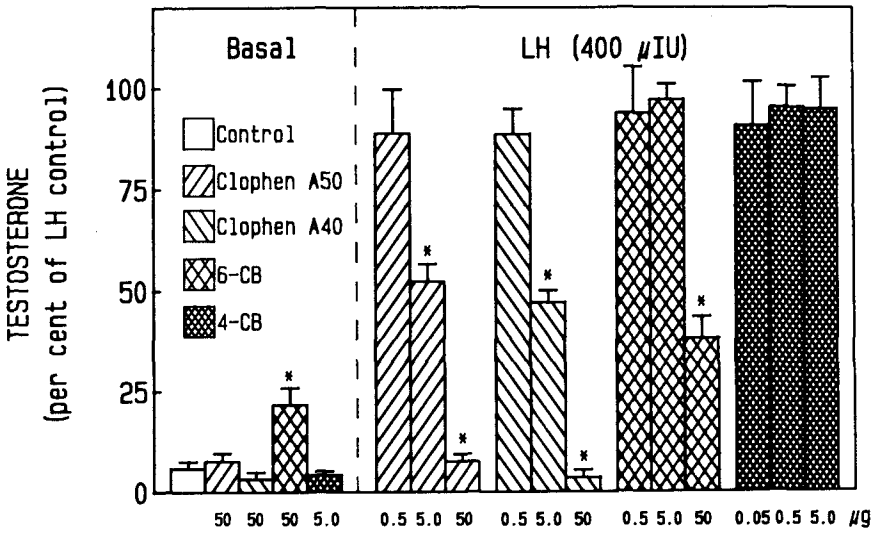


Figure 1. Effects of different polychlorinated biphenyls on testosterone production by mouse Leydig cells. Leydig cells were incubated for 3 hr in the absence or presence of LH (400 μIU). The testosterone concentrations are calculated per number of added cells. Bars indicate the mean ± S.D. of five experiments. The statistical difference between controls and treatments is indicated by asterisk, * $P<0.01$.

reveal any significant differences between the two groups receiving the lower doses and the controls. After the highest concentration of the two Clophens was added to the suspension no "normal-appearing" cells could be found, explaining why there was no testosterone production. Technical mixtures of PCBs have been shown to be cytotoxic in experiments with Chinese hamster cells (Rogers et al. 1983). The Clophen doses causing cytotoxicity in this study were similar to those causing cytotoxicity in the hamster cells. It was surprising that 3,3',4,4'-tetrachlorobiphenyl showed no activity of any kind - not even affecting cell viability - since it has been shown to be a very toxic substance - e.g. in chick embryos (Brunström and Darnerud 1983).

The results presented here suggest that 2,2',4,4',5,5'-hexachlorobiphenyl can stimulate the basal synthesis of testosterone. In contrast there was no evidence that any of the PCBs can affect LH-stimulated testosterone synthesis, since the effects shown can be explained solely on the basis of decreased cell viability.

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