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Quantitative Alterations in the Liver and Adrenal Gland in Pregnant Rats Induced by Pyralene 3000

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Polychlorinated biphenyls (PCBs) are among the most widespread environmental pollutants known in the world. The half-life of PCBs is very long and, therefore, once released into the environment, they accumulate in food chains and tissues of various mammals, including man. Their presence can cause numerous toxic effects, e.g., hepatotoxicity, immunotoxicity, dermatotoxicity, neurotoxicity, and disorders of the reproductive system, among others (Parkinson and Safe, 1987). These effects depend on the distribution route in the organism, the rate of metabolism and excretion. Their characteristics are closely associated with the number and position of the chlorine atoms in the molecule (Sipes and Schnellmann, 1987).

Previous studies of trichlorobiphenyl distributions in various tissues demonstrated that low chlorinated trichlorobiphenyls do not accumulate in endocrine organs, whereas higher chlorinated biphenyls, such as hexa- and octachlorobiphenyl, are deposited and retained in the adrenal gland (Brandt, 1977). A selective distribution of radiolabelled tetrachlorobiphenyl to the zona fasciculata, accompanied by morphometric evidence of the hypertrophy of the zona fasciculata, was also noted (Durham and Brouwer, 1990).

The purpose of this study was to examine changes in the tissue structure of the pregnant rat liver and adrenal gland induced experimentally by Pyralene 3000 administration. We chose this commercial low chlorinated PCB because it was in use in Slovenia and, discharged from the electroindustrial plants, caused a serious incidence of environmental pollution in the region of Bela Krajina (Brumen et al., 1984). Our further aim was to research the transplacental influences of Pyralene 3000 in rats.

MATERIALS AND METHODS

Pyralene 3000 was obtained from Prodelec (Paris, France). It presents a PCB technical mixture similar to Aroclor 1242, and the estimated mass percentages for izomers Cl-2, Cl-3, Cl-4 and Cl-5 are 10.8%, 58.4%, 30% and 0.8%, respectively.

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Adult female Wistar rats, weighing 230-250 g, were mated overnight with males. Vaginal smears were taken in the morning and the presence of sperm was considered as day 0 of gestation. Fourteen rats were given 60 mg/kg of Pyralene 3000 in 1 ml neutral olive oil by intraperitoneal injection on days 7 and 9 of the pregnancy. Six control animals were injected with vehicle only. Rats were sacrificed on the day 21 of pregnancy, by deep ether anesthesia and fetuses were delivered by caesarean section. The number of fetuses, their body and liver weights were recorded. The fetuses were then placed into Bouin's solution. Maternal body weight was also recorded and liver and adrenal glands were removed and weighed. The absolute liver volumes (Vo) were also measured. The organs were preserved in Bouin's solution for histologic and morphometric study. The specimens were processed and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (H&E). Periodic acid-Schiff (PAS) staining and PAS-Amylase controls were also done for the detection of glycogen (Drury and Wallington, 1980).

For the morphometric analysis of the liver tissue structure, the H&E stained sections were used. Fifty adjacent fields on serial tissue sections were counted for morphometric measurements using stereological technique. The multipurpose test system M42 (Weibel, 1979) with 21 test lines enclosed in a frame was applied at 40x magnification. The end points of test lines served as markers for volume estimation. Every point (P) that fell on hepatocyte cytoplasm (Pc), hepatocyte nuclei (Pn) and sinusoids (Ps) were recorded. The volume densities (Vv) of particular structure within the total parenchymal volume were then calculated as $V_{v(c,n,s)} = P_{(c,n,s)}/Pt$; (Pt = 2100). The surface density of hepatocyte nuclei (S_{vn}) was derived from counts of the intersection points of the surface contour of nuclei (In) with test lines (Lt) of known length ($S_{vn} = 2\ In/Lt$). From the above data the following parameters then were calculated: absolute volume of hepatocyte cytoplasm ($V_c = V_{vc} \times V_o$); absolute volume of hepatocyte nuclei ($V_n = V_{vn} \times V_o$); absolute volume of sinusoids ($V_s = V_{vs} \times V_o$); absolute surface of hepatocyte nuclei ($S_n = S_{vn} \times V_o$); and the volume density ratio nuclei/cytoplasm (V_{vn}/V_{vc}) of hepatocytes.

A linear ocular micrometer was used for the determination of the ratio cortex/medulla of adrenal glands, and within the cortex, the ratios between the zones. All data were statistically evaluated by the Student's t-test and significance was ascribed to $p<0.05$.

RESULTS AND DISCUSSION

Pregnant rats in treatment and control groups were clinically normal during the course of the experiment. There were no significant differences in the average number of fetuses which was 10.6 ± 0.9 in control group and 10.7 ± 1.9 (mean $\pm$ SD) in the treated group.

Table 1 indicates that our protocol of low chlorinated commercial Pyralene 3000 administration increased both maternal and fetal liver/body ratios. Studies by Takagi et al. (1976, 1986) showed that the average amount of lower chlorinated PCBs transferred from the dams to the fetuses after peroral treatment was less than 0.028% of the unexcreted dose. In pregnant rats this was approximately 30% of the

Table 1. Effects of Pyralene 3000 on body and organ weights of mothers and fetuses.

	Control Group	Treated Group
Final Body Wt. (g)	328.4 ± 34.8	331.0 ± 43.9
Corrected Body Wt. (g)	291.6 ± 14.4	287.0 ± 31.4
Adrenal Gland Wt. (g/100 g CBWt.)	0.030 ± 0.01	0.026 ± 0.01
Maternal Liver Wt. (g/100 g CBWt.)	3.5 ± 0.4	4.1± 0.6 *
Fetal Liver Wt. (g/100 g body wt.)	6.6 ± 0.7	7.5 ± 1.2 *

Data are mean ± SD (n=6 in the control group of female adults; n=14 in the treated group of female adults; and n=18 for fetuses from control group; n=42 for fetuses from treated group). Corrected body weight (CBWt.) is the final body weight minus fetuses weight. *Significantly different from control group ($p<0.05$).

dose applied. When the higher chlorinated PCBs were administered, 0.03% of the absorbed PCB was transferred. Pyralene 3000 contains 42% chlorine by weight; therefore, we may estimate that a relatively low concentration of PCBs (<1.0 mg/kg of body weight) was sufficient to cause changes in the liver/body ratios in the fetuses.

A significant increase in the absolute volume of the hepatocyte cytoplasm was established (Table 2) in the treated group. In addition, a moderate but non-significant increase in the absolute volume and surface of hepatocyte nuclei was observed. Similarly, 5-days of phenobarbital treatment in rats has been reported to cause liver hypertrophy via enlargement of the hepatocyte cytoplasm with an insignificant effect on nuclei (Stäubli et al., 1969). The obtained hepatocyte nuclei/cytoplasm volume density ratio was significantly lower in the treated group of animals.

In our experiment the quantitative alterations were, therefore, observed primarily for the cytoplasm of hepatocytes. This could be the consequence of an increased amount of the smooth endoplasmic reticulum and of the mitochondria. According to Parkinson and Safe (1987), industrial PCB mixtures have the abilities to exhibit properties of both phenobarbital (PB) and 3-methylcholanthrene (MC) type xenobiotics, and thus to induce cytochromes P-450a - P450-e in the rat liver. The PB inducible cytochromes P-450 are mainly located in the perivenous zone and MC inducible cytochromes in the periportal and midzonal zones (Baron et al.,1981). PB-type inducers increase the content of smooth endoplasmic reticulum, especially

Table 2. Effect of Pyralene 3000 on volume densities of hepatocytes in female rat liver.

	Control Group	Treated Group
Absolute volume of hepatocyte cytoplasm (Vc; mL)	7.4 ± 1.0	9.3 ± 1.4 *
Absolute volume of sinusoids (Vs; mL)	1.9 ± 0.3	1.7 ± 0.3
Absolute volume of hepatocyte nuclei (Vn; mL)	0.51 ± 0.1	0.58 ± 0.1
Absolute surface of hepatocyte nuclei (Sn; mm^2)	319.6 ± 38.4	351.2 ± 61.6
Nuclei/cytoplasm volume density ratio (V_{Vn}/V_{Vc})	0.074 ± 0.007	0.060±0.003 *

Data are mean ± SD (n=14 in the treated group; n=6 in the control group). *Significantly different from control group ($p<0.05$).

in the perivenous hepatocytes (Gillette et al.,1987). However, there are many drugs which produce smooth endoplasmic reticulum hypertrophy in the hepatocytes (Ghadially, 1977). Smooth endoplasmic reticulum hypertrophy could be regarded as an adaptive response by which hepatocytes enhance their ability to handle different drugs.

The analysis of PAS and amylase-stained liver sections showed a general depletion of glycogen in both periportal and perivenous zones of the livers of treated animals (Figure 1). The depletion of glycogen particles was previously described for intoxication with phenobarbital (Tanikawa, 1968; Stäubli et al., 1969), as well as for other hepatotoxicants, e.g., for carbon tetrachloride-induced cirrhosis in rats (Krahenbuhl et al., 1991). The described depletion of glycogen particles in hepatocytes was, therefore, not specific; however, it could serve as an indicator for the enhanced activity of enzymes in the cytosol and smooth endoplasmic reticulum, and for an accelerated transition from the fed to the fasted liver state.

Morphometric analysis of the adrenal gland indicated that the cortex-medulla ratio was not altered by PCB administration. However, when the zonation of the cortex was analysed, PCB treatment caused an increase and decrease in the zona fasciculata and the zona reticularis, respectively (Table 3). Since significant differences were not found in relative adrenal gland weight or in the cortex/medulla ratio between the groups, we may conclude that the relative reduction of zona reticularis observed in the adrenal cortex of treated rats was associated with the enlargement of the zona fasciculata.

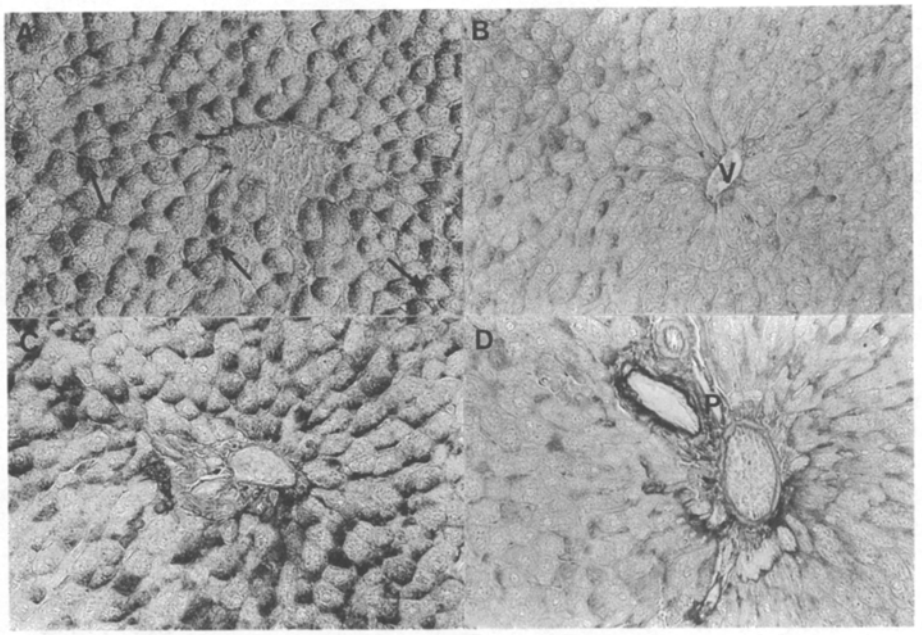

Figure 1. Light micrographs of perivenous (panels A,B) and periportal (panels C,D) regions of control (panels A,C) and PCB-treated (panels B,D) livers from female rats. Arrows (panel A) indicate glycogen particles. V, central vein; P, portal triad. PAS staining, x 400.

Table 3. Effect of Pyralene 3000 on the proportion of individual zones in the adrenal gland cortex in female rats.

	Control Group	Treated Group
Z. glomerulosa (%)	5.8 ± 0.7	6.1 ± 0.7
Z. fasciculata (%)	59.9 ± 2.0	64.0 ± 3.6 *
Z. reticularis (%)	34.3 ± 2.0	29.9 ± 3.9 *

Data are mean ± SD (n=14 in the treated group; n=6 in the control group)
* Significantly different from the control group ($p<0.05$).

Recent studies have shown that PCBs are localized in zona fasciculata of the adrenal cortex (Durham and Brouwer; 1990), the primary site of adrenal production of corticosterone. Accumulation of hydrophobic xenobiotics may alter the catalytic activities of the cytochrome P-450 mediated system. PCB compounds of Aroclor 1254 diminish the activity of P-450 C21-hydroxylase and enhance the activity of P-450 17α-hydroxylase. This selective effect may lead to a decrease in corticosteroids and increased androgen production by the adrenal cortex (Goldman and Yawetz, 1992). Because of this enzyme disproportion, a higher ACTH secretion could

occur. Prolonged periods of higher ACTH levels can cause similar morphological alterations in the adrenal cortex as PCB administration (Nussdorfer, 1986). Durham and Brouwer (1990) have also shown that after tetrachlorobiphenyl administration, hypertrophy of the zona fasciculata and its parenchymal cells results from increases in the mitochondrial and endoplasmic reticulum compartments. We, therefore, suggest that the observed morphological differences could be indirectly caused by elevated ACTH secretion as well as directly by PCBs.

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