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Subchronic toxicity of 3,3',4,4'-tetrachlorobiphenyl in the rat liver: An electron microscope study

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Summary. Ultrastructural effects of 3,3'4,4'-tetrachlorobiphenyl (PCB) congener #77 on the liver were evaluated following its feeding to Sprague-Dawley weanling rats. Treatment diets were prepared by dissolving the congener in 4% corn oil. Ten animals, either male or female, in each group were placed on the respective diets containing 10, 100, 1,000 and 10,000 ppb congener for 13 weeks. Ten animals of each sex served as the control that had only the oil added to the diets. In the congener-exposed animals the alterations consisted of a marked increase in the profiles of smooth endoplasmic reticulum, and in the heightened number of lipid droplets in many parenchymal cells. Several mitochondria showed abnormalities such as dumbbell shapes, and in others, the cristae were oriented parallel to the long axis of the organelle. Peroxisomes were numerous in the 10 ppb group and apparently had increased numerically in the liver of animals from the higher dose groups. Females were notably more affected by the congener when compared to their male counterparts. The results indicated that the compound is mildly toxic, and alteration in structure and function can be noted at the lowest dose used (10 ppb congener exposure). It is concluded that congener #77 may be moderately toxic and it may affect the overall health of the exposed animal.

Key words: PCB congener #77, Rat liver, Electron microscopy

Introduction

Polychlorinated biphenyls (PCBs) are persistent environmental contaminants (Kasza et al., 1976), which are among the most abundant chlorinated hydrocarbon pollutants known worldwide (Norback and Allen, 1972). The contamination is the result of widespread commercial use of these compounds for their properties of heat resistance, adhesiveness, thermoplasticity, chemical stability, and low volatility (Norback and Allen, 1972; Azatori et al., 1991). A potential health hazard exists for humans, and domestic and wild animals because of the PCBs long half-life and capacity to bioaccumulate in the aquatic and terrestrial food chains (Clarke et al., 1984; Safe, 1984, 1990; Azatori et al., 1991). The degree of toxicity of a PCB congener is determined by the number of chlorines on the biphenyl rings (Hansell and Ecobichon, 1974; Safe, 1984, 1990). Several studies (Fishbein, 1974; Kasza et al., 1978; Lin et al., 1979; MacLellan et al., 1994) have reported the ultrastructural alterations in the liver of mammals administered PCB. Hepatotoxicity induced by PCBs is known, in general, to be more severe in the females than in the males (Wassermann et al., 1979; Clarke et al., 1984; MacLellan et al., 1994). The present study was undertaken to determine the existence of ultrastructural lesions in the liver of the rats fed for 13 weeks 3,3'4,4'-tetrachlorobiphenyl, PCB congener #77. Preliminary results of this work have been presented in a science forum by Singh et al. (1994). The work is part of a comprehensive investigation in which the toxic potential of eight PCB congeners is being examined.

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Materials and methods

Compound

PCB congener #77 was purchased from Accustandard Chemicals, New Haven, CT and had a stated purity of >99.1%.

Animal treatment

One hundred weanling Sprague-Dawley rats (40-50 g bw) were randomly distributed into 10 groups each of 10 male or female animals. The animals were placed individually in stainless steel cages, and were given Rodent Chow (Ralston Purina Company, St. Louis, MO) and water, *ad libitum*. The acclimatization period lasted for one week. The experimental diets were prepared by

dissolving the congener in 4% corn oil. The animals were placed on respective diets comprised of 10, 100, 1,000 and 10,000 ppb of the congener. One group each of males and of females that received diets with corn oil only, served as the controls. Animal-room was maintained at a temperature of 22±1 °C with a relative humidity of 50±10%. The fluorescent lighting was alternated on a 12-hour basis. The animals were anaesthetized using 3.5 ml/kg bw Equithesin (Jensen-Salsbery Laboratory, Kansas City, MO); the animals were exsanguinated from the abdominal aorta, 13 weeks after the onset of dosing.

Electron microscopy

Liver samples were taken from each animal, and fixed in 2% glutaraldehyde (0.1M, 436 mOsM, phosphate-buffered at pH 7.3). The samples were post-fixed in 2% osmium tetroxide prepared in the same buffer as used for the fixative. Samples were dehydrated through a graded series of ethanols, cleared in propylene oxide and embedded in Epon (Singh et al., 1981). Semithin (ca. 1 μm) sections of the embedded tissues were prepared with a Reichert-Jung ultramicrotome, mounted on glass slides, and stained with 1% toluidine blue. Favourable areas in the specimens were selected by light microscopy for thin sections. The thin sections of samples from 3-5 rats of each group of gold interference color were cut on the ultramicrotome, and double contrasted with uranyl acetate prepared in 50% ethanol, and lead solution (Sato, 1968). The sections were examined and photographed in a Hitachi H-7000 electron microscope that was operated at 75 kV.

Results

The control groups

The liver ultrastructure from both the male and female animals was normal. Typical spherical nuclei were circumscribed by nuclear envelopes and contained prominent nucleoli. Profiles of smooth endoplasmic reticulum (SER) tubules were dispersed throughout the cytoplasm in moderate amounts. Characteristic arrays of rough endoplasmic reticulum (RER) cisternae occurred in the hepatocytes (Fig. 1). Numerous mitochondria bounded by inner and outer membranes were present in the cytoplasm (Fig. 1). Inner mitochondrial membranes were oriented in a typical manner, i.e., horizontal to the long axis of the organelle, and the inner chamber contained a finely granular electron dense matrix. The peroxisome images bounded by a single membrane, and containing a granular matrix with nucleoid were present. The lipid droplets, and glycogen granules, which occurred as α or ß particles, were randomly distributed in the cytoplasm (Fig. 1). Liver cells from one male animal contained many lipid droplets. What follows

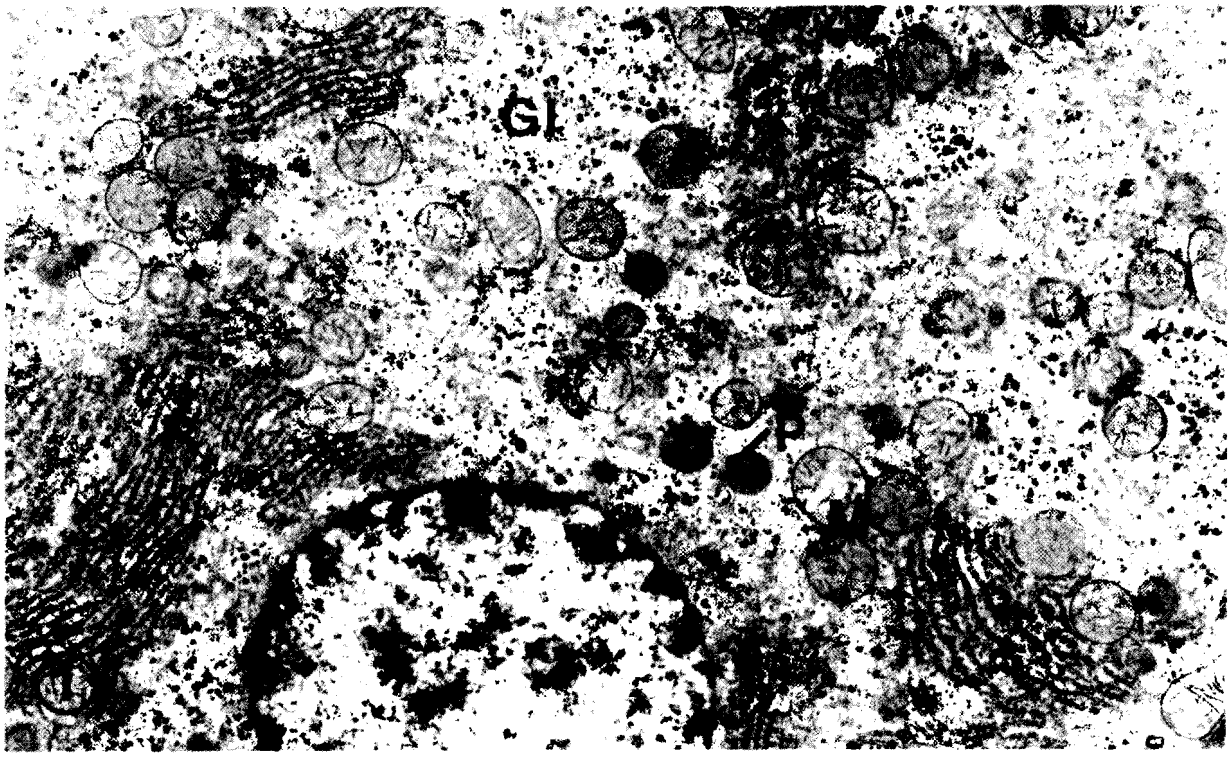

Fig. 1. Electron micrograph of a portion of hepatocyte from a male rat of the control group. A normal distribution of rough endoplasmic reticulum (R), mitochondria (M), peroxisome (P) and glycogen particles (Gl) is illustrated. x 10,000

is a description of a spectrum of lesions that was observed albeit in a majority of the examined parenchymal cells.

Male animal dose groups

10 ppb PCB #77

The hepatocyte images from the animals of the group demonstrated a numerical augmentation in SER profiles with a concomitant reduction in the number of glycogen particles. Peroxisomes were numerically elevated in comparison with those in the liver of animals from the control group (Fig. 2). Mitochondrial aberrations comprised alteration in the orientation of cristae that were arranged parallel to the longitudinal axis of the organelle in one animal. Lipid droplets were moderately augmented in many hepatocytes.

100 ppb PCB #77

The parenchymal cell images of the liver from the animals of this group revealed many electron-lucent areas in the cytoplasm where the SER had notably increased (Fig. 3). Mitochondrial cristae were abnormally orientated and were parallel to the long axis of the organelle. In many cells, the number of lipid droplets had increased.

1,000 ppb PCB #77

The images of hepatocytes from the animals of this group displayed many zones of proliferated SER. The altered morphology in the hepatocyte mitochondria was similar to that described for the organelle in the preceding dose groups, and was noted in one animal. A heightened number of peroxisomes was observed. The lipid droplets in the cells were markedly increased in number and in size, sometimes appearing larger than the nucleus.

10,000 ppb PCB #77

Micrographs of the hepatocytes from the animals treated in this group showed large regions of proliferated SER which were characterized as a «moth-eaten» appearance of the cytoplasm. Mitochondrial abberations were similar to those described for the organelle in the preceding dose groups, save that the altered mitochondria were seen in one animal. The number of peroxisomes in the hepatocytes had also augmented. As well, lipid droplets had numerically heightened.

Female Animal Dose Groups

10 ppb PCB #77

The images of hepatocytes from the animals of this

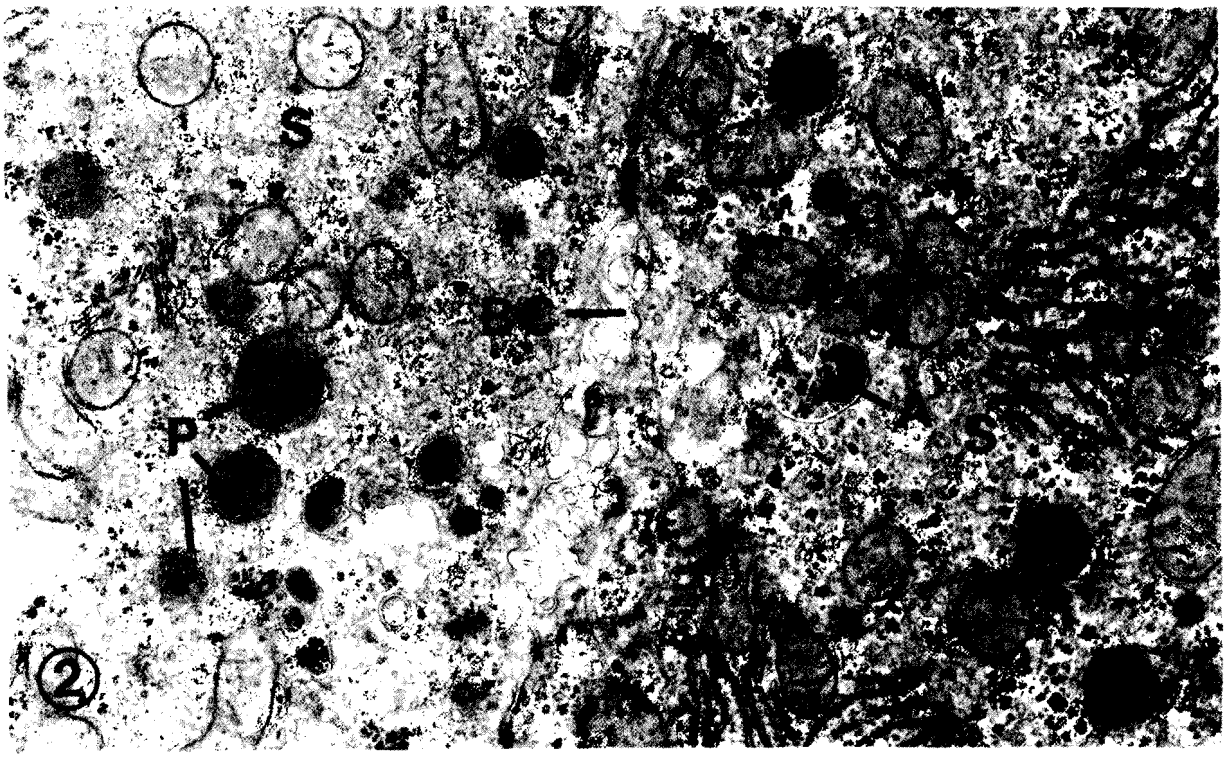

Fig. 2. Micrograph of portions of the hepatocytes from a male of the 10 ppb congener group. Many peroxisomes (P) are depicted in the image. S denote regions of proliferated SER. A= Autophagolysosome; BC= Bile canaliculus. x 16,000

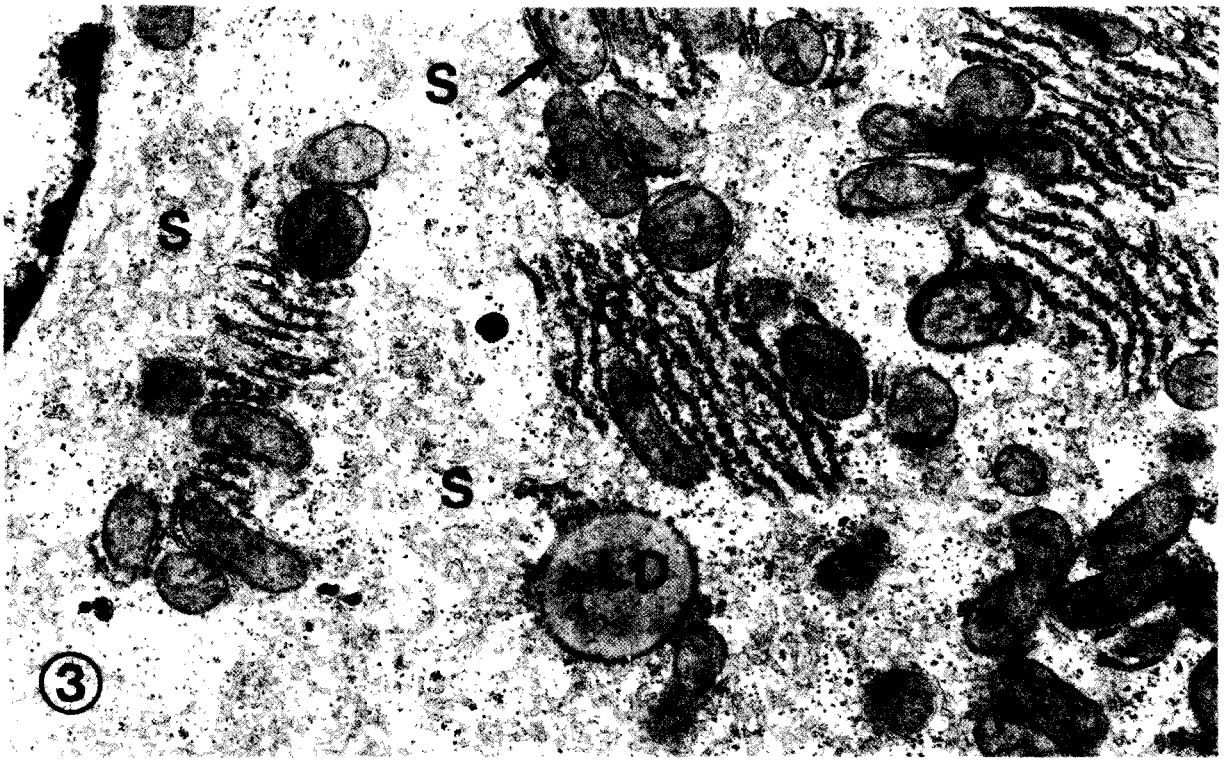

Fig. 3. Micrograph of a portion of hepatocyte from a male of the 100 ppb congener group. Many regions comprising SER profiles (S) occupy the field. Arrows indicate mitochondria containing abnormally-oriented cristae. LD= Lipid droplet; R= RER. x 21,000

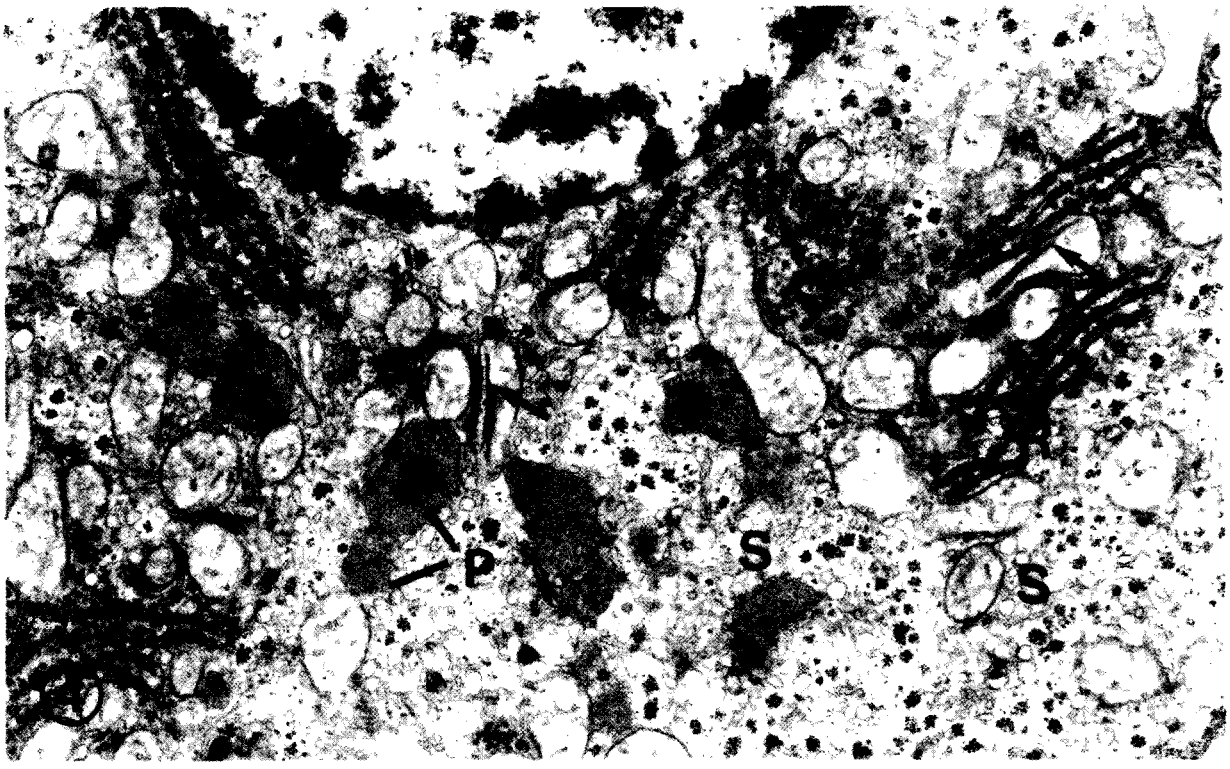

Fig. 4. Micrograph of a portion of hepatocyte from a female of the 1,000 ppb congener group. Note the presence of many peroxisomes (P) in a small field. Arrows point to longitudinally-oriented cristae in mitochondria. R= Cisternae of rough endoplasmic reticulum; S= Profiles of smooth endoplasmic reticulum. x 21,000

group contained more profiles of SER in comparison with that in the cells of the females from the control group. Mitochondrial shape was normal but some of them were altered to include cristae in arrays parallel to the long axis of the organelle. Peroxisomes were slightly elevated in number.

100 ppb PCB #77

The hepatocyte images from animals of this group revealed abundant SER profiles resulting in electron-lucent (moth-eaten) zones in the cytoplasm. Alterations in mitochondrial shapes included dumbbell, branching and cup-like configurations. Peroxisomes were slightly increased in number. Lipid droplets were elevated in number in comparison with those noted in the cells of animals from the preceding group.

1,000 ppb PCB #77

Liver parenchymal cells from the animals of this group contained proliferated SER that gave the characteristic moth-eaten appearance to the cytoplasm. Mitochondrial alterations were similar to those described for the organelle in the lower dose groups, showing cristae parallel to the long axis of the organelle in one animal (Fig. 4). Peroxisomes apparently had numerically increased over those in the low-dose level groups. The number of lipid droplets in the cells had heightened in one animal.

10,000 ppb PCB #77

Hepatocyte alterations were most prominent in the animals of this group. Augmentation of the SER profiles was most apparent. Mitochondrial changes were similar to those in the female animals of the preceding dose groups, although relatively more parallel cristae were noticed in the organelles as depicted in Figure 5. Many peroxisomes and lipid droplets were present.

Discussion

The results of the present study demonstrate a mild toxicity of the PCB congener #77 to the rat liver. The SER proliferation, mitochondrial abnormalities, heightened number of peroxisomes, and numerical elevation of lipid droplets were the most conspicuous hepatocyte lesions noted in the treated animals. These alterations were dose-dependent and were most evident in the rats receiving 10,000 ppb of congener, the highest dose level used in the study.

The proliferation of SER in the hepatocytes is a non-specific response, and it also results from administration to the animal of a halogenated hydrocarbons. Numerous studies have cited the proliferation of SER following administration, among others, of PCB compounds (Kimbrough et al., 1972; Hansell and Ecobichon, 1974;

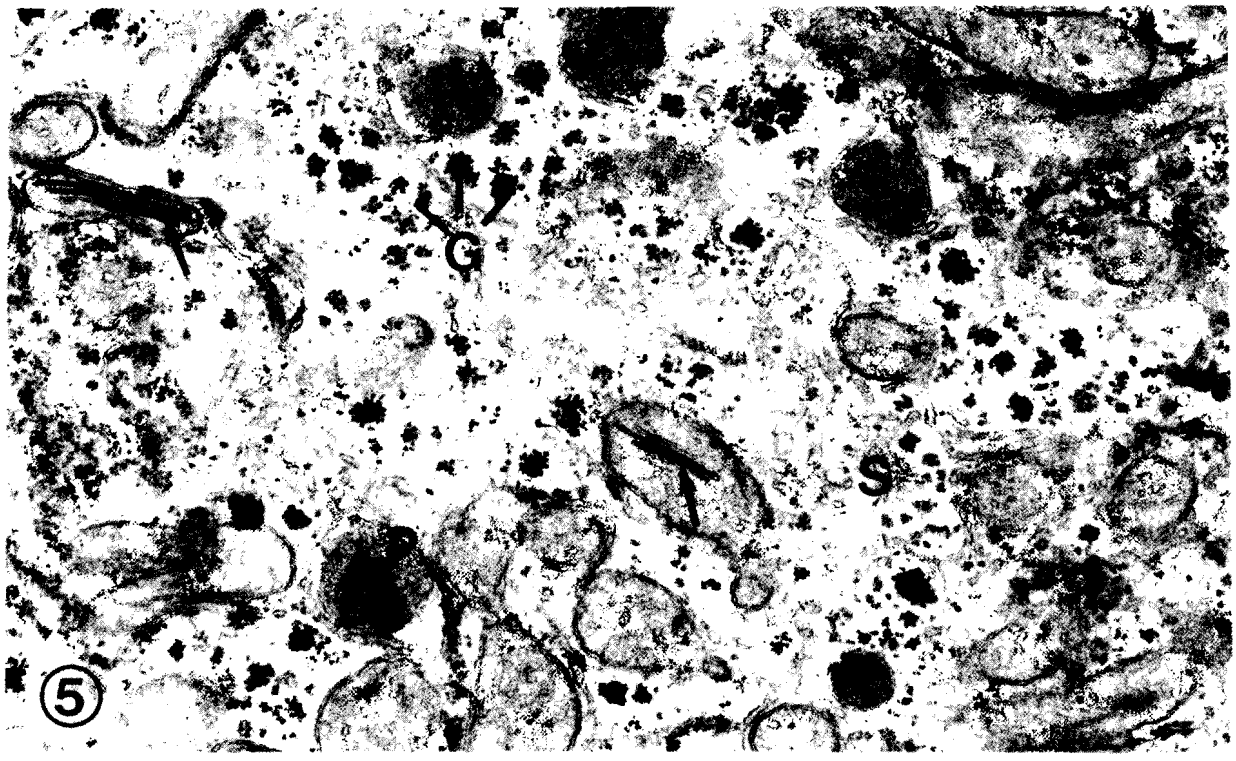

Fig. 5. Micrograph of a small field from a hepatocyte of a female of the 10,000 ppb congener group. Profiles of smooth endoplasmic reticulum (S), mitochondria with longitudinally-oriented cristae (arrows), and peroxisomes are illustrated. G= Glycogen rosettes. x 31,000

Kasza et al., 1978; Baumann et al., 1983; MacLellan et al., 1994), tetrachlorodibenzodioxin (Schecter et al., 1984, 1985), dichlorodiphenyltrichloroethane (Hansell and Ecobichon, 1974; Jonsson et al., 1981), and chlorinated diphenyl ethers (Chui et al., 1985). SER proliferation noted in the present study was consistent with the alterations reported by us on earlier studied PCB congeners #126 and #118 under similar test conditions (MacLellan et al., 1994). This proliferation was also in accord with the observations of other authors studying PCBs in rats (Gillette et al., 1987a,b; Elangbam et al., 1991). Ghadially (1988) relates the SER hypertrophy to an adaptive response by which the animal metabolizes and tolerates xenobiotics at quantities which otherwise would be fatal; SER is believed to be the seat of enzymes involved in xenobiotic metabolism.

Nishizumi (1970) reported an inverse relationship between the amounts of SER and RER present in the liver parenchymal cells, however, the RER profiles of the treated animals were not modified in our study.

In the present study many profiles of altered mitochondria were noted. These mitochondrial alterations are in agreement with the studies by Nishizumi (1970), and by Lin et al. (1979) who showed conformational changes in mitochondria of mice and rat hepatocytes. The mitochondria serve as the major source of cellular ATP (Weiss, 1988), and changes to their ultrastructure may result in decreased energy and metabolic activity. Increases in the cytoplasmic lipid droplet contents noted in the treated animals of the present study may be attributable to the inability of the mitochondria to function properly with respect to the oxidative metabolism normally fuelled by fatty acids (Schecter et al., 1984). The inner membranes of the hepatic mitochondria form cristae that normally lie in a transverse plane to the long axis of the organelle (Ghadially, 1988). Our study revealed that these cristae were oriented parallel to the long axis in many mitochondria. Similar findings on the modification of the cristae orientation were reported by MacLellan et al. (1994) in the study of congeners #126 and 118.

Modifications of the mitochondrial cristae and of enzyme activity in the hepatocytes have been reported following PCB administration (Schecter et al., 1984; Durham and Brouwer, 1989a,b). Hanaki (1985) found that the mitochondrial cristae of the adrenal gland change their shape in relation to the action of steroidogenesis or other physiological states; however, the mechanism of alteration of the cristae is unclear.

Heightened number of peroxisomes were observed in the animals treated with congener #77 in the current study. Hepatic peroxisomes are thought to be involved in the disposal of hydrogen peroxide; in the metabolism of purines, lipids, and alcohols; in the oxidation of reduced NAD; and gluconeogenenesis (Weiss, 1988). Cheville (1976) reviewed the role of peroxisomes as a protective mechanism to oxidize hydrogen peroxide produced in the event of cellular injury. Peroxisomes were reported to increase numerically in the hepatocytes of rats fed chlorobiphenyls (Nishizumi, 1970), with the greatest response coming from substances composed of highly chlorinated chemcials and those possessing a chlorine in the 4 and /or 4' position (Hansell and Ecobichon, 1974). Ghadially (1988) states that the alterations in peroxisomes are sex and species specific, where clofibrate caused alterations in male rat hepatocytes that were unaltered in females receiving the same dose. In the current study male rats did not appear to be more adversely affected than their female counterparts.

In the present study, lipid droplets were numerically augmented in the animals from the 10 ppb congener group, the lowest dose level used, and appeared to increase in a dose-related fashion in both the male and female animals. Similar observations were reported in our studies on congeners #126 and 118 (MacLellan et al., 1994). Various physiological and pathological conditions may cause the size, shape and number of lipid droplets to change in liver parenchymal cells. An augmentation over the normal lipid content of hepatocytes represents a disruption in the normal physiology of cells. In the present study changes described in the hepatocytes of the treated animals exposed to the lowest treatment group (10 ppb congener) indicate that this compound may potentially cause alterations at even lower doses. It is concluded that the congener may be moderately toxic, and it may affect the over-all health of the exposed animal.

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