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Toxicity of 2,2',4,4',5,5'-hexachlorobiphenyl in the rat liver: An electron microscope study

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Summary. Morphological effects on the liver of Sprague-Dawley rats administered orally 2,2',4,4',5,5'-hexachlorobiphenyl (PCB), congener #153, were analyzed. Treatment diets were prepared by dissolving the congener in 4% corn oil. Ten animals of either sex in each group were placed on the respective diets that contained 50, 500, 5,000, or 50,000 ppb congener. Ten animals of each sex served as the control that had only the oil added to the diets. Thirteen weeks after the onset of dosing, the animals were euthanized and liver samples were obtained from the animals and prepared for electron microscopy.

Animals exposed to the congener showed (in a dose-related manner) a marked increase in smooth endoplasmic reticulum profiles, and in the number of lipid droplets in many parenchymal cells. Mitochondrial abnormalities such as dumbbell shapes, and in others, the cristae that were oriented parallel to the long axis of the organelle were present. The magnitude of morphologic alterations did not reveal gender differences. The results indicate mild hepatotoxicity of the congener in the rat.

Key words: Hexachlorobiphenyl, Liver, Ultrastructure, Rat

Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous and persistent environmental contaminants (Kasza et al., 1976), ranking among the most abundant hydrocarbon pollutants in the world (Norback and Allen, 1972). Environmental contamination resulted from the widespread use of these compounds for commercial use due to their properties of heat resistance, adhesiveness, thermoplasticity, and chemical stability (Norback and Allen, 1972; Aztori et al., 1991). A potential health risk persists for humans, and animals because of the long half-life and bioaccumulation of PCBs in the aquatic and terrestrial food chains (Clarke et al., 1984; Safe, 1984, 1990; Atzori et al., 1991).

The number and position of chlorine atoms on the biphenyl rings are known to alter the degree of PCB toxicity (Hansell and Ecobichon, 1974; Safe, 1984, 1990). Several studies (Fishbein, 1974; Kasza et al., 1978; Lin et al., 1979; MacLellan et al., 1994a,b) on the ultrastructural alterations in the liver of mammals administered PCB have appeared. This study was undertaken to ascertain the hepatocyte lesions in rats fed 2,2',4,4',5,5'-hexachlorobiphenyl (PCB), congener #153, for 13 weeks. The work is part of a comprehensive investigation in which liver lesions induced by feeding various congeners to rats are analyzed

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Materials and methods

Compund

PCB congener #153 was obtained from Accustandard Chemicals, New Haven, CT with a stated purity of > 99.1%.

Animal treatment

One hundred weanling Sprague-Dawley rats (40-50 g bw) were distributed randomly into 10 groups each of 10 male or female animals. The animals were individually housed and were given Rodent Chow (Ralston Purina Company, St. Louis, MO) and water, ad libitum. Housing conditions were maintained at a temperature of 22±1 °C (relative humidity 50±10%), with alternate 12-hour light/dark cycles. The experimental diets were prepared by dissolving the congener in 4% corn oil. The animals were placed on the respective diets comprised of 50, 500, 5,000 and 50,000 ppb of the congener. Control groups of each sex received diets that contained corn oil. The animals were anaesthetized using 3.5 ml/kg bw Equithesin (Jensen-Salsbery Laboratory, Kansas City, MO), and were exsanguinated from the abdominal aorta, 13 weeks after the onset of dosing.

Electron microscopy

Liver samples from each animal, were fixed in 2% glutaraldehyde (0.1M, 436mOsM, 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 in graded series of ethanol, cleared in propylene oxide and embedded in Epon. Semi-thin (ca. 1μm) 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 (Singh et al., 1981). The thin sections of samples from three rats of each group of pale interference color were cut on the ultramicrotome, and double contrasted with uranyl acetate prepared in 50% ethanol, and lead solution (Sato, 1968). Thin sections were examined and photographed in a Hitachi H-7000 electron microscope that was operated at 75kV.

Results

The control groups

Ultrastructure of the liver from both genders of rats revealed characteristics typical of normal hepatocytes as depicted in Figure 1. Distinctive spherical nuclei contained prominent nucleoli. Tubular profiles of smooth endoplasmic reticulum (SER) were dispersed throughout the cytoplasm. Characteristic arrays of rough endoplasmic reticulum (RER) cisternae occurred in the cells. Mitochondria were numerous; their inner mitochondrial membranes projected perpendicularly to the long axis of the organelle, and the inner chamber contained matrix. Peroxisomes limited by a single membrane contained a finely granular matrix with nucleoid present in many cells. Lipid droplets (not illustrated) appeared to be more numerous in the cells of the males than in those of the female counterparts. Glycogen granules were randomly located in the cytoplasm.

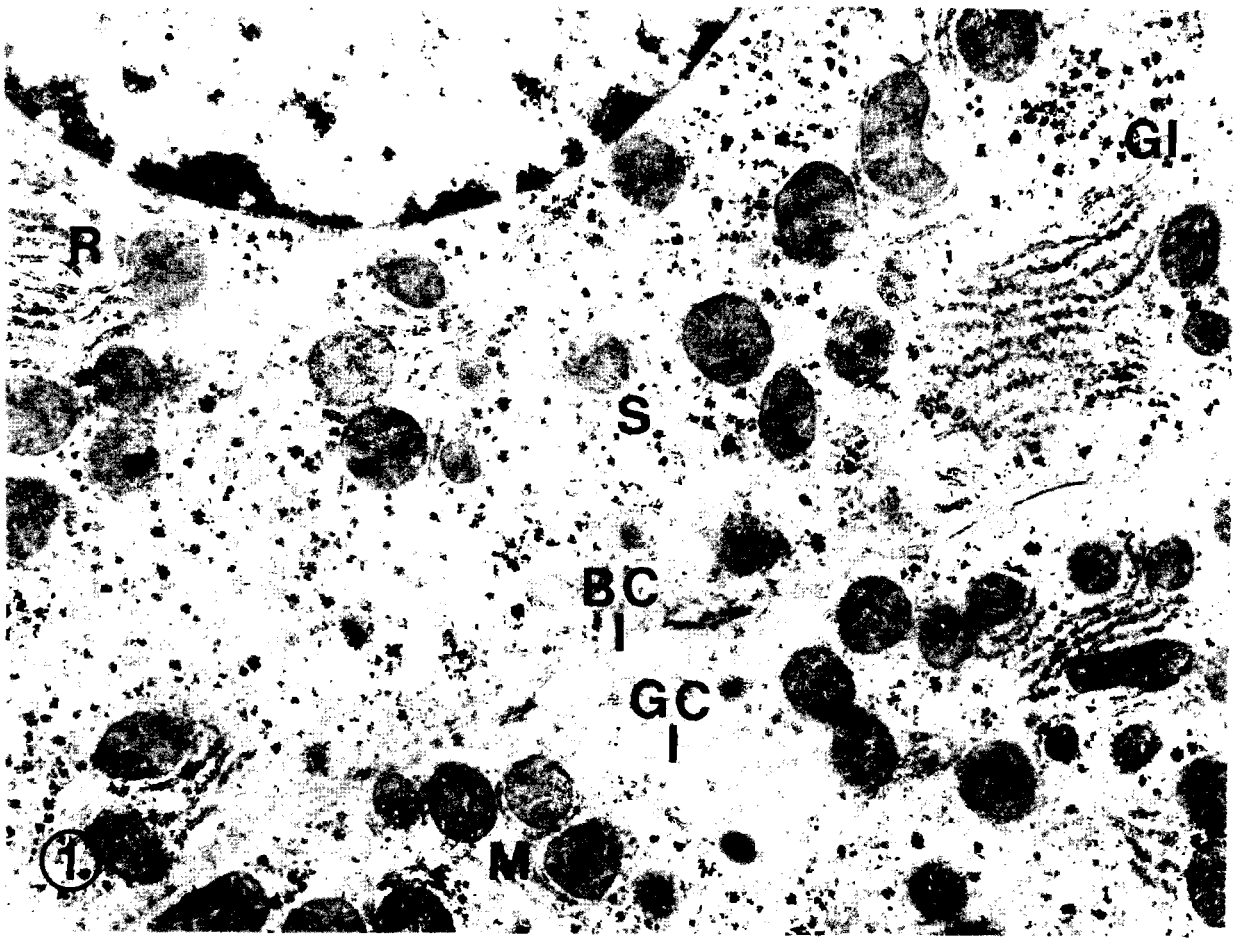

Fig. 1. Electron micrograph of a portion of liver from a male rat of the control group. Characteristic profiles of rough (R) and smooth (S) endoplasmic reticulum, Golgi complex (GC), and typical mitochondria (M) are depicted. BC= Bile canaliculus; GI= Glycogen particles. x 14,000

Male animals dose groups

50 ppb PCB

The hepatocyte images from the animals of this group demonstrated mitochondrial aberrations that included alteration in the orientation of cristae which were arranged parallel to the longitudinal axis of the organelle in one animal (Fig. 2); a few branching and cup-shaped organelles were present in the organ. Lipid droplet number was moderately augmented in many hepatocytes.

500 ppb PCB

The parenchymal cell images from the animals of this group revealed many electron-lucent areas in the cytoplasm where the SER had noticeably increased. Mitochondrial cristae were abnormally oriented, and were parallel to the long axis of the organelle. The number of peroxisomes was elevated in one animal, and lipid droplets had increased numerically in many parenchymal cells as illustrated in Figure 3.

5,000 ppb PCB

The images of hepatocytes in the animals from 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. An elevated number of peroxisomes was observed. Lipid droplets in the cells had markedly increased in number. Augmented number of peroxisomes was noted in one animal.

50,000 ppb PCB

Micrographs of hepatocytes in the animals from this group showed large regions of proliferated SER as the characteristic moth-eaten appearance of the cytoplasm. Mitochondrial aberrations were similar to those described for the organelle in the preceding dose groups, save that the altered cristae were observed in only one animal. The number of peroxisomes had increased in

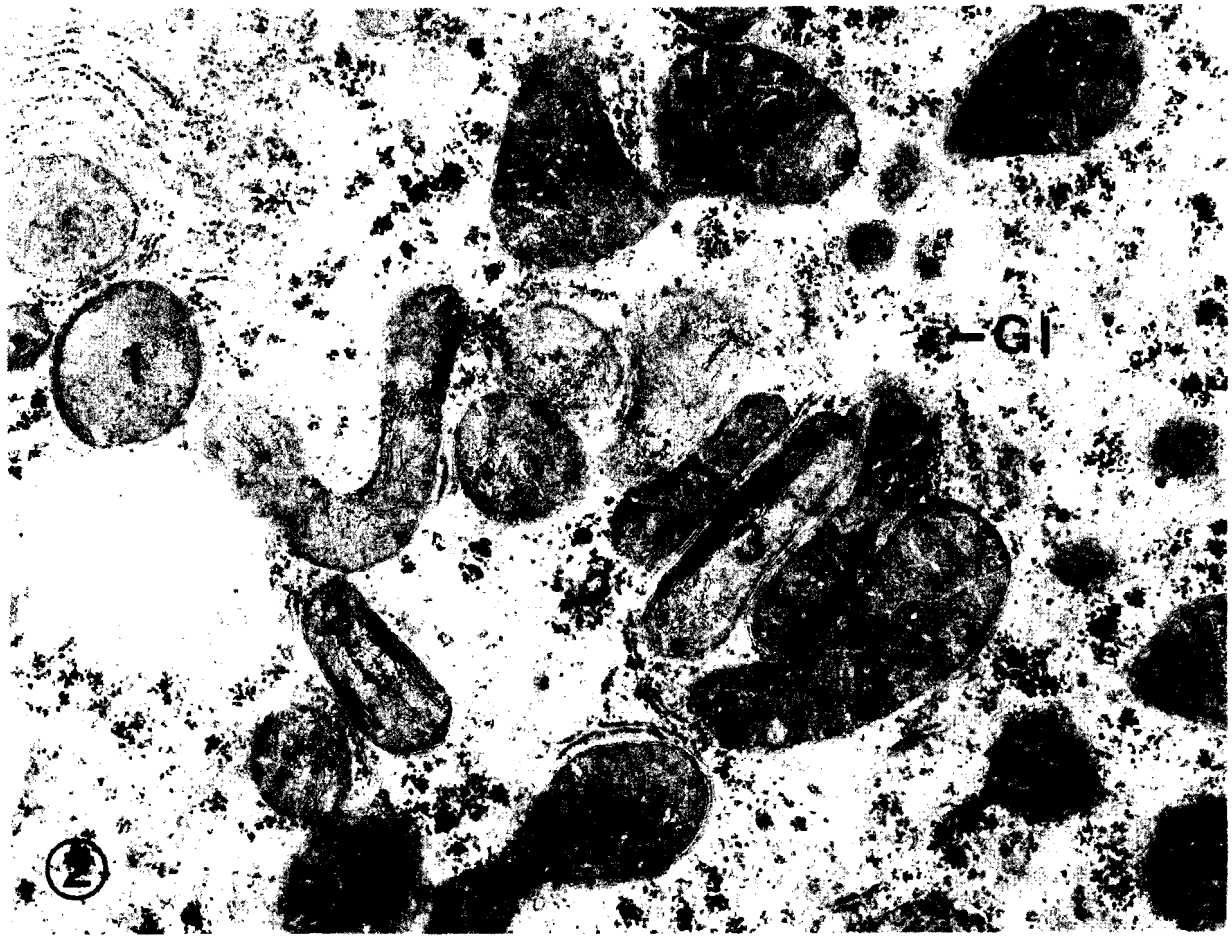

Fig. 2. Micrograph of portions of hepatocyte from a male of the 50 ppb group. Altered orientation of cristae in mitochondria (1,2,3) is demonstrated. Gl= Glycogen rosetts. x 24,000

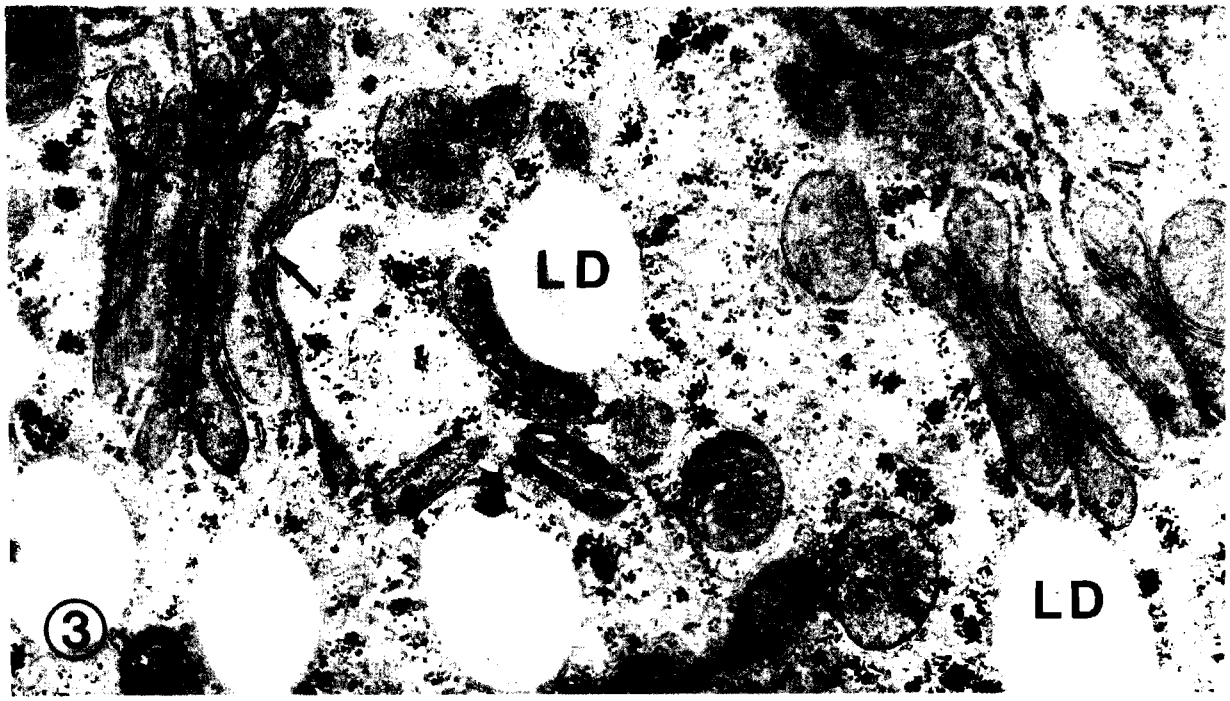

Fig. 3. Micrograph of a portion of hepatocyte from a male of the 500 ppb group. Mitochondria with longitudinally oriented cristae (arrows), and several lipid droplets (LD) are depicted. x 23,000

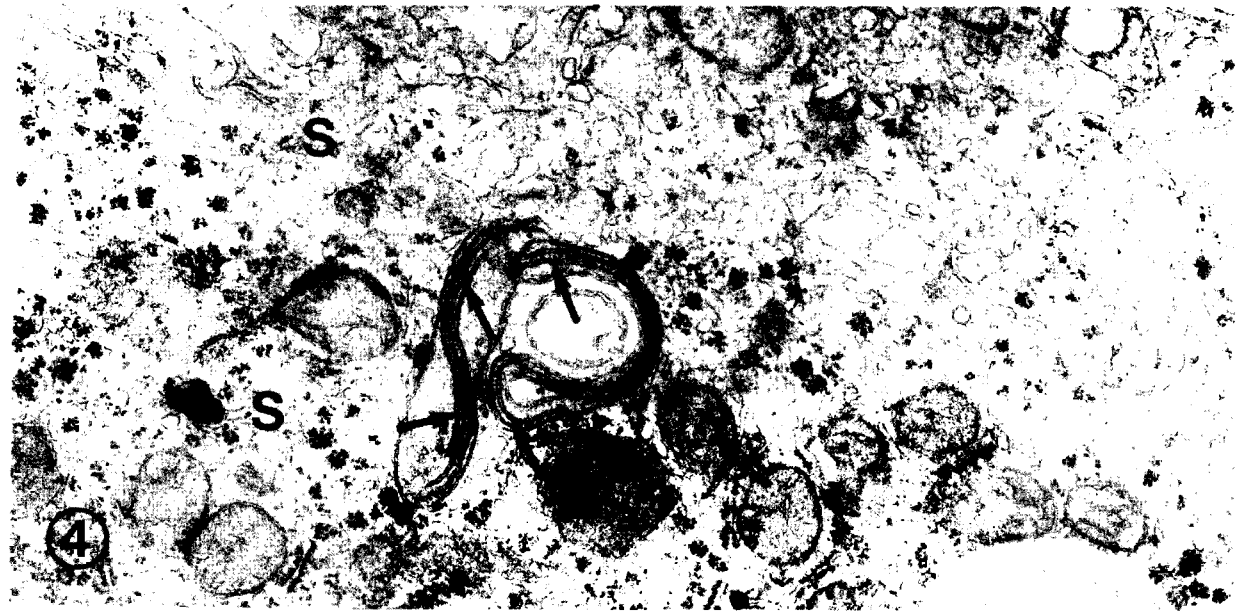

Fig. 4. Micrograph of a portion of hepatocytes from a female of the 50 ppb group. Arrows point to «S»-shaped cristae in mitochondria. S= Smooth endoplasmic reticulum profiles. x 25,000

some hepatocytes. As well, lipid droplets had increased both in numbers, as well as in size up to that of the cell nucleus.

Female animals dose groups

50 ppb PCB

The images of hepatocytes contained many profiles of proliferated SER in comparison with that in the cells of the females from the control group. Mitochondrial alterations were noted that comprised aberrant shapes, and others contained cristae oriented in «S»-shapes, or parallel to the long axis of the organelle as shown in Figure 4.

500 ppb PCB

The hepatocyte images revealed abundant SER profiles resulting in electron-lucent zones in the cytoplasm. Alterations in mitochondrial shapes included dumbbell, branching and cup-like configurations; in addition several mitochondria had parallel cristae. Peroxisomes in the cells had numerically increased. Lipid droplets had slightly increased in number in comparison with those noted in the cells of the animals from the preceding group.

5,000 ppb PCB

The parenchymal cells contained proliferated SER profiles that gave the characteristic moth-eaten appearance to the cytoplasm. Mitochondrial alterations were similar to those described for the organelle in the preceding dose groups. Peroxisomes had relatively increased in number over those in the low-dose level groups.

50,000 ppb PCB

Hepatocyte alterations were most prominent in the animals of this group. Augmentation of the SER profiles was most apparent in the images. Mitochondrial changes were similar to those in the female animals of the

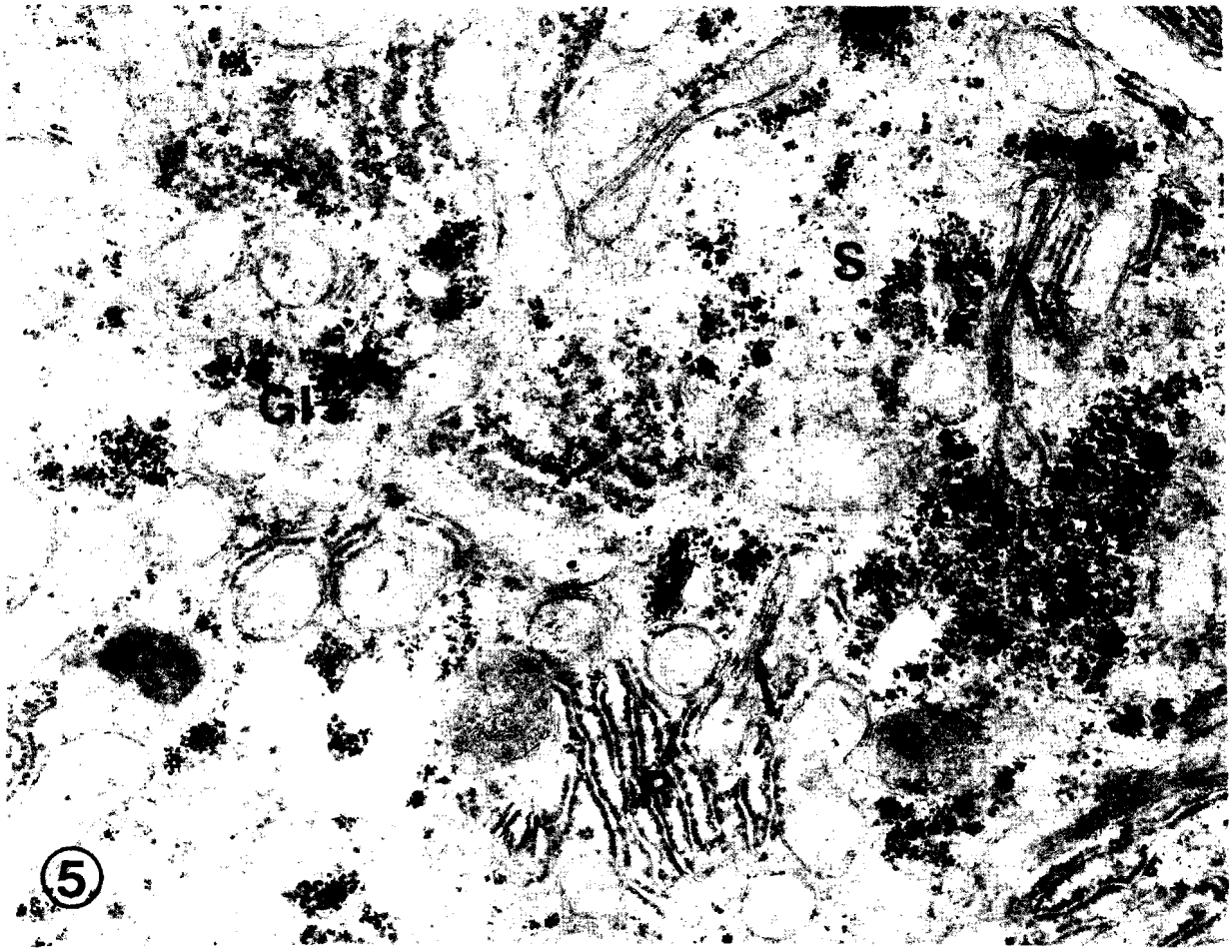

Fig. 5. Micrograph of a portion of hepatocyte from a female of the 5,000 ppb group. Profiles of smooth (S) and rough (R) endoplasmic reticulum, mitochondria with longitudinally-oriented cristae (arrows), and glycogen rosettes (GI) are illustrated. x 28,000

preceding dose groups, although the organelles were more affected. Peroxisomes were slightly elevated in number.

Discussion

Results of the present study demonstrate hepatotoxicity of the PCB congener #153 to Sprague-Dawley rats. Mitochondrial abnormalities, SER proliferation, and numerical elevation of lipid droplets were the most conspicuous lesions in the parenchymal cells. Morphological alterations to hepatocytes were dose-dependent and were most evident in the animals receiving 50,000 ppb congener.

Halogenated hydrocarbons produce a non-specific response which results in an augmentation in the profiles of SER in hepatocytes. Ghadially (1988) relates the hypertrophy of the SER to an adaptive response by which the animal metabolizes and tolerates xenobiotics at doses which normally would be fatal. Several studies have cited the proliferation of SER after administration of PCB compounds (Kimbrough et al., 1972; Hansell and Ecobichon, 1974; Kasza et al., 1978; Baumann et al., 1983; MacLellan et al., 1994a,b), tetrachlorodibenzodioxin (Schecter et al., 1984, 1985), dichloridiphenyltrichloroethane (Hansell and Echobichon, 1974; Jonsson et al., 1981), and chlorinated diphenyl ethers (Chui et al., 1985). SER proliferation noted in the present study was consistent with changes reported in our earlier studies on PCB congeners #126, 118 and 77 under similar test conditions (MacLellan et al., 1994a,b). This alteration was also in agreement with the observations of other authors that studied PCBs in rats (Gillette et al., 1987; Elangbam et al., 1991).

Nishizumi (1970) reported an inverse relationship between the amounts of SER and RER present in liver

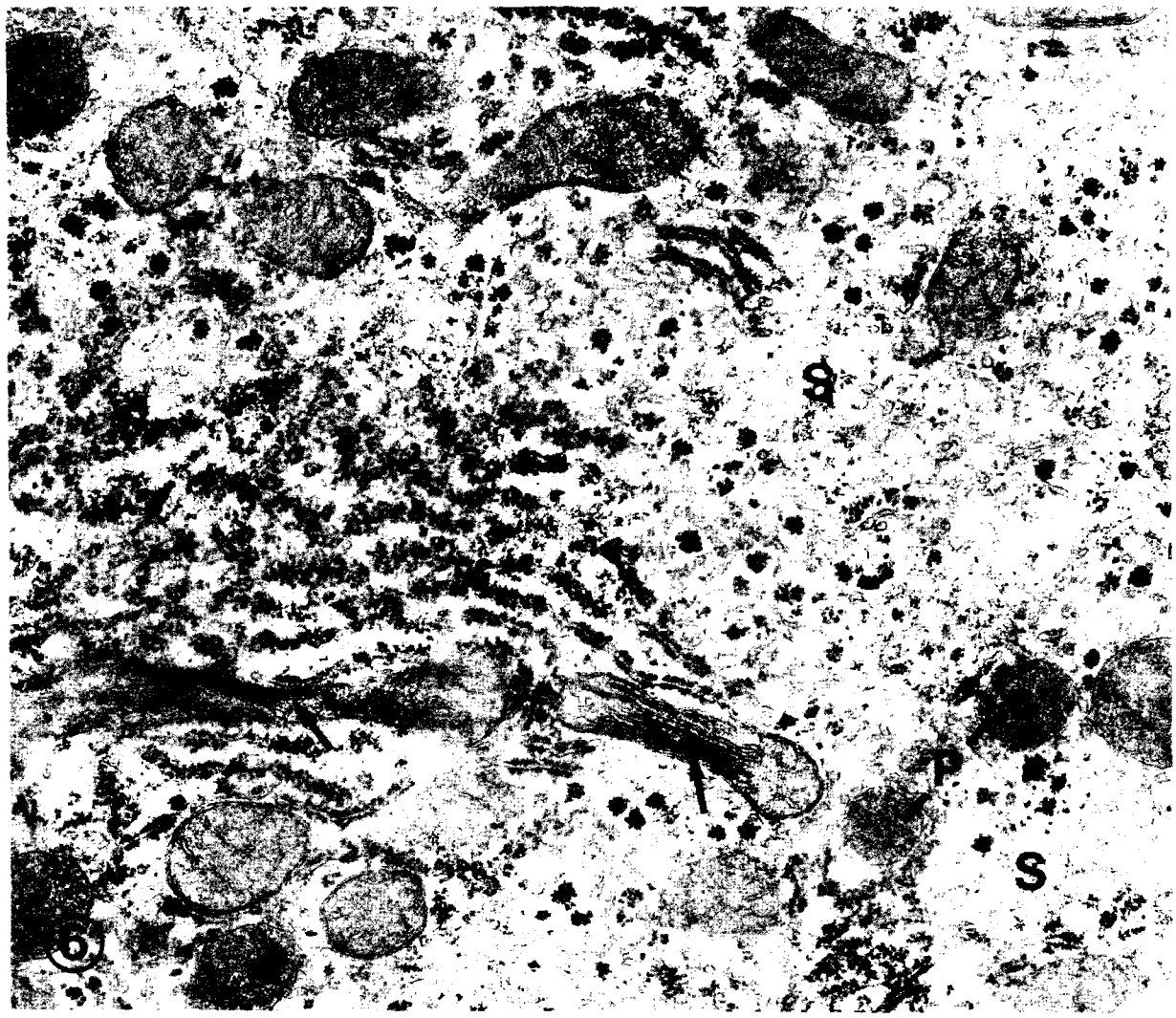

Fig. 6. Micrograph of a portion of hepatocyte from a female of the 50,000 ppb group. A striking proliferation of SER (S) with a reduction in glycogen particles is depicted. Arrows indicate abnormally oriented mitochondrial cristae. P= Peroxisome. x 29,000

parenchymal cells, however, the RER profiles of the treated animals in our study appeared unaltered.

Mitochondrial alterations were present in this study at the lowest dose group (50 ppb). Mitochondria serve as the major source of cellular ATP (Weiss, 1988), and changes to their ultrastructure may result in abnormal energy levels and metabolic activity. Increases in cytoplasmic lipid and serum triglycerides commonly noted in PCB treated animals 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). In the present study, many altered mitochondria were noted. These mitochondrial alterations are consistent with the observations of Nishizumi (1970), and Lin et al. (1979). The inner membrane of the mitochondria which form the cristae normally lie in a transverse plane to the long axis of the organelle (Ghadially, 1988). Our study revealed mitochondrial cristae which were oriented parallel to the long axis. Similar findings were reported by us (MacLellan et al., 1994a,b) in the study of PCB congeners #126, 118 and 77. Alterations in mitochondrial cristae and enzyme activity have been associated with PCB-induced morphologic alterations in hepatocytes (Schecter et al., 1984; Durham and Brouwer 1989a,b). The significance of these mitochondrial alterations is unknown. An increase in the number of peroxisomes was noted in the 50,000 ppb group. Hepatic peroxisomes are thought to be involved in the disposal of hydrogen peroxide, metabolism of purines, lipids, and alcohols, oxidation of reduced NAD, and gluconeogenesis (Weiss, 1988). Alterations in number and structure of peroxisomes have been reviewed by Ghadially (1988) due to clofibrate, a hypolipidaemic agent used to lower serum cholesterol, and Cheville (1976) due to salicylate drugs. Cheville (1976) reviewed the role of peroxisomes as a protective mechanism to oxidize hydrogen peroxide produced in the event of cell injury. Peroxisomes or microbodies, were reported to increase in mice fed chlorobiphenyl (Nishizumi, 1970). Peroxisomes were reported to increase in number in the hepatocytes of rats fed chlorobiphenyls, with the greatest response coming from substances composed of highly chlorinated chemicals 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, based upon a finding that clofibrate caused alterations in male rat hepatocytes whereas hepatocytes from females receiving similar dose were unaffected. Schecter et al. (1984) has reported on the occurrence of peroxisomes in various tissues in PCB-exposed animals.

Lipid droplets were slightly augmented in the male 50 ppb group and appeared to increase in a dose-related fashion in both the male and female animals. Various physiological and pathological conditions may cause the size, shape and number of lipid droplets to change within a hepatocyte. An augmentation over the normal lipid content of hepatocytes represents a disruption in physiology of the cells.

In our earlier work on congeners #126, 118, and 77 (MacLellan et al., 1994a,b), hepatocyte lesions of similar type were revealed as described in the present study. In addition, gender differences were noted where the female rats were detected to be more sensitive, particularly to congener #118 than the males unlike in the present work. However, congener #153 dose levels were the highest among the congeners studied by us; the congener levels administered were 500 folds of that used for congener #126, 5-25 folds of that for congener #118, and five folds of that for congener #77. If a general comparison is made on the bases of qualitative morphologic data, congener #153 turns out to be least toxic among the PCB congeners analyzed thus far. Work on the quantitative facet of the aforementioned congeners is in progress.

We conclude that congener #153 is mildly toxic, and may affect the over-all health of the exposed rat.

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