Morphological Changes in Livers of Rats Fed Polychlorinated Biphenyls

Light Microscopy and Ultrastructure

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Male and female Sherman strain rats were fed polychlorinated biphenyls Aroclor 1260 and Aroclor 1254 at 0, 20, 100, 500 and 1,000 ppm in their diet. Rats received the dietary levels for eight months. Light microscopic changes consisted of hypertrophy of the liver cells, inclusions in the cytoplasm, brown pigment in Kupffer cells, lipid accumulation, and, at the higher dietary levels, adenofibrosis. Ultrastructural changes of the livers of exposed animals consisted of : increase in smooth endoplasmic reticulum ar atypical mitochondria. Lipid vacuoles were oc casionally surrounded by concentric membrane The epithelial component of adenofibrosis co sisted of goblet cells and cells that resemble the epithelium which lines the bile ducts. In ger eral, the effect of Aroclor 1254 on the liver wa more pronounced than that of Aroclor 1260.

Polychlorinated biphenyls (PCBs) are widely distributed in the environment.[1] They are marketed in the United States under the trade name Aroclor followed by a four digit number with the last two digits indicating the percent of chlorine. They were first marketed in the 1930s. Jones and Alden[2] reported a case of chloracne in a worker who had been heavily exposed to a chlorinated biphenyl. Miller[3] reported pathological changes in animals exposed to a commercial chlorinated biphenyl. He studied rats, rabbits, and guinea pigs, and found liver damage in all series of experiments, as well as skin changes in the animals receiving subcutaneous injections or applications of the material to the skin. Liver damage was most pronounced in the guinea pig. The rat livers had intercellular hyaline bodies in addition to the fatty degeneration that was observed in all three species. Bennett and his co-workers[4] also reported that Aroclors affected the livers of rats. Von Oettingen[5] discusses these earlier findings in his book *"The Halogenated Hydrocarbons: Toxicity and Potential Dangers."* After the Second World War, the PCBs were increasingly used for moisture proofing, sealing, impregnation, and vap suppression in prolonging the residual life c insecticides and as fire retardants. They ca be found in synthetic resins, synthetic an natural rubber, cellulose resins, paint, var nish, wax, asphalt, and allyl starch. The have also been used in printing inks, textil dyes, and as heat transfer fluid. In 196 an outbreak of poisoning that involved a least 600 people occurred in western Japa where rice bran oil was contaminated wit Kanechlor 400, a PCB.[6] The contaminatio of the oil occurred because PCBs used a heat exchangers in the manufacturing proc ess leaked into the oil through pinholes i the pipes. This incident prompted Nishizu mi[7] to study the effect of PCBs on the liver of mice and monkeys. He found a large amount of acidophilic material in the cytoplasm of hepatocytes and fatty vacuoles Using electron microscopy, he observed "myelin figures" in the cytoplasm, and an increase in smooth endoplasmic reticulum and lipid droplets.

More recently, PCBs leaked into fishmeal from a heat exchanger of a processing plant in North Carolina. The contaminated fishmeal was fed to adult chickens and breeders. The contamination was discovered when the eggs from the breeders did not hatch. Some of the chickens also exhibited symptoms of chick edema.[8] The fact that PCBs can produce chick edema has been described repeatedly.[9-11]

Contaminants may be at least partially responsible for some of these toxic effects.

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Chlorinated dibenzofuran and chlorinated naphthalenes have been found as contaminants in two European commercial PCBs.[12] It is possible that other PCBs are also contaminated with these or other highly toxic substances.[13] The effect of two PCBs, Aroclor 1254 and Aroclor 1260, on Sherman strain rats, was studied in our laboratory to gain better insight into the toxicity of these commercial products. The Aroclor 1260 was from the same lot as that studied by Vos and Koeman.[11]

Materials and Methods

Sherman strain rats were used in these experiments. The animals were caged individually and weighed weekly. To determine the single dose oral LD_{50}, Aroclor 1254 and 1260 were given in peanut oil to groups of ten rats/dosage level by stomach tube as described by Gaines.[14] Survivors were observed for a minimum of 15 days after treatment. The method of Lichtfield and Wilcoxon[15] was used to calculate the LD_{50} values.

When the Aroclors were fed in the diet they were dissolved in ether and mixed with cornstarch. The ether was allowed to evaporate and the Aroclor-cornstarch mixture was mixed into increasing amounts of ground chow. In the feeding studies, 3 to 4-week-old male and female rats were distributed in groups of ten rats of each sex according to a table of random numbers. Their food consumption was measured during the second, fifth, 11th, 22nd, and 30th week of exposure to the dietary levels of 0, 100, 500, and 1,000 ppm, and at similar intervals in the groups fed 20 ppm and their controls. The rats fed 20 ppm were set up at a later date than the other dietary levels. For

Table 1.—Mean Body Weight Gain and Liver Weights of Rats Fed Aroclor 1260*

Sex	Dietary Level (ppm)	Dosage Level (mg/kg/day)	Weight Gain (gms)	Liver Weights (gms)	Relative Liver Weights: Fraction of Body Weight (%)	Relative Liver Weights: Difference From Controls
M	0	0	451	14.56	2.59	...
M	100	6.5	463	18.87	3.23	$P<.001$
M	500	32.8	398	22.01	4.25	$P<.001$
M	1,000	71.4	351	22.46	4.91	$P<.001$
F	0	0	256	11.23	3.12	...
F	100	7.2	253	12.20	3.40	$P>.10$
F	500	38.2	185	12.67	4.52	$P<.001$‡
F	1,000†	72.4	148	15.85	6.35	...
M	0	0	435	12.77	2.39	...
M	20	1.4	455	16.63	3.02	$P<.001$
F	0	0	226	11.94	3.74	...
F	20	1.6	233	12.68	3.86	$P>.50$

* In diet for eight months; ten rats/group. † Only two animals survived. ‡ For the actual liver weight $P>.05$.

Table 2.—Mean Body Weight Gain and Liver Weights of Rats Fed Aroclor 1254*

Sex	Dietary Level (ppm)	Dosage Level (mg/kg/day)	Weight Gain (gms)	Liver Weights (gms)	Relative Liver Weights: Fraction of Body Weight (%)	Relative Liver Weights: Difference From Controls
M	0	0	484	14.14	2.44	...
M	100	6.8	459	16.01	2.92	$P<.025$
M	500	36.4	315	24.48	5.95	$P<.001$
F	0	0	244	10.59	3.19	...
F	100	7.5	235	11.86	3.62	$P>.05$†
F	500	37.6	158	18.98	7.47	$P<.001$
M	0	0	453	13.96	2.53	...
M	20	1.4	439	15.38	2.90	$P<.025$
F	0	0	238	11.21	3.34	...
F	20	1.6	249	12.49	3.72	$P<.025$

* In diet for eight months; ten rats/group.
† Equal numbers of rats fed 100 ppm and 20 ppm, respectively, for same length of time did not show significant increase in actual liver weight ($P<.1$ and $>.05$); however, the increase in relative liver weight was significant $P<.01$.

this reason, a second group of controls for each sex was added to the experiments (Tables 1 and 2). All animals that were fed PCB as well as their controls were used in reproduction studies. The results of the reproduction studies will be reported separately. At autopsy, the tissues were fixed in a buffered 4% solution of formaldehyde (formalin). They were stained with hematoxylin-eosin for light microscopic study. Selected liver sections were cut on the cryostat and stained with Oil red O. Perls' stain for iron and a PAS stain were also performed on a few selected livers. Tissue from the livers of two male and two female rats at each dietary level except 20 ppm were minced and fixed in chilled 5% buffered glutaraldehyde for two hours, postfixed for two hours in 1% osmic acid (osmium tetroxide), dehydrated through changes of alcohol and propylene oxide, and embedded in resin. Sections were cut with a glass knife, stained with lead citrate and uranyl acetate, and examined with the electron microscope. In a preliminary experiment, Aroclor 1254 at the dietary level of 1,000 ppm for three months killed most of the rats. Tissue from this preliminary experiment was examined only under the light microscope.

Results

There was considerable variation, in both sexes, in the acute, oral, toxic effects of Aroclor 1260 and 1254 at various dosage levels. It was impossible to calculate LD_{50} values. The data indicated that the LD_{50} values were about 4,000 to 10,000 mg/kg for both mixtures. Autopsies of rats killed by either mixture revealed hemorrhage into the lung, stomach, and pancreas. Foci of ulceration surrounded by a severe inflammatory reaction were observed primarily in the duodenum and occasionally in the glandular part of the stomach.

When the material was fed in the diet over a period of time, porphyria was observed in a number of animals poisoned with either mixture. The liver fluoresced most consistently under the UV light. Occasionally, fluorescence of bones, serum, and urine was observed. A more detailed study of this phenomenon will be reported later.

When Aroclor 1260 was fed daily to groups of ten male and ten female rats each

Table 3.—Light Microscopic Findings in the Liver of Rats Fed Aroclor 1260*

Dietary Level (ppm)	Sex	Enlarged Hepatocytes	Inclusions in Cytoplasm	Adenofibrosis	Increased Lipid	Foamy Cytoplasm	Pigment
0	M	...	...	...	...	...	...
20	M	10	9	...	...	3	...
100	M	10	9	...	10	...	...
500	M	10	4	...	10	7	1
1,000	M	10	6	2	10	9	2
0	F	...	...	...	...	...	...
20	F	3	...	...	...	...	...
100	F	7	2	1	10	...	1
500†	F	9	5	1	9	8	6
1,000‡	F	6	...	4	7	4	2

* In their diet for eight months; ten rats/group. † Only nine animals were studied. ‡ Only seven animals were studied.

Table 4.—Light Microscopic Findings in the Liver of Rats Fed Aroclor 1254*

Dietary Level (ppm)	Sex	Enlarged Hepatocytes	Inclusions in Cytoplasm	Adenofibrosis	Increased Lipid	Foamy Cytoplasm	Pigment
0	M	...	...	...	...	...	...
20	M	7	1	...	...	1	...
100	M	10	7	1	10	10	3
500	M	10	2	10	10	4	2
0	F	...	...	...	...	...	...
20	F	...	...	...	...	...	1
100	F	10	7	7	10	9	10
500†	F	9	2	9	9	2	7

* In their diet for eight months; ten rats/group.
† Only nine tested since one rat that died showed too much autolysis.

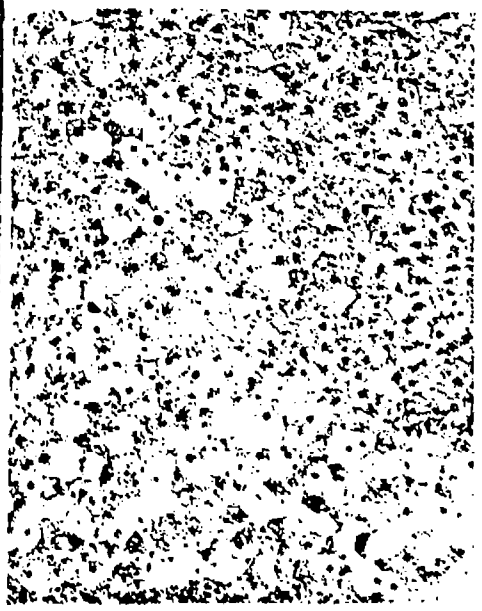


Fig 1.—Liver section of male rat fed 500 ppm Aroclor 1260 for eight months. Note foamy cytoplasm of many of hepatocytes (hematoxylin-eosin ×125).

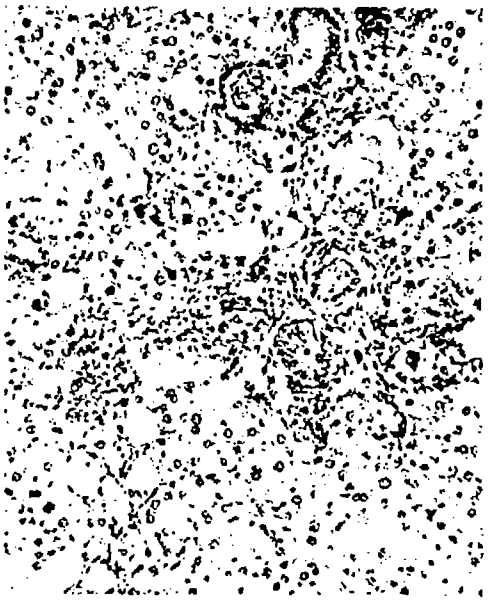
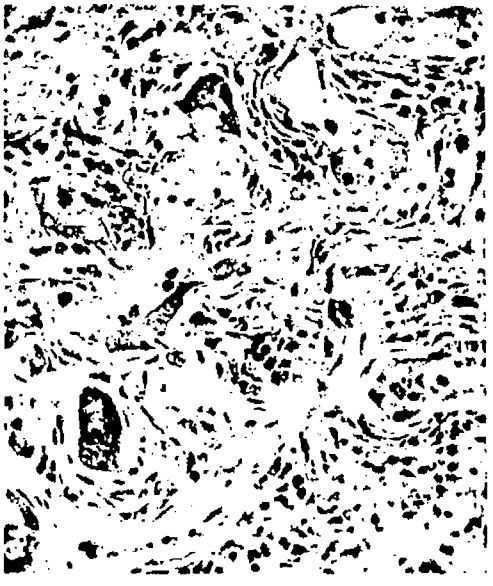


Fig 2.—Section of liver from male rat fed 1,000 ppm Aroclor 1254 for eight months illustrating adenofibrosis. Note area of fibrosis which surrounds ductules of glandular cells. Cellular debris, polymorphonuclear leukocytes, or mucus can be observed in lumen of ductules (hematoxylin-eosin ×125).

at dietary levels of 0, 20, 100, 500, and 1,000 ppm for eight months, none of the control rats and none of the male rats fed the mixture died. One female fed 100 ppm died after six months exposure and two females fed 500 ppm died after one and two months exposure. Eight females fed 1,000 ppm died within two to six months after onset of the experiment. The rats fed 500 and 1,000 ppm Aroclor 1260 in their diet gained less weight than the controls. At autopsy, the livers of all male rats fed Aroclor 1260 weighed significantly more than the livers of the controls (Table 1). The livers of the female rats were larger also but a significant weight difference was not observed, except that at 500 ppm the percent of body weight of the livers was increased as a result of the reduced weight gain of these animals (Table 1).

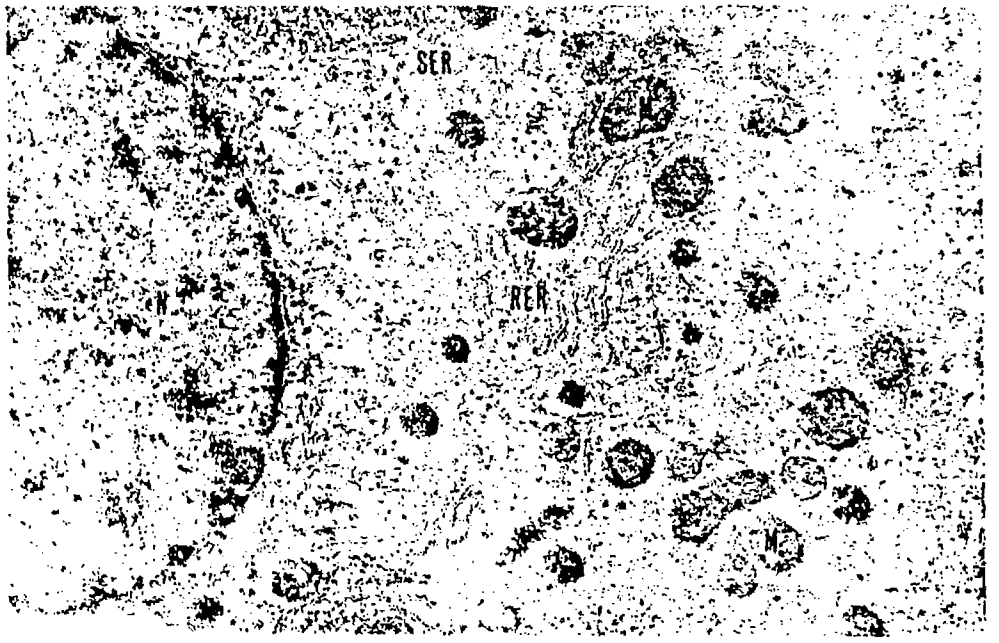
Grossly, the livers of the exposed animals were soft and often yellowish brown or dark olive. Grayish-white, firm, glistening areas, measuring up to 0.5 cm in diameter, were seen on cross section in a number of livers. The dark livers showed pink-orange fluorescence under the UV light.

Fig 3.—Area of adenofibrosis as in Fig 5 from female rat fed 500 ppm Aroclor 1254 for eight months. Area was examined under electron microscope (Fig 8-10) (toluidine blue ×300).



The incidence of the various light microscopic findings of the livers are given in Table 3. They consisted of hypertrophy of the individual liver cells. Often the nuclei were hyperchromatic and showed pleomorphism, and occasional mitotic figures were observed. The cytoplasm had either large vacuoles or appeared foamy (Fig 1). Eosinophil inclusions identical to those described in the liver of rats that have been exposed to DDT,[10] mirex,[17] or dieldrin[18] were also observed. The Kupffer cells and perivascular macrophages of some livers contained a brown pigment. Frozen sections of livers containing the brown pigment fluoresced pink under UV light which is consistent with the presence of porphyrin. Some of the pigment showed a positive staining reaction for iron with Perls' stain as well. In addition to these findings, some livers also exhibited focal areas of fibrosis which contained glandular structures (Fig 2 and 3) that either formed strands or dilated cystic ducts that were filled with cellular debris (polymorphonuclear leukocytes) or a mucus-like PAS-positive substance. The glandular cells had a pale, scanty, basophilic cytoplasm and usually a hypochromatic nucleus. The areas of adenofibrosis corresponded to the grayish-white, glistening areas described grossly. The glandular epithelium appeared stratified in some areas. Electron microscopic study revealed (Fig 4 to 10) an increase in smooth endoplasmic reticulum (Fig 5) in the hepatocytes of all but the control rats (Fig 4). Many large and small lipid vacuoles and occasional atypical mitochondria were also seen. At the dietary level of 100 ppm, dark and light cells, dilated bile ducts, and granular cytoplasmic inclusions were additional findings (Fig 6). At the dietary level of 500 and 1,000 ppm, concentrically arranged membranes which surrounded lipid vacuoles were also observed. They have been previously described in livers of rats fed DDT and dieldrin.[18] The cytoplasm of many cells had a loosened-up, "moth eaten" appearance (Fig 7), with occasional vacuoles containing a few strands of electron-dense material. These cells corresponded to the hepatocytes with foamy cytoplasm observed under the light microscope. Widening of intercellular spaces, occasional phagocytized red blood cells, and an increase in collagen were observed. The nodular, grayish-white areas (Fig 8) that represented

Fig 4.—Section from normal male rat liver. N, nucleus; M, mitochondria; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum (lead citrate, uranyl acetate ×13,490).



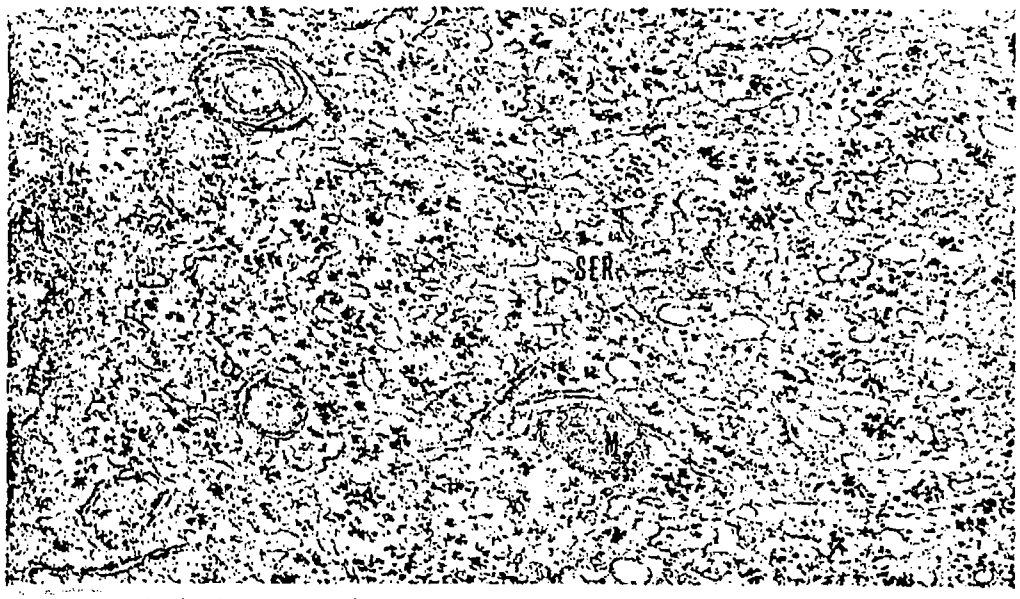
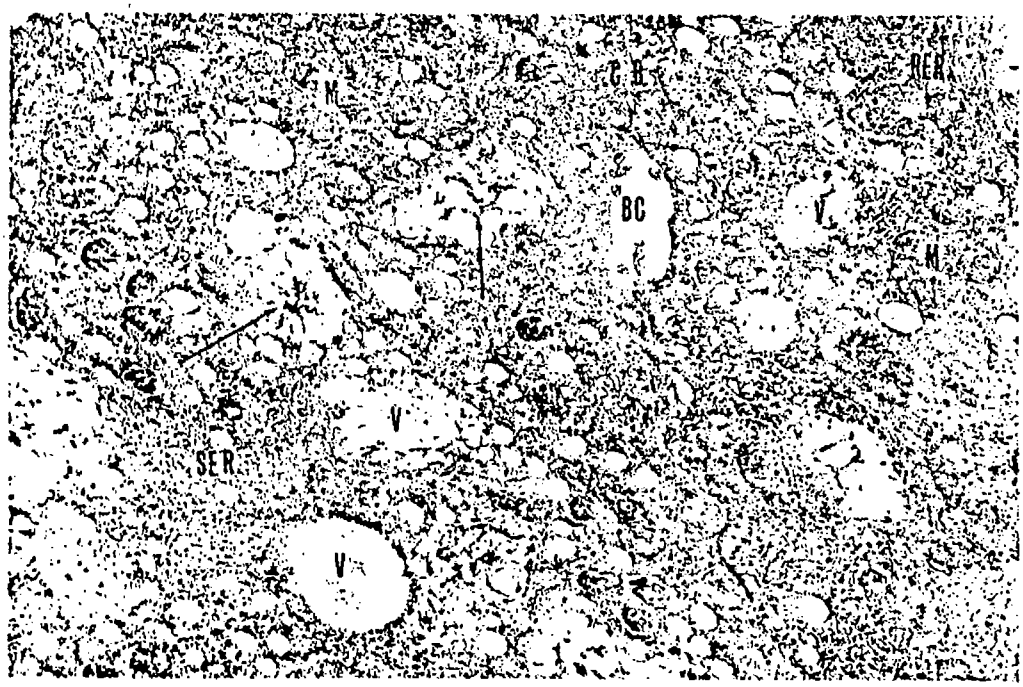


Fig 5.—Section from male rat liver fed 100 ppm Aroclor 1260 for eight months. Note large amount of smooth endoplasmic reticulum. SER, smooth endoplasmic reticulum; M, mitochondria; N, nucleus (lead citrate, uranyl acetate ×34,200).

Fig 6.—Section from liver of female rat fed 500 ppm Aroclor 1260 for eight months. Note small and large vacuoles within cytoplasm, some filled with lipid and others with strands of dark granular material (arrows). V, vacuoles; M, mitochondria; CB, cell border; BC, bile canaliculus; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum (lead citrate, uranyl acetate ×13,490).



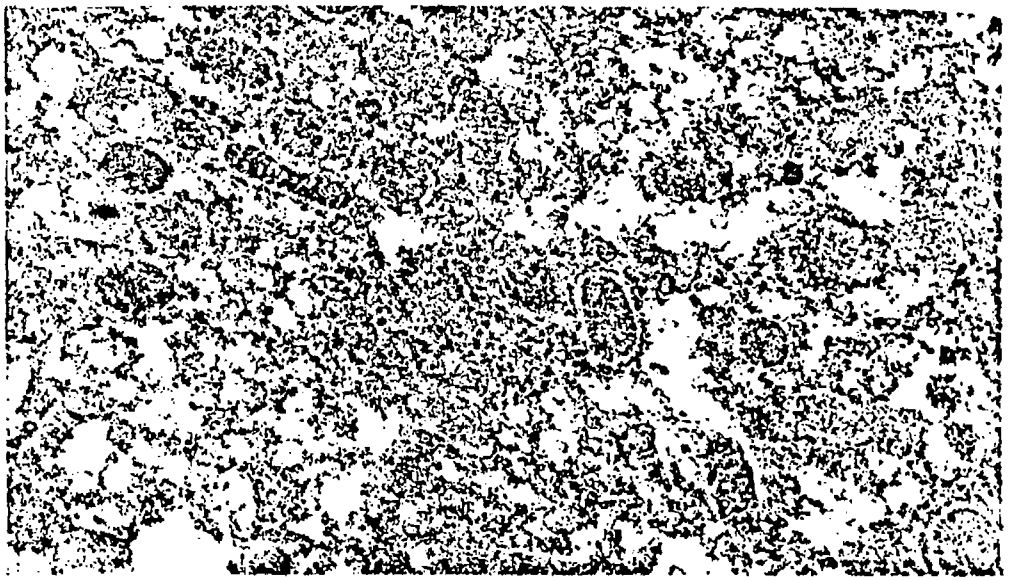
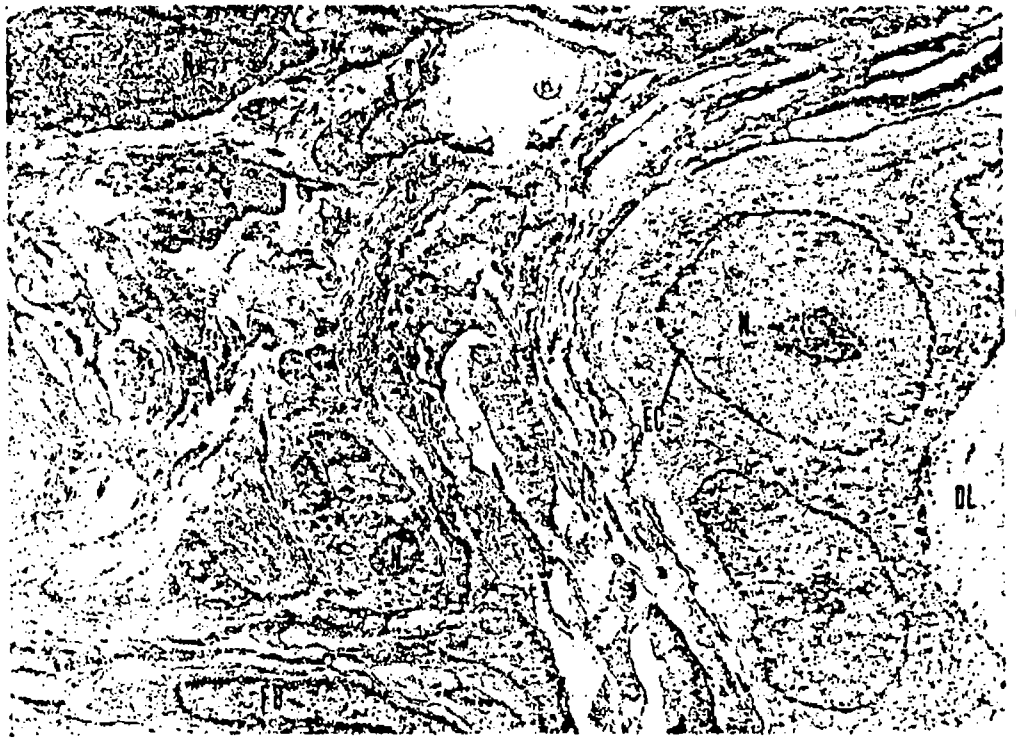


Fig 7.—Section from liver of male rat fed 1,000 ppm Aroclor 1260 for eight months. Note "moth-eaten" appearance of the cytoplasm (lead citrate, uranyl acetate ×13,490).

Fig 8.—Section from liver of female rat fed Aroclor 1254 for eight months. Note large amount of collagen, fibroblasts, and epithelial cells lining a ductule. C, collagen; FB, fibroblast; N, nucleus; EC, epithelial cell (arrow); DL, lumen of ductule (lead citrate, uranyl acetate ×5,320).



extensive foci of adenofibrosis consisted of fibroblasts and a great deal of collagen that surrounded rosettes of epithelial cells. These cells were cuboidal or columnar in shape. The free surface was lined by microvilli, and cells contained large, pale nuclei. The cytoplasm was granular, with many ribosomes and a few endoplasmic membranes. Some of these liver duct cells contained a great deal of mucus and resembled goblet cells similar to those observed in the intestinal tract (Fig 9). The cytoplasm contained tonofilaments and, particularly near the duct lumen, the boundaries between adjacent cells were marked by prominent terminal bars (Fig 10).

In a preliminary study in which 1,000 ppm Aroclor 1254 was fed in the diet to ten male and ten female rats, five of the males and eight of the females died. At autopsy of the exposed animals, the livers were pale and enlarged and contained firm, grayish-white, glistening areas. One male rat that died after 49 days exposure showed only slight enlargement of the liver cells. The hepatocytes of all the other exposed rats were enlarged, vacuolated often with hyperchromatic—sometimes enlarged and sometimes pyknotic—nuclei which occasionally displayed mitotic figures. Varying degrees of fibrosis around the bile ducts were observed. Hyperplasia and proliferation of glandular cells simulating bile ducts were also seen in these areas. In some areas, the sinusoids were dilated and contained cellular debris, lined by small "compressed" hepatocytes. A brown pigment was seen within the macrophages. A few livers stained with Oil red O showed a large amount of red staining (lipid) material. The animals that died not only had liver lesions but also suffered from severe pulmonary congestion and, in a few instances, from massive hemorrhages into the soft tissue or the peritoneal cavity.

When 500, 100, 20, and 0 ppm Aroclor 1254 were fed to the rats for eight months, two males and one female rat died at 500 ppm, but none died at the lower dietary levels. The rats fed 500 ppm Aroclor 1254 gained less weight than the controls. The

Fig 9.—Section from same liver as Fig 8. Note goblet cell containing mucus and degenerated cells within lumen of ductule. C, collagen; EC, epithelial cell; N, nucleus; CB, cell border (arrow); DL, lumen of ductule (arrow); MU, mucus goblet cell (lead citrate, uranyl acetate ×11,400).

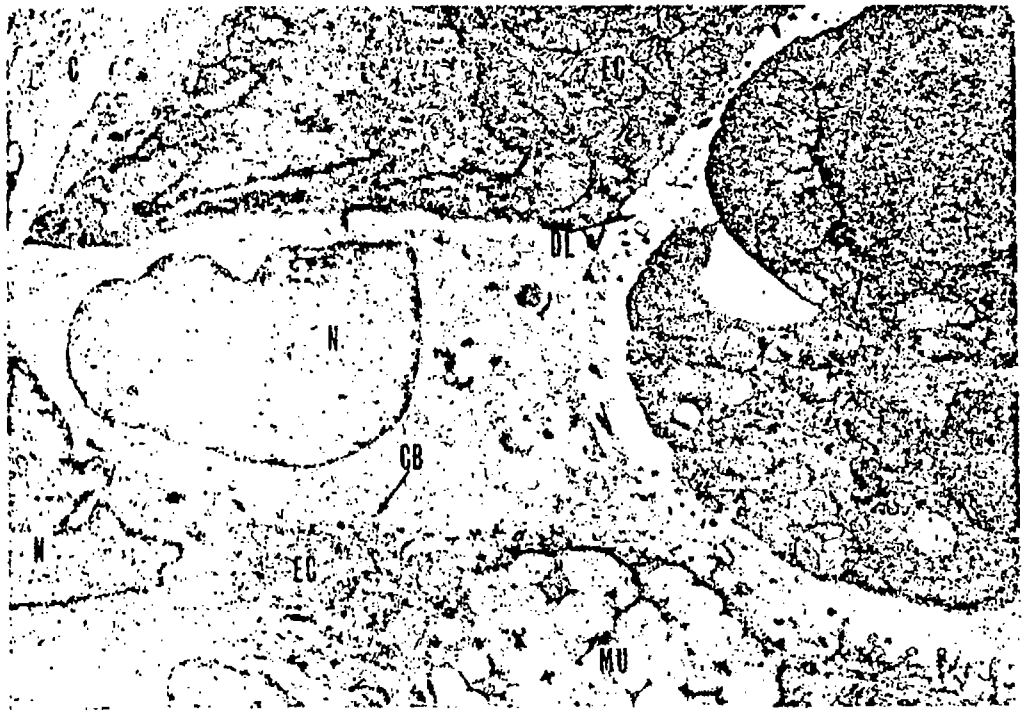


Fig 10.—Higher magnification of two epithelial cells from Fig 9 showing T, tonofilaments; D, desmosomes; N, nucleus; M, mitochondria; CB, cell border (lead citrate, uranyl acetate ×34,200).

livers of the exposed groups were larger than those of the controls (Table 2). This was significant in all but the female rats fed 100 ppm. At autopsy, the livers of the exposed animals, particularly at the higher dietary levels, were soft, yellowish-brown, or dark olive. Firm, grayish-white, glistening areas were seen, especially in those fed higher PCB levels. Occasionally, the liver surface was nodular. These livers also fluoresced when exposed to UV light. The light microscopic findings are given in Table 4. The observed changes in the livers of exposed rats were quite similar to those described for Aroclor 1260, except that all male rats at 500 ppm and one male rat at 100 ppm had areas of adenofibrosis in their liver, while none of the males demonstrated this at the respective dietary levels of Aroclor 1260. In the females, the incidence of adenofibrosis was much higher at the same dietary levels of Aroclor 1254 than in those fed Aroclor 1260. The electron microscopic examination of the different liver changes summarized in Table 4 corresponded to those already described for Aroclor 1260.

The main difference between the two compounds was the much higher incidence of adenofibrosis at a lower dietary level of Aroclor 1254. When fed over a period of time, Aroclor 1260 seemed to be more toxic to female rats. Such a sex difference could not be established for Aroclor 1254.

Comment

Aroclor 1260 and 1254 have a definite effect on the liver. This effect is more pronounced with Aroclor 1254 when all morphologic changes of equivalent dietary levels of Aroclor 1254 and 1260 are compared. All dietary levels tested so far showed an increase in the size of the individual liver cells and often an increase in the liver weights. Accumulation of fat was observed at 100 ppm, and it was very pronounced at the higher dietary levels. The incidence of cytoplasmic inclusions was higher at the lower dietary levels. This is probably due to the fact that at the higher dietary levels most of the cytoplasmic components have been replaced by lipids. The pigment observed in the Kupffer cells showed a positive prussian blue reaction and represented, at least in part, hemosiderin. The fluorescence of the livers under UV light was caused by the presence of porphyrin, which resulted in the dark discoloration of the liver.

An additional finding in the livers was the presence of grayish-white, glistening areas which microscopically corresponded to adenofibrosis. This lesion consisted of glandular, often mucus-producing, epithelial cells forming ductules. They were surrounded by fibrosis. Synonyms for this lesion are cholangiofibrosis, bile duct proliferation, bile duct adenomatosis, and fibroadenoma. Stewart

and Snell[19] give an excellent description of adenofibrosis of the liver of rats. The authors concluded that "adenofibrosis is a contracting lesion built up by the incorporation and transformation of normal (hepatic cords) and pathologic (pseudotubules) components of the surrounding liver. Thus far there is no convincing evidence that adenofibrosis is a precancerous lesion." Adenofibrosis, for instance, has been described in livers of rats fed butter yellow (p-dimethylaminoazobenzene).[20] Recently, Reuber[21] fed hamsters 2-acetamidofluorene or 2-diacetamidofluorene and produced adenocarcinomas. He thought that some of these carcinomas originated from bile ducts. The author interpreted adenofibrosis as cholangiofibrosis, suggesting that adenofibrosis in the rat was a precancerous lesion and was identical to the lesions he observed in hamsters which, in his opinion, also represented a precancerous lesion. He postulated that the observed cholangiocarcinomas developed from the adenofibrosis. Glaser and his colleagues[22] produced a highly malignant transplantable tumor, derived from the liver of a hamster fed 2-acetylaminofluorene, that did not appear to be malignant in the donor but showed changes similar to the adenofibrosis described in this paper. It is possible that the malignant lesion was not simply derived from the adenofibrosis-type lesion but from cells that already manifested irreversible preneoplastic changes that were not microscopically detectable.

Miller[3] found fatty livers, liver necrosis, and inclusions in the cytoplasm of the hepatocytes in rats that had been exposed to a biphenyl with 42% chlorination. He did not mention adenofibrosis in his report. However, he studied a different biphenyl and his animals were exposed to the chemical for much shorter periods of time. Nishizumi[7] studied the effect of a biphenyl with 48% chlorination in monkeys and mice. The longest exposure time was 26 weeks. He described enlargement of the liver, fatty changes, granular cytoplasm of the hepatocytes, increase in size of Kupffer cells and hepatocytes, and a brown pigment in some of these cells, but did not observe adenofibrosis. At some stages of exposure he observed, with the electron microscope, an increase in the smooth endoplasmic reticulum, variation in the appearance of mitochondria, and an increase in the number of microbodies, as well as "myelin figures" which seem to be similar to the concentrically arranged membranes that have been described in rats after exposure to chlorinated hydrocarbons.[16] The increase in liver weights and the observation of inclusions in the cytoplasm has been reported for several persistent insecticides.[16-18] To our knowledge adenofibrosis has not been produced with chemicals such as DDT, dieldrin, pyrethrum, piperonyl butoxide, and mirex. Unlike Nishizumi's findings,[7] an increase in the number of microbodies was not observed in the rats fed PCBs in this study.

The increase in smooth endoplasmic reticulum has also been observed with other chemicals, and it is not surprising that chlorinated biphenyls induce microsomal enzymes.[23-25]

Study of the adenofibrosis under the electron microscope shows the epithelial component of the lesion consisting of two types of cells—goblet cells and epithelial cells with granular cytoplasm, tonofilaments, and desmosomes. The latter cells resemble the epithelial cells lining the bile ducts rather than hepatocytes. The epithelial cells are reminiscent of "oval" cells which are thought to represent ductular cells. However, the oval cells are smaller and have a hyperchromatic nucleus.[26] Whether they develop originally from bile duct epithelium or from other cells in the liver needs further investigation. The fact that adenofibrosis has not been observed in previous feeding studies with PCBs may have a variety of reasons such as the length of exposure, the animal species or rat strain, and the composition of PCB given to the rats. Contamination of PCBs with traces of other chemicals such as chlorinated dibenzodioxins, chlorinated dibenzofurans, or chlorinated naphthalenes may also be responsible. Vos and Koeman[11] checked Aroclor 1260 of the same lot and also did not demonstrate any contaminants. However, preliminary chemical analyses of this lot of Aroclor 1254 suggest possible contamination with a chlorinated dibenzofuran. This is still under investigation.[27]

The significance of adenofibrosis will be further investigated in order to establish whether it is an irreversible lesion and whether it is transplantable. From experi-

ence with DDT and other chlorinated hydrocarbons, it can be deduced that the liver enlargement, as expressed by hypertrophy of the liver cells and the observation of inclusions in the cytoplasm, is reversible after cessation of exposure.

The observed porphyria, which was also noted by Vos and Koeman,[11] and the effect of PCBs on the liver, particularly the adenofibrosis, make it necessary to reevaluate our present concept of the toxicity of PCBs. Additional information is also needed on the interaction of PCBs with substances such as alcohol, chlorinated hydrocarbons, sex hormones, lead, and, of course, the various contaminants that may or may not be present in the PCBs. The quality of the diet may have an impact on the effect of PCBs. The findings described in this paper support the conclusion of Lichtenstein et al[28]—that the toxicity of the PCBs decreases as the level of chlorination increases.

Richard L. Moore assisted with the animal tests. Estelle C. Gray did the statistical analysis. Linda W. Anderson and Annie R. Alford prepared the tissues.

Aroclor 1254 (lot No. AK-38) and Aroclor 1260 (lot No. AK-3) were provided gratis by Monsanto Chemical Co, St. Louis, Mo.

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