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Evaluation of Ultrastructural Hepatic Response to Environmental Toxicants in Wild Cotton Rats (*Sigmodon hispidus*)

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The cytochrome P-450-mediated microsome monooxygenase is involved in the metabolism of endogenous substrates, such as steroid hormones, as well as xenobiotics, including many drugs and carcinogens (Conney 1982; Payne et al. 1987). The induction of cytochrome P-450 enzymes to certain chemicals plays a significant role in the rate of metabolism of these compounds and could lead to the development of a biological monitoring system of sufficient sensitivity to discriminate environmental quality differences for a wide variety of pollution conditions (Payne et al. 1987). There is a correlation between the alteration in structure and content of organelles and biochemical changes observed in the subcellular fractions (Fouts and Rogers 1960; Weibel et al. 1969; Staubli et al. 1969; Toftgard et al. 1986; Woods and Fowler 1986).

Hepatic lobules are composed of hepatocytes organized in three microcirculatory zones (periportal, midzonal, and centrilobular). The hepatocytes in each of these zones contain enzymes which are involved in various biochemical reactions. The predominant location of the mixed-function oxidation system in the liver lobule is the centrilobular zone (Farber and Fisher 1979). Ultrastructural changes in the hepatocytes not only correlate with biochemical events of detoxification but also with toxic effects of a parent compound or its metabolites. The objective of this study was to characterize the ultrastructural alterations in the liver of wild cotton rats (Sigmodon hispidus) following exposure to polychlorinated biphenyls (PCB) contaminated habitat.

MATERIALS AND METHODS

An industrial site near Pryor, Oklahoma (PO), a known PCB-contaminated area was selected for the field study. This site is located in Mayes County, Oklahoma. According to information provided by the United States Corps of Engineers (1987), the site has Aroclor 1254 (PCB mixture) concentration in the soil greater than 800 PPM. The contaminated site was dominated by tall-grass cover. An uncontaminated control site with an ecologically similar habitat to PO was selected approximately 0.50 km Southwest of PO.

Cotton rats were live-trapped for 3 consecutive days, using an 3 x 22 trapping grid with 5 m spacing between trap stations and 10.2 x 10.2 x 22.9 cm Sherman aluminum traps baited with rolled oats. Individual trap stations were identified with labeled flags. The third day's captives were weighed, sexed, and returned to the laboratory for further evaluation. Body weights were used as an index of age: 0-59.9g=Juvenile, 60-

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110g=Subadult, over 110g=Adult (Glass and Slade 1980).

All rats were fasted overnight with water provided ad libitum, immobilized by cervical dislocation, and exsanguinated by severing the aorta. The cotton rats were necropsied, and their livers quickly removed, weighed and volumes determined (Elias and Hyde 1980). Each carcass (excluding liver, kidneys, spleen, portions of intestine, stomach, adrenals, reporductive organs and brain) was labeled and submitted to the United States Corps of Engineers for PCB analysis. Total hepatic cytochrome P-450 levels were assayed by the method of Omura and Sato (1964). Microsomal protein was determined on solubilized microsome samples (Smith et al. 1985). Liver, kidney, adrenal, pancreas, representative samples of intestinal areas, reproductive organs, and brain were fixed in 10 % neutral buffered formalin for histopathology. Liver tissue for transmission electron microscopic examination was minced to approximately 1-mm cubes and fixed in Karnovsky's fixative. Tissue was rinsed in three changes of cacodylate-H2O-sucrose buffer for 30 min (10 min each rinse), post-fixed in a 1:1 solution of osmium tetroxide and 0.27 M cacodylate buffer for one hr. Tissue was rinsed in three changes of cacodylate-H2O-sucrose buffer for 30 min (10 min each rinse). Dehydration consisted of consecutive 10 min rinses each of 50%, 70% , 90% reagent grade ethyl alcohol followed by three 10 min rinses in 100% ethyl alcohol and 10 min three rinses in propylene oxide. Tissue was then allowed to remain for 24 hrs in 1:1 propylene oxide and DER resin. The tissue was embedded in fresh 100 % DER and polymerized at 60°C with 5-8 Hg vacuum for 48 hrs. Thick (55 nm) sections were cut with glass knives on a Sorvall MT 6000 Ultracut ultramicrotome, mounted on glass microscope slides, and stained with Mallory's stain. Centrilobular hepatocytes were selected for thin sectioning. Thin (0.55nm) sections were stained with uranyl acetate and lead citrate and examined with a Jeol 100C XII transmission electron microscope (Jeol Ltd, Tokyo, Japan).

RESULTS AND DISCUSSIONS

The population of the cotton rats in the Pryor site including the uncontaminated was relatively low. Nine cotton rats (6 contaminated and 3 uncontaminated) were collected from the pryor study site. Except for a one cotton rat (body weight 47.67g) from contaminated site, all cotton rats were subadults. At necropsy, major gross lesions in the contaminated rats were confined to the liver. The livers were friable and enlarged with accentuated lobular patterns. Additionally, one rat had multiple irregular white foci of 1-3 mm in diameter in the central hepatic lobe. These hepatic gross changes were not observed in the uncontaminated rats. The other organs from both contaminated and uncontaminated rats were essentially normal. Liver weight, liver volume, liver-weight to body-weight ratio and liver volume to body-weight ratio are presented in the Table 1. Despite limited number of cotton rats, both liver-weight to body-weight and liver volume to body weight were significantly ($P < 0.05$) increased in the contaminated rats.

This increase was associated with increased activity of total hepatic cytochrome P-450. The total hepatic cytochrome P-450 in the contaminated rat was 144 % that for the uncontaminated cotton rat. Except for one cotton rat (PCB concentration < 0.5 ppm), PCB concentration of PO rats (1.42 to 12.36 ppm) greatly exceeded the concentrations in CO rats (< 0.5 ppm). There were substantial light microscopic changes in the livers of contaminated rats. These alterations included mild to moderate enlargement of centrilobular and midzonal hepatocytes including enlarged nuclei and increased cytoplasmic lipid droplets. Within a single hepatocyte, these lipid droplets ranged from numerous fine droplets to one or more larger droplets (Fig. 1). The periportal hepatocytes were minimally affected. The liver from one rat from the contaminated site had multiple, locally extensive foci of coagulation necrosis. The livers of the uncontaminated rats were essentially normal (Fig. 2). Incidental extrahepatic lesions seen in both contaminated and

Table 1. Body weights, liver weights, Liver volume and total hepatic cytochrome P-450 levels from contaminated and uncontaminated cotton rats

Measurement	Uncontaminated site (n=1M+2F)	PCB Contaminated site (n=3M+3F)
Body weight (g)	93.06±3.97	74.73±7.84
Liver weight (g)	2.990.24	4.52±0.53
Liver volume (cubic cm)	2.78±0.25	4.15±0.50
Liver weight ---------- X100 Body weight	3.25±0.41	6.22±0.64 *
Liver volume ---------- X100 Body weight	3.02±0.41	5.71±0.61 *
P-450 (nmoles/mg protein)	1.70±0.21	2.45±0.26 (144%)

All data are expressed as Mean±SE
Number in parenthesis indicates the percentage of the control value
* Statistically different from uncontaminated ($P < 0.05$)
n= Number of rats, M=male, F=female

uncontaminated rats included mild interstitial nephritis, intestinal strongyloidiasis, and cestodiasis and focal interstitial mononuclear cell infiltrations in the pulmonary interalveolar septa.

Ultrastructurally, the most striking feature of centrilobular hepatocytes of the contaminated rats was the proliferation of smooth endoplasmic reticulum (SER). The cytoplasm was filled with SER, which displaced the mitochondria (Fig. 3). The proliferation of SER consisted of both vesicular and tubular membranes. The rough endoplasmic reticulum (RER) was disorganized with long profiles woven between mitochondria and in one case encircling the nucleus. The rough endoplasmic reticulum were dilated and continuous with the proliferated SER. Additionally, RER had fewer than normal attached ribosomes. Membrane-bound lipid droplets of various sizes were in the cytosol, and most were enclosed by SER. The presence of lipid droplets varied among rats; two rats had substantially more lipid droplets compared to cells from other rats.

Compared with contaminated rats, the centrilobular hepatocytes of uncontaminated rats had less SER, uniformly distributed mitochondria (Fig. 4), and fewer membrane-bound lipid droplets. However, one rat had minimal mitochondrial degeneration (one or two mitochondria involved) characterized by microconcretions.

The phenomenon of increased liver weight associated with induction of microsomal enzymes has been observed in rabbits with dermal application of Aroclor (PCB mixture). Similarly, hepatomegaly, liver weight increases and increased friability have been report-

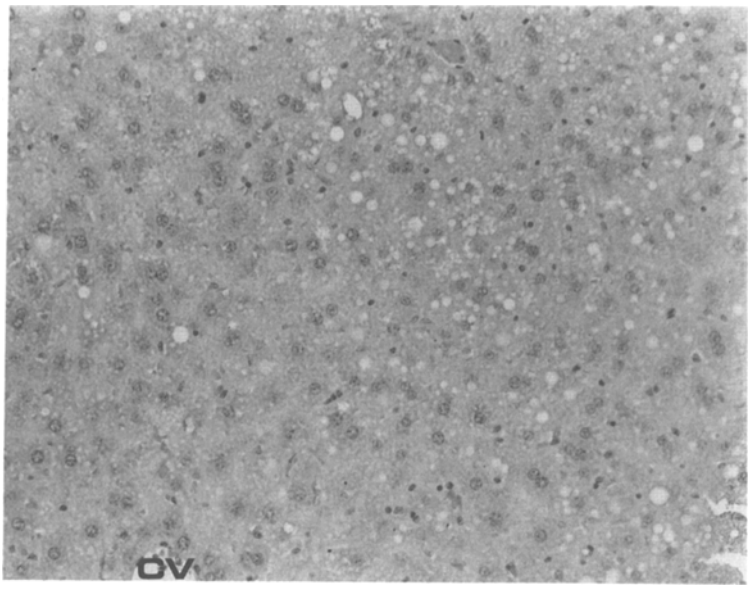

Figure 1. Liver from a contaminated cotton rat. Swollen centrilobular hepatocytes with numerous cytoplasmic lipid droplets. Central vein (CV). 12.5 X.

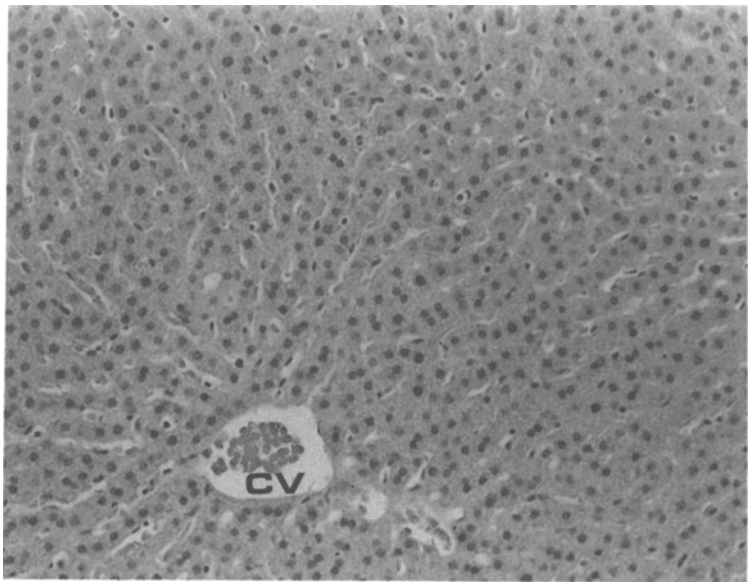

Figure 2. Liver from an uncontaminated cotton rat. Normal hepatocytes. Central vein (CV). 12.5 X.

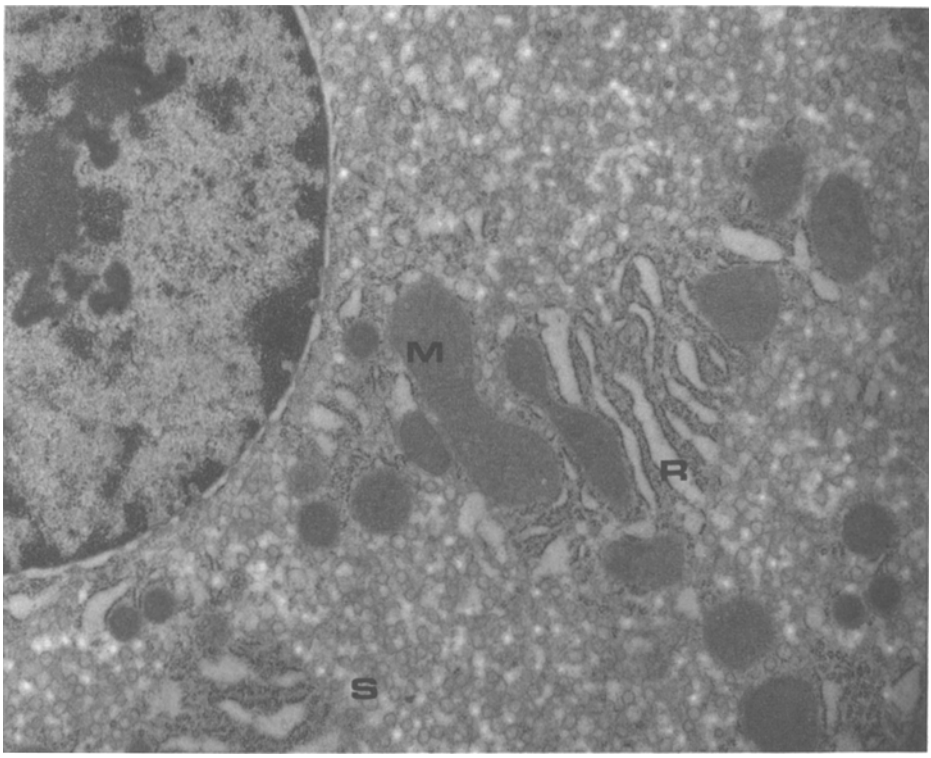

Figure 3. Centrilobular hepatocyte from a contaminated cotton rat. Note proliferation smooth endoplasmic reticulum (S). Mitochondria (M); rough endoplasmic reticulum (R). 10,000 X.

ed in rats fed polybrominated biphenyls (Sleight and Sanger 1976; Raber et al.1986). This increase in the liver weight was related to hypertrophy of the hepatocytes with increased organelle (SER) content. These findings are in agreement with significantly increased liver weight and volume of the contaminated rats in the present study.

The proliferation of SER is a reversible response to a variety of exogenous substances including PCB and polybrominated biphenyls (PBB) and is a morphological reflection of enhanced enzyme activity (Sleight and Sanger 1976; Farber and Fisher 1979; Render et al. 1982). Increased SER following PCB treatment, has been reported in the livers of mice, monkeys, guinea pigs, and rats (Nishizumi 1970; Kimbrough et al. 1972; Vos and Notenboom-ram 1972). Biotransformation enzymes of the mixed-function oxidation system are associated with electron transport chains on the SER (Farber and Fisher 1979); therefore, proliferation of the SER results in increased activity of the MFO enzymes. Since detoxification of PCB and PBB is dependent on the MFO enzymes, the logical result of PCB-induced hepatotoxicity would be proliferation of SER in hepatocytes containing substantial mixed-function oxidation capability. Thus, the zonal distribution of the PCB-induced increase in SER, is greater in centrilobular hepatocytes (Farber and Fisher 1979).

Increased SER originates from continuous growth and budding from preexisting cisternae

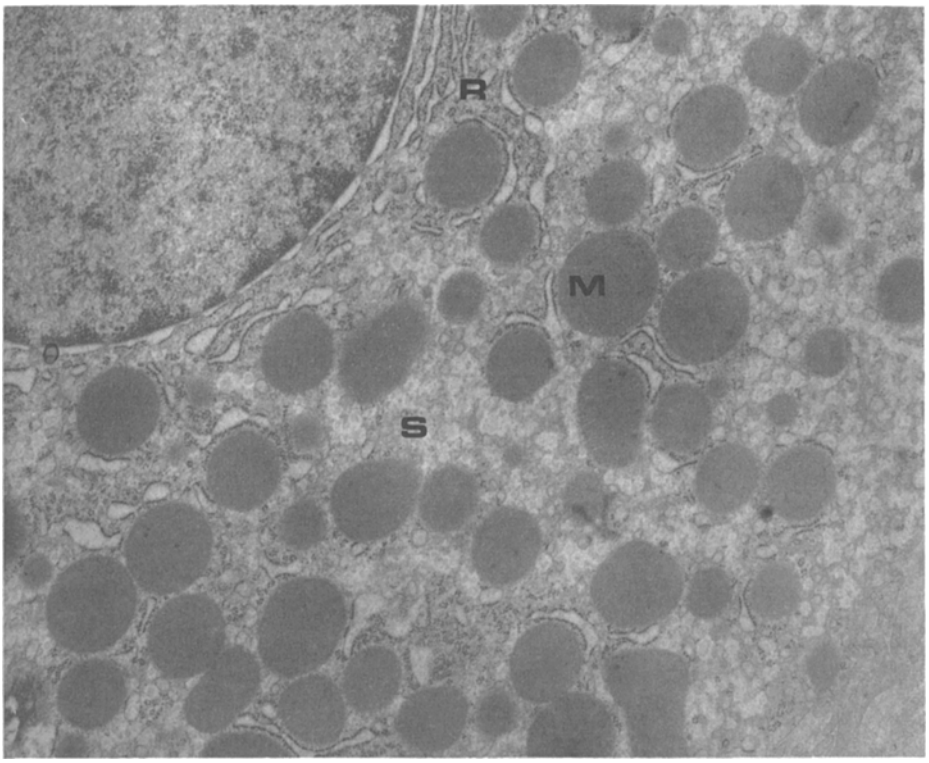

Figure 4. Electron micrograph of centrilobular hepatocyte from an uncontaminated cotton rat. Diffusely scattered mitochondria (M) and network of endoplasmic reticula of rough (R) and smooth (S) types. 10,000 X.

of RER (Orrenius and Ericsson 1966). Vos and Notenboom-Ram (1972) have reported numerous ultrastructural changes in rabbit hepatocytes due to PCB treatment. Included in their observations were degranulation of RER in hepatocytes from animals after dermal application of PCB for 28 days. In the present study, the ribosomes in many hepatocytes from contaminated cotton rats were affected similarly. According to Farber and Fisher (1979), the dispersal of ordered aggregates of ribosomes and detachment of ribosomes from RER can occur under various conditions, including anoxia, ischemia, and hepatotoxcosis. This phenomenon has been associated with inhibition of protein synthesis during liver injury induced by carbon tetrachloride and ethionine. Ethionine-induced ultrastructural and biochemical changes can be reversed by the administration of a metabolic antagonist of ethionine such as methionine or adenine.

Intracytoplasmic membrane-bound lipid droplets were found throughout the cytosol and varied in number and size. In PCB-treated rats, fatty livers were produced as a result of the decreased availability of non-esterified fatty acids for hepatic mitochondrial oxidation (Mehlman et al. 1974).

Polychlorinated biphenyls have been identified as widespread, persistent environmental contaminants. They have been detected in fish (Brunn and Manz 1982; Zabik et al.

1982), birds (Barbehenn and Reichel 1981), and humans (Safe 1984). The PO contaminated cotton rats were exposed to known PCB polluted site for an unknown period. Their whole body PCB content confirmed the bioavilability of the toxicant. How PCB entered or enters the body is still unknown. Most likely, the mode of exposure would be through ingestion. However, inhalation through dust filled air and percutaneous absorption are also possible. Additionally, transfer of PCB from maternal to fetal and suckling rats has been reported (Takagi et al. 1986) whereby the highest concentration of PCB was in the fetal placenta followed by the fetal liver, heart, skin, muscle, blood, lung and brain and the dam's milk. In conclusion, the ultrastructural evaluation of liver together with hepatic microsomal assay, hepatic light microscopy, liver weight and volume from wild cotton rats provide a meaningful method of biomonitoring for environmental contamination.

REFERENCES

Barbehenn KR, Reichel WL (1981) Organochlorine concentrations in bald eagles : Brain / body lipid relations and hazard evaluation. J Environ Health 8:325-330

Brunn H, Manz D (1982) Contamination of native fish stock by hexachlorobenzene and polychlorinated biphenyl residues. Bull Environ Contam Toxicol 28:599-604

Conney AH (1982). Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons : G. H. A. Clowes Memorial lecture. Cancer Res 42:4875-4917

Elias H, Hyde DM (1980). An elementary introduction to stereology (quantitative microscopy). The American Journal of Anatomy 159:411-446

Farber E, Fisher MM (1979) Toxic injury of the liver. Part A. Marcel Dekker, New York, pp. 486

Fouts JR, Rogers LA (1960) Morphological changes in the liver accompanying stimulation of microsomal drug metabolizing enzyme activity by phenobarbital, chlordane, benzyprene or methylcholanthrene in rats. J Pharm Exp Ther 147:112-119

Glass GE, Slade NA (1980) Population structure as a predictor of spatid association between Sigmodon hispidus and Microtus ochrogaster. J Mammol 61:473-485

Kimbrough RD, Linder RE, Gaines TB (1972) Morphological changes in livers of rats fed polychlorinated biphenyls. Light and ultrastructure. Arch Environ Health 25:354-364

Mehlman MA, Yin L, Nielsen CR (1974) Metabolic changes in "frozen-clamped" livers of rats caused by ingestion of polychlorinated biphenyls. Toxicol Appl Pharmacol 27:300-307

Nishizumi M (1970) Light and electron microscope study of chlorobiphenyl poisoning in mouse and monkey liver. Arch Environ Health 21:620-632

Omura T, Sato R (1964) The carbon-monoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem 239:2370-2378

Orrenius S, Ericsson JLE (1966) Enzyme-membrane relationship in phenobarbital induction of synthesis of drug metabolizing enzyme system and proliferation of endoplasmic membranes J Cell Biol 28:181-198

Payne JF, Fancey LL, Rahimtula AD, Porter EL (1987) Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. Comp Biochem Physiol 86:233-245

Raber BT, Carter JW (1986) Localization of ultrastructural alterations induced in rat liver by dietary polybromobiphenyls (FireMaster BP-6). Arch Environ Contam Toxicol 15:725-732

Render JA, Aust SD, Sleight SD (1982) Acute pathologic effects of 3,3',4,4',5,5'-hexabromobiphenyl in rats: Comparison of its effects with FireMaster BP-6 and 2,2',4,4',5,5'-hexabromobiphenyl. Toxicol Appl Pharmacol 62:428-444

Safe S (1984) Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) :

Biochemistry, toxicology, and mechanism of action. Crit Rev Toxicol 13:319-395
Sleight SD, Sanger VL (1976). Pathologic feature of PBB toxicosis in the rat and guinea pig. J Am Vet Med Assoc 169:1231-1235
Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzno MD, Fujimoto EK, Goek NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinochoninic acid. Anal Biochem 150: 76-85
Staubli W, Hess R, Weibel ER (1969) Correlated Morphometric and Biochemical studies on the liver II. Effects of phenobarbital on rat hepatocytes. J Cell Biol 42:92-112
Takagi Y, Aburada S, Hashimoto K, Kitaura (1986) Transfer and distribution of accumulated (14C) polychlorinated biphenyls from maternal to fetal and suckling rats. Arch Environ Contam Toxicol 15:709-715
Toftgard R, Nilsen OG, Carlstedt-Duke J, Glaumann H (1986) Polychlorinated terphenyls : Alterations in liver morphology and induction of cytochrome P-450. Toxicology 41:131-144
United States Army Corps of Engineers (1987), Southwestern Division, Tulsa district : Fort Gibson lake, Oklahoma, Phase I - Remedial investigation / feasibility study, March Vos JG, Notenboom-Ram E (1972) Comparative toxicity of 2,4,5,2',4',5'-Hexachlorobiphenyl and a Polychlorinated biphenyl mixture in rabbits. Toxicol Appl Pharmacol 23:563-578
Weibel ER, Staubli W, Gnagi HR, Hess FA (1969) Correlated Morphometric and Biochemical studies on the liver cell I. Morphometric model, stereologic methods, and normal morphometric data for rat liver. J Cell Biol 42:68-91
Woods JS, Fowler BA (1986) Alteration of hepatocellular structure and function by thallium chloride : ultrastructural, morphometric and biochemical studies. Toxicol Appl Pharmacol 83:218-229
Zabik ME, Merril C, Zabik MJ (1982) PCBs and other xenobiotics in raw and cooked carp. Bull Environ Contam Toxicol 28:710-715

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