Histol Histopath (1993) 8: 35-40

Histology and Histopathology

Ultrastructure of granulosa lutein cells from rats fed hexachlorobenzene

I.J. MacPhee[1], A. Singh[2], G.M. Wright[2], W.G. Foster[3] and N.N. LeBlanc[1]
Departments of [1]Biology, and of [2]Anatomy and Physiology, University of Prince Edward Island, Charlottetown, Canada; and [3]Environmental and Occupational Toxicology Division, Health Protection Branch, Tunney's Pasture, Ottawa, Canada

Summary. Corpora lutea from Sprague-Dawley rats that were orally administered 0.0 (control), 1.0, 10.0, and 100.0 mg/kg hexachlorobenzene (HCB) for 21 days were analyzed by electron microscopy. Granulosa lutein cells (GLC) from animals of the 10.0 mg group showed differences from the cells of animals that served as the controls. Golgi complexes and smooth endoplasmic reticulum appeared more conspicuous, possibly due to dilation resulting from hyperactivity. Free polysomes seemed more prominent in the cells of the 10.0 mg group. The GLC architecture from animals of the 1.0 and 100.0 mg groups was similar to that of the corresponding cells in the control group. Since smooth endoplasmic reticulum is involved in the synthesis of steroid hormones, and that free polysomes are engaged in synthesis of cytoplasmic proteins, it is suggested that HCB at a dose of 10.0 mg/kg given for 21 days may alter the synthetic activity of the GLC of the rat.

Key words: Corpus luteum, Ultrastructure, Hexachlorobenzene, Rat

Introduction

Hexachlorobenzene (HCB) is a persistent organohalogen that is widely distributed in the environment. This organic compound was used as a fungicide on crops in the United States (US Environmental Protection Agency, 1972), and is produced as a waste in the production of some chlorinated solvents and pesticides. The fungicides pentachloronitrobenzene, tetrachlorobenzene, and the herbicide «Dacthal» have been shown to be contaminated with HCB. HCB has been found in atmospheric dust in France (Mestres et al., 1978), and in the waters of the Great Lakes area of North America (Strachan and Huneault, 1979). The compound's presence in plastic water bottles used in Canada and the U.S. by campers and cyclists is of great concern (Rourke et al., 1977). Furthermore, HCB is reported to be a reproductive toxin (Foster et al., 1992a,b) in addition to being immunotoxic and carcinogenic to experimental animals (Krewski et al., 1986).

This compound has been shown to cause the alteration of cell shape in ovarian surface epithelium (Babineau et al., 1991; Sims et al., 1991) as well as reproductive failure in mammals. HCB is known to increase the mortality rate of litters of Sprague-Dawley rats, and to pass from the mother to the litter (Siegel-Scott and Johnson, 1986). For these reasons, and its high rate of bioaccumulation, HCB has been classified as an environmental hazard (Bro-Rasmussen, 1986). We have reported (Foster et al., 1992b) augmented circulating levels of progesterone in the same HCB-exposed rats used in the current study.

The objective of this study was to determine the effects, if any, of HCB on the ultrastructure of corpora lutea, specifically, on the granulosa lutein cells (GLC), from Sprague-Dawley rats. This was achieved by comparing the tissue from the treated animals to that from the animals of the control group.

Materials and methods

Sprague-Dawley rats, weighing approximately 250 g were used in this study. The rats were maintained at the Animal facility of the Health Protection Branch, Ottawa under environmental conditions of 12-12 hour light dark cycle and relative humidity of 50%. Access to food and water was on an *ad libitum* basis. Twenty-four animals, that were equally divided into four groups, were acclimatized for seven days prior to dosing by gavage with HCB (0.0, 1.0, 10.0, and 100 mg HCB/kg b.w./day), mixed in corn oil. Animals were dosed daily for 21 days. Health of the animals was monitored daily by examining the mucous membranes, coat, body weight, and food and water consumption. On day 18 of the experiment, the animals were given, to induce

Offprint requests to: Dr. A. Singh, Atlantic Veterinary College, Charlottetown, PE, C1A 4P3, Canada

ovulation a 10 IU of pregnant mare serum gonadotropin that was administered by subcutaneous injection, followed by 15 IU of hCG (human chorionic gonadotropin) on day 20. The rats were euthanized on day 22, by giving 0.5 ml sodium pentabarbital. The left ovary was collected and prepared for ultrastructural examination. Pieces of the ovary were placed in 2% glutaraldehyde fixative, prepared in phosphate buffer saline (PBS), pH 7.3, and post-fixed in 1% osmium tetroxide in PBS. Specimens were processed using conventional methods for transmission electron microscopy (Singh et al., 1981). Thin sections were contrasted with a saturated solution of uranyl acetate prepared in 50% ethanol, and with a lead solution (Sato, 1968). The sections were examined in Hitachi-7000 or Hitachi-600 electron microscopes. Photographs were taken on Kodak EM film 4489, and prints were made on Kodak Brilliant paper.

Results

The control group

The images of GLC were irregularly shaped with frequent infoldings of the plasma membrane. The nuclei of the cells were ovoid, and contained both euchromatin and heterochromatin, the former being in higher proportion (Fig. 1). A conspicuous nucleolus was typical of the cells. Several profiles of Golgi complex, as illustrated in Figure 1, occurred in the cells, and smooth endoplasmic reticulum (sER) was found throughout the cytoplasm. The cells contained rough endoplasmic reticulum, and free ribosomes in the form of polysomes. The mitochondria contained tubular cristae, and the lipid droplets were plentiful in the cells.

The 1.0 mg HCB group

Profiles of the nuclei of the GLC in animals of this group were similar to those described in the preceding section. Thus, they retained the typical ovoid shape, and contained euchromatin, heterochromatin, and prominent nucleoli. Similarly, mitochondria were abundant, and polysomes and a large number of lipid droplets were distributed in the cytoplasm. In comparison with features of the cells from the animals of the control gorup, Golgi complex and sER, like other organelles in these cells appeared to be unaffected.

The 10.0 mg HCB Group

The cells from the animals of this group were altered

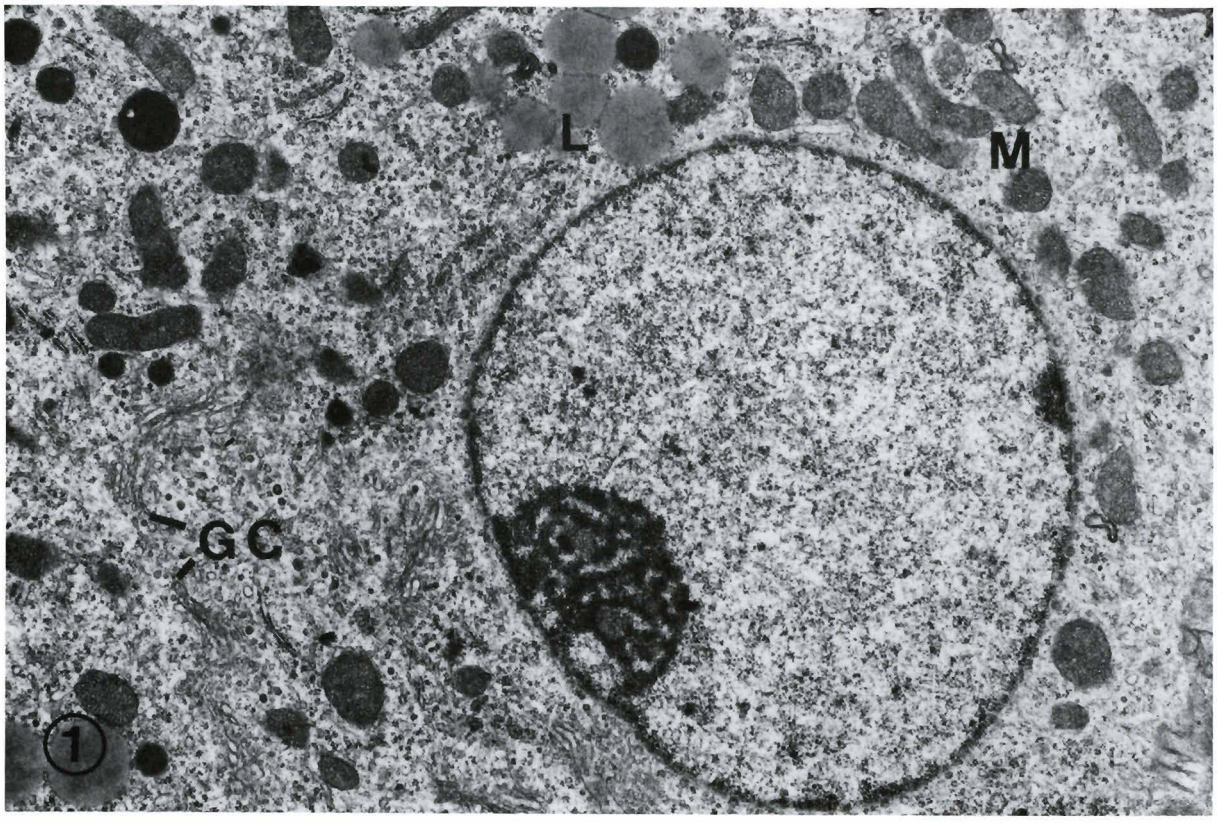

Fig. 1. Electron micrograph of a portion of typical granulosa lutein cell from a rat of the control group to depict its characteristics. Ovoid nucleus contains a prominent nucleolus. Several profiles of Golgi complexes (GC), mitochondria (M), and lipid droplets (L) are illustrated. x 13,000

(Figs. 2, 3). The profiles of sER appeared more distinct and dilated as were the images of the Golgi complex. Similarly, polysomes' images were more conspicuous in the cells of the animals in this group than in the cells of the corpus luteum of animals from the preceding groups. The abundance of mitochondria, the number of lipid droplets, and the profiles of rough endoplasmic reticulum in the GLC of the treated group seemed similar to those in the cells of animals from the control group. As well, the plasma membrane had the characteristic interdigitations (Fig. 3) among the cells. The appearance of nuclei of the GLC resembled that of the cells from animals in the preceding groups as depicted in Figure 1.

Table 1. The cell features examined in the rat granulosa lutein cells.

CELL FEATURES	CONTROL	1.0 mg	10.0 mg	100.0 mg
Plasma membrane interdigitations	Typical	*	*	
Nucleus	Ovoid			
Nucleolus	Prominent			
Rough reticulum	Typical			
Mitochondria	Typical	*	*	
Lipid droplets	Normal	*	*	
Smooth reticulum	Typical	*	Dilated**	
Golgi complex	Typical	*	Dilated**	
Polysomes	Typical	*	Pronounced **	*

*: similar appearance to that in the Control; **: Indicates modified cytoplasmic organelles.

The 100 mg HCB Group

The GLC from animals of this group were indistinguishable from the corresponding cells of the animals from the control group.

A summary of the various cell components studied, and their manifestations in the GLC from animals of the various HCB groups is included in Table 1.

Discussion

In the present study, 18 Sprague-Dawley rats that were distributed in three groups were given oral doses of HCB in concentrations of 1, 10, and 100 mg/kg, daily, for 21 days, respectively. The effects of the compound on the GLC were determined by comparing the ultrastructure of the corpora lutea of rats from the

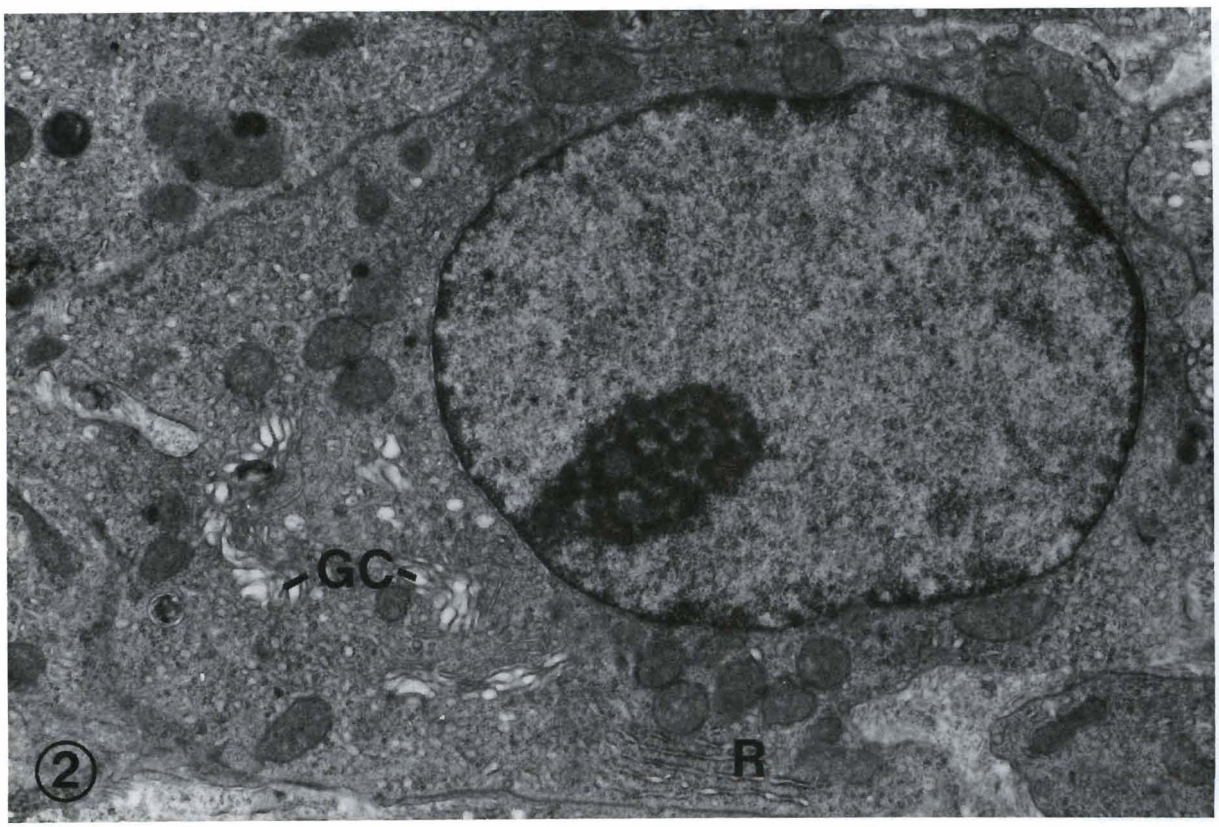

Fig. 2. Micrograph of a portion of corpus luteum from a rat fed 10.0 mg/kg body weight hexachlorobenzene for 21 days. Dilated Golgi complexes (GC) are demonstrated in a granulosa lutein cell that contains an unaltered nucleus. R = rough reticulum cisternae. x 13,000

control group with those from animals that were administered HCB. The GLC from the 10.0 mg HCB group only, appeared to be altered in that the sER and polysomes were prominent, and the cisternae of the Golgi complex were dilated.

In the present study, nuclei of the GLC remained ovoid in all the observed groups. In the examined lutein cells from animals of the control and experimental groups, the euchromatin was in far more abundance than heterochromatin in the nucleus, indicating a high synthetic activity in these cells (Fawcett, 1986). It has been shown (Babineau et al., 1991) that the surface epithelial cells of the monkey ovary were affected after treatment with HCB, such that the nuclei were irregular in shape and located near the apical surface of the cells. However, in the present study, HCB showed no effect on either the nuclear shape or chromatin definition of the GLC. The nucleoli in the GLC of the animals from all groups remained prominent.

The sER was more prominent possibly due to dilation

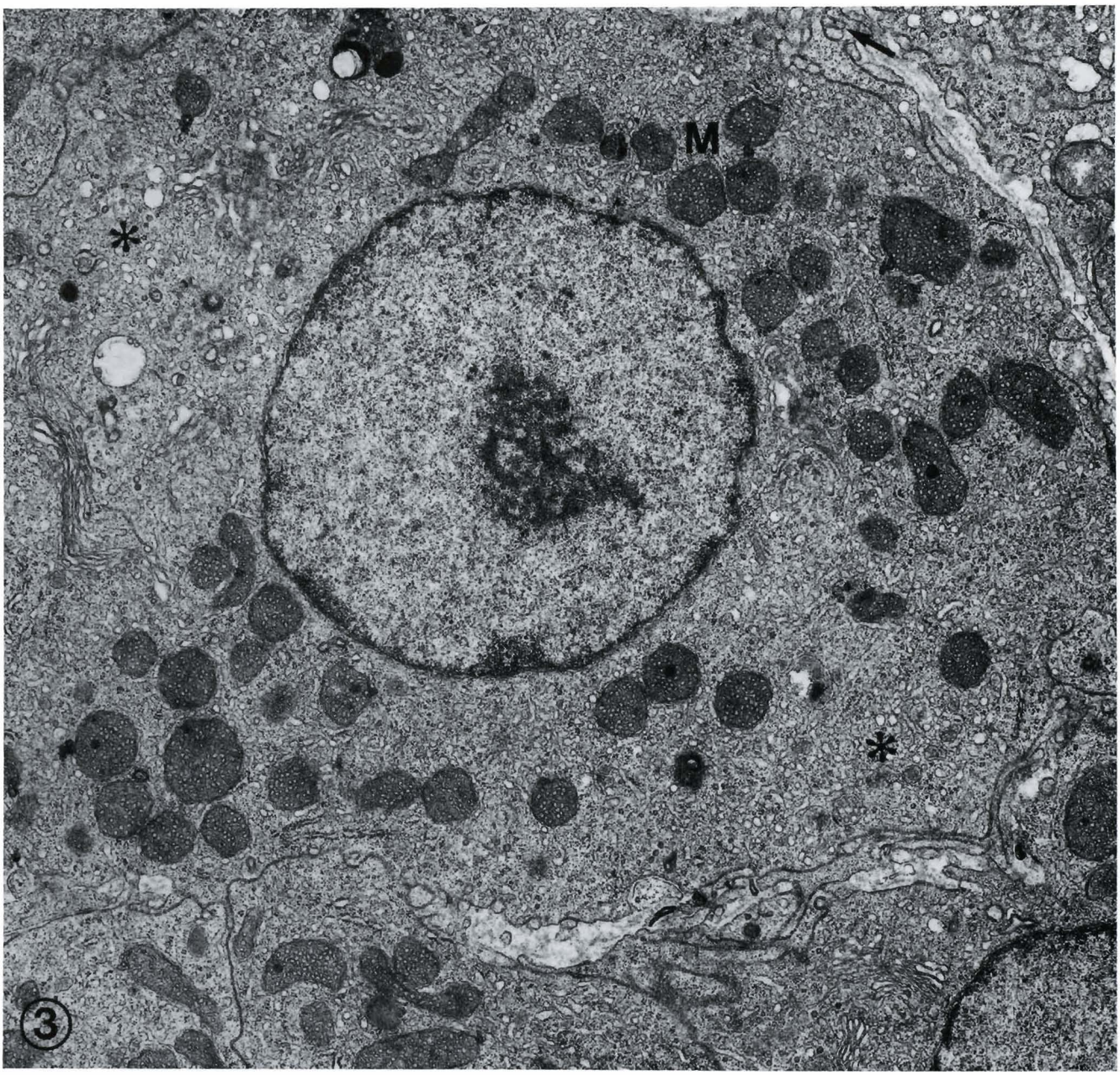

Fig. 3. Micrograph of a portion of corpus luteum from another animal of the same group as for the preceding illustration. Note the profiles of smooth endoplasmic reticulum (*) that dominate the field in a granulosa lutein cell. Characteristic tubular cristae, sectioned transversely, are seen in mitochondria (M). Typical GLC-GLC interdigitations are indicated by an arrow. x 13,000

of cisternae in the GLC of the of animals of the 10.0 mg group, suggesting that these cells may be more synthetically active than the GLC from the animals in the control, 1.0, or 100.0 mg groups. The augmentation of sER is typical of cells involved in the production and secretion of steroid hormones such as cells of the corpus luteum (Yamada and Ishikawa, 1960; Enders, 1962; Enders and Lyons, 1964). It is known that the GLC synthesize steroids from acetate via cholesterol, and that the necessary enzymes for this process are indeed found in the sER (Srere et al., 1948; Morris and Chaikoff, 1959; Werbin and Chaikoff, 1961; Armstrong et al., 1964). The prominent sER noted in the present study, is similar to that found in the sER of hepatic cells of rats that were treated with HCB (Kuiper-Goodman et al., 1977). It seems likely that HCB also induces prominence of sER in the GLC of rat corpora lutea. In a sister publication (Foster et al., 1992b), elevated levels of circulating progesterone were reported in the same HCB-treated rats of the 1.0, 10.0 and 100.0 mg groups which led to the proposal that HCB induces increased synthetic activity of the sER, probably HCB-exposure alters steroidogenesis before a correlation with morphologic evidence can be demonstrated.

The GLC from the animals of the 10.0 mg group contained an abundance of free polyribosomes. These nonmembrane-bound organelles are involved in the synthesis of cytosolic proteins. The presence of large quantities of these organelles are linked to the cells undergoing rapid growth (Ghadially, 1988). Since the GLC are the former granulosa cells which have become luteinized and grown in size, the synthetic activity associated with this abundance of polysomes semms to contribute to volume of the GLC from animals in the 10.0 mg HCB group. Since the findings on the occurrence of many polysomes in rapidly growing cells were noted in tumour cells (Ghadially, 1988); the possibility of HCB affecting the growth of the GLC cannot be ruled out. It seems likely that the oral administration of HCB affects the degree of protein synthesis in the GLC cytoplasm which may result in increased volume or growth of the cell.

The Golgi complex, an organelle involved in post-translational modifications of secretory proteins (Ross and Romrell, 1989), appeared to be affected by the HCB treatment in the 10.0 mg group. In the cells that produce protein-rich secretions, the contents of the rough reticulum cisternae are moved to the Golgi complex where they are condensed, modified, and packaged to form secretory granules (Ghadially, 1988). This organelle can be looked upon as an expression of cellular differentiation and functional maturity of the cell (Ghadially, 1988). According to Ghadially (1988), hypertrophy of the Golgi complex, noted by an increase in the number of observed profiles, can usually be correlated with an increased secretory activity. When compared to the GLC of the control group, the Golgi complex profiles in the cells were more distinct in the corresponding cells from the animals of the 10.0 mg group. It would seem that the GLC of animals in the 10.0 mg group are synthetically more active than the GLC from animals of either the control group or other two treated groups resulting in an increase production of the proteinaceous substances to be excreted by the cell. Difference between the Golgi complex of the GLC from the animals in the control, 1.0 and 100.0 mg groups could not be detected. This indicates that there is no dose-related effects of HCB on the GLC of rats, since these cells from animals of the 100.0 mg group did not show prominent or elevated numbers of the organelles than the GLC from animals of the 10.0 mg group.

Subsequently analysis of the GLC revealed no apparent alterations in the mitochondria due to the addition of HCB, since unaltered mitochondria with tubular cristae were found in the control, 1.0, 10.0, and 100.0 mg groups.

Lipid droplets, another characteristic of luteal cells (Kelly et al., 1984), were detected in the GLC from the animals of all four of the examined groups. These inclusions are storage sites for triglycerides that are used in the production of steroid hormones. If there was an increase in steroid hormone synthesis, the amount of lipid droplets in the GLC would be expected to drop. No apparent differences in these inclusions were detected. Treatment with HCB did not affect the organization of the plasma membrane of the treated cells. The irregular foldings, normally found in luteal cells (Adams and Hertig, 1969) were also present in the GLC of animals from all three of the treated groups.

There were no dose-related effects of HCB on the ultrastructure of GLC of the treated rats indicated by the fact that corpora lutea in only the animals from the 10.0 mg group (but not those in animals from the 1.0 or 100.0 mg groups) contained altered GLC. A plausible explanation for this is that the 1.0 mg dosage of HCB was not large enough to cause any effects, the 10.0 mg dosage was near the optimum concentration, and 100.0 mg was too high a concentration to be metabolized by the cells. However, it is apparent that oral administration of HCB at 10.0 mg/kg level alters the appearance of cellular organelles involved in the synthesis of cytoplasmic proteins and steroid hormones in the GLC of the rat corpus luteum.

References

Adams E.C. and Hertig A.T. (1969). Studies on the human corpus luteum. I. Observations on the ultrastructure of development and regression of the luteal cells during the menstrual cycle. J. Cell Biol. 41, 697-715.

Armstrong D.T., O'Brien J. and Greep R.O. (1964). Effects of luteinizing hormone on progestin biosynthesis in the luteinized rat ovary. Endocrinology 75, 488-500.

Babineau K.A., Singh A., Jarrell J.F. and Villeneuve D.C. (1991). Surface epithelium of the ovary following oral administration of hexachlorobenzene to the monkey. J. Submicrosc. Cytol. Pathol. 23, 457-464.

Bro-Rasmussen F. (1986). Hexachlorobenzene: an ecotoxicological

profile of an organochlorine compound. IARC Sci. Publ. 77, 231-242.

Enders A.C. (1962). Observations on the fine structure of lutein cells. J. Cell Biol. 12, 101-113.

Enders A.C. and Lyons W.R. (1964). Observations on the fine structure of lutein cells. II. The effects of hypophysectomy and mammotrophic hormones in the rat. J. Cell Biol. 22, 127-141.

Fawcett D.W. (1986). A textbook of histology. 11th ed. Saunders. Philadelphia. pp 1-35.

Foster W.G., McMahon A., Jarrell J.F. and Villeneuve D.C. (1992a). Hexachlorobenzene (HCB) suppresses circulating progesterone concentrations during the luteal phase in the cynmolgus monkey. J. Appl. Toxicol. 12, 13-17.

Foster W.G., Pentick J.A., McMohan A. and Lecavalier P.R. (1992b). Ovarian toxicity of hexachlorobenzene (HCB) in the superovulated female rat. J. Biochem. Toxicol. 7, 1-4.

Ghadially F.N. (1988). Ultrastructural pathology of the cell and matrix. 3rd ed. Vol. 1. Butterworths, London. pp 330-340.

Kelly D.E., Wood R.L. and Enders A.C. (1984). Bailey's textbook of microscopic histology. Williams and Wilkins. Baltimore. pp 723-742.

Krewski D., Colin D. and Villeneuve D.C. (1986). Environmental risk assessment: Hexachlorobenzene. In: Hexachlorobenzene: Proceedings of an international symposium. Morris C.R. and Cabral J.R.R. (eds). International Agency for Research on Cancer. Lyon. pp 621-628.

Kuiper-Goodman T., Grant D.L., Moodie C.A., Korsrud G.O. and Munro I.C. (1977). Subacute toxicity of hexachlorobenzene in the rat. Toxicol. Appl. Pharmacol. 40, 529-549.

Mestres R.F.C., Chevalier C. and Vigo G. (1978). Search for micropollutants in atmospheric dust samples taken in Laguedoc. Trav. Soc. Pharm. Montepellier. 38, 273-282.

Morris M.D. and Chaikoff I.L. (1959). The origin of cholesterol in liver, small intestine, adrenal gland, and testis of the rat dietary versus endogenous contributions. Biol. Chem. 234, 1095-1097.

Ross M.H. and Romrell L.J. (1989). Histology: A text and atlas. 2nd ed. Williams and Wilkins. Baltimore. pp 28-33.

Rourke D.R., Mueller W.F. and Yang R.S.H. (1977). Identification of hexachlorobenzene as a contaminant in laboratory plastic wash bottles. J. Assoc. Off. Anal. Chem. 60, 233-235.

Sato T. (1968). A modified method for lead staining of thin sections. J. Electron Microsc. 17, 158-159.

Siegel-Scott C. and Johnson A.E. (1986). A risk analysis of hexachlorobenzene-related reproductive outcomes. IARC Sci. Publ. 77, 629-634.

Sims D.E., Singh A., Donald A., Jarrell J. and Villeneuve D.C. (1991). Alteration of primate surface epithelium by exposure to hexachlorobenzene: a quantitative study. Histol. Histopath. 6, 525-529.

Singh A., Valli V.E.O., Ritter L. and Villeneuve D.C. (1981). Ultrastructural alterations in the liver of rats fed photomirex (8-monohydromirex). Pathology 13, 487-496.

Srere P.A., Chaikoff I.L. and Dauben W.B. (1948). The in vitro synthesis of cholesterol from acetate by surviving adrenal corticoid tissue. J. Biol. Chem. 176, 829-833.

Strachan W.M.J. and Huneault H. (1979). Polyclorinated biphenyls and organochlorine pesticides in Great Lakes precipitation. J. Great Lakes Res. 5, 61-68.

US Environmental Protection Agency (1972). Compedium of Registered Pesticides, Vol. 3, Insectidices, Ascaricides, Molluscicides and Antifouling Compounds. US Environmental Protection Agency. Washington D.C.

Werbin H. and Chaikoff I.L. (1961). Utilization of adrenal gland cholesterol for synthesis of cortisol by the intact normal and ACTH-treated guinea pig. Arch. Biochem. 93, 476-482.

Yamada E. and Ishikawa T.M. (1960). The fine structure of the corpus luteum in the mouse ovary as revealed by electron microscopy. Kyushu J. Med. Sci. 11, 235-239.

Accepted July 25, 1992