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Changes Induced by Hexachlorocyclohexane Isomers in Rat Liver and Testis

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Hexachlorocyclohexane(HCH) is the most common pesticide used in India and other countries. The technical product is a mixture of several stereoisomers of which the γ-isomer is responsible for the insecticidal action. The β-isomer is a very persistent constituent of technical HCH and is known to be highly toxic to mammals (Ulmann 1973). Biochemical effects of γ-HCH and technical HCH in mammals, such as tissue enzyme level changes and induction of hepatic drug metabolizing enzyme system have been documented (Ulmann 1973). Various dietary concentrations of technical HCH, γ-HCH and β-HCH have been known to increase liver wt/body wt ratio in various experimental animals (Ulmann 1973; Srinivasan and Radhakrishnamurty 1983).

In the present study, the chemical composition of these HCH isomers induced enlarged livers was analysed and the relative contribution of water, protein, fat, glycogen and nucleic acid to changes in liver weight was examined. The functional capacity of dietary β- and γ-HCH induced enlarged livers was investigated by studying the serum protein profile. In addition, the liver was examined histologically for abnormalities. Since recent reports have proclaimed reproductive effects produced by dietary organochlorine pesticides (De Bruin 1976), influence of dietary HCH isomers on testis was also examined here.

MATERIALS AND METHODS

Pure grade β- and γ-HCH isomers obtained from K & K Labs, USA were used in this study. Young male albino rats (Wistar strain) weighing 65-70g were fed basal diet containing 800 ppm β- or γ-HCH for two weeks(Srinivasan and Radhakrishnamurty 1977). The animals were sacrificed on the 15th day. Blood was collected by heart puncture and serum was obtained by centrifugation at 1000 X g for 10 min. Liver and testis were quickly excised, washed with cold physiological saline and used for estimation of various biochemical parameters. Sub-

cellular fractions of liver were obtained according to Hogeboom(1955). Light microscopic observations of liver or testis were made on thin paraffin sections previously fixed in 10% formalin or Bouin's solution and stained with haematoxylin-eosin.

Liver moisture content was determined by drying weighed tissue samples at 80° C to a constant weight. Total nitrogen (Oser 1965), protein content (Lowry et al.1957), DNA (Schneider 1957) and glycogen (Oser 1965) were determined by standard procedures. Total fat in liver and testis samples was extracted and purified by the method of Folch et al.(1957) and estimated gravimetrically. Phospholipids and cholesterol were determined according to Henry (1966) and Searcy and Bergquist(1960). acylglyceride values were computed by subtracting the sum of phospholipids and cholesterol from total fat.

Total serum proteins, albumins and globulins were estimated by the biuret method (Reiner 1953). Electrophoresis of serum proteins was carried out on 10% polyacrylamide gels (Davis 1964) and stained with amido black. Intensities of protein bands were measured in a Chromoscan Microdensitometer using #66 filter and relative percentages of protein fractions were computed.

The formulae used for calculations of various cellular parameters were (Winick and Noble 1965):

$$\text{Number of cells}(10^6) = \frac{\text{Organ DNA}(\mu g)}{6.2}; \quad \text{Cell wt.}(\mu g) = \frac{\text{Organ wt.}(g)}{\text{No. of cells}(10^6)};$$

$$\text{Protein per cell}(ng) = \frac{\text{Organ prot.}(mg)}{\text{No. of cells}(10^6)}; \quad \text{Fat per cell}(ng) = \frac{\text{Organ fat}(mg)}{\text{No. of cells}(10^6)}$$

Statistical evaluation of analytical data was done by Student's 't' test (Snedecor and Cochran 1967), and a 'P' value of less than 0.05 was considered significant while comparing values of HCH isomer group with control.

RESULTS AND DISCUSSION

Hepatic weights were about 190 and 150% of controls following dietary β-HCH and γ-HCH treatment (Table 1). Although liver weight was increased, no differences were detected in moisture, nitrogen, protein and glycogen on a tissue weight basis between control and HCH isomer groups. The liver fat content was significantly higher in HCH fed groups, the values being 62.8 and 53.0 mg/g liver in contrast to 45.0 mg/g in control liver. DNA content per unit tissue was decreased in livers of β- and γ-HCH fed animals by about 27 and 14% respectively, compared to controls. When expressed on a body wt.basis the increases in the total organ weights by HCH isomers corresponded to substantial increases in total organ protein and fat. Whole liver DNA was significantly higher in HCH isomer treated animals than controls. Although liver enlargement in response to xenobiotics

Table 1. Effect of dietary HCH isomers on liver weight and major constituents of liver.

Constituent	Control	/3-HCH	γ-HCH
Liver weight (g/100g body wt)	5.6±0.3	10.4±0.6*	7.4±0.5*
Moisture (mg/g fresh wt)	757±16	739±20	758±12
Total nitrogen (mg/g fresh wt)	34.3±1.0	36.5±1.1	33.8±0.6
Protein (mg/g fresh wt)	189±2.7	195±4.4	193±4.9
(mg/100g body wt)	1059±19	2030±52*	1427±35*
Fat (mg/g fresh wt)	45.0±1.1	62.8±2.4*	53.0±1.5*
(mg/100g body wt)	252±6.2	653±26.4*	392±9.3*
DNA (mg/g fresh wt)	1.85±0.04	1.35±0.04*	1.58±0.05*
(mg/100g body wt)	10.4±0.3	14.0±0.4*	11.7±0.4*
Glycogen (mg/g fresh wt)	34.4±3.7	34.2±6.5	23.6±5.6
(mg/100g body wt)	193±18	356±58	174±34

Values are mean ± SEM of six animals in the control group and of nine animals in HCH isomer groups.
* Statistically significant compared to control value.

has been well documented, the specific mechanism of enlargement is unknown. In the present study, quantitative determinations of liver protein, lipid, glycogen, DNA and moisture indicated that liver enlargement involves addition of new tissue, rather than simple fluid accumulation. Zufarov et al.(1975) have reported increases in liver RNA, protein, glycogen and total fat content in technical HCH fed rats. Although the data indicate a significant increase in hepatic lipid deposition, it is not sufficient enough to be the sole causative agent for the observed increases in liver/body wt ratio.

Among the changes in other hepatic parameters in response to dietary HCH isomers (Table 2), are increased number of cells in the livers of /3- and γ-HCH treated animals. Cell weights were also significantly higher in the two HCH treated groups (37 and 17% higher than controls). Both protein and fat content per cell were higher in /3- and γ-HCH fed rats. The belief that increased cell size contributes to liver enlargement is supported by the observation of decreased liver DNA per unit tissue. Nevertheless, a certain extent of hyperplasia is also present in livers of HCH isomer treated

Table 2. Effect of β- and γ-HCH feeding on hepatocyte parameters.

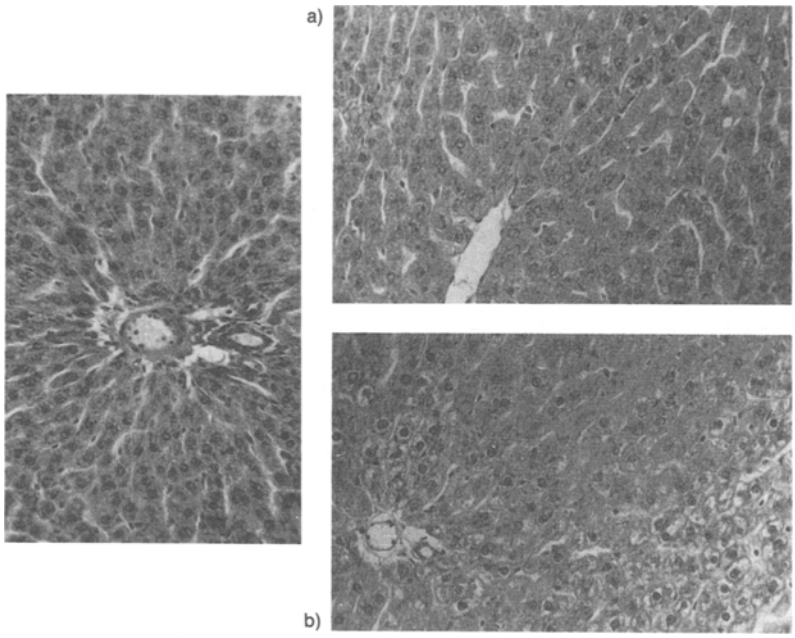
Parameter	Control	β-HCH	γ-HCH
No.of liver cells/ 100g body wt. (10^6)	1665±38	2256±71*	1884±55*
Cell wt. (ng)	3.36±0.15	4.61±0.27*	3.93±0.20*
Protein/cell (pg)	636±9	900±20*	758±19*
Fat/cell (pg)	151±4	290±11*	208±6*

Values are mean ± SEM of six animals in the control group and of nine animals in HCH groups.
* Statistically significant compared to control value.

animals as evident by increased cell number. Since our visual observation indicated that the extent of binucleated cells in a given number of hepatocytes was the same in the control and treated groups, cell number was just calculated based on DNA content. Schulte-Hermann et al. (1971) have attributed increases in liver mass following γ-HCH treatment to both hypertrophic and hyperplastic changes.

Among the protein contents of different subcellular fractions of liver, significant increases as compared to controls were observed only in microsomal fraction of both isomer exposed groups and were 27% and 34% respectively (Table not given). The increase in protein of liver microsomal fraction by HCH exposure was expected because HCH isomers are potent microsomal enzyme inducers (Srinivasan and Radhakrishnamurty 1983). The lack of change in the protein levels of whole liver indicates that the increase in liver wt/body wt ratio is likely to be due to an increase in cell mass and not in cell density.

The most predominent change revealed by microscopic examination of liver sections of β- and γ-HCH treated rats was hypertrophy of hepatocytes (Fig.1). Congestion of blood vessels in the sinusoidal region was also observed. The hypertrophy was associated with highly vacuolated cytoplasm. Focal necrotic regions were also observed in the livers of HCH isomers treated animals (Fig.1), some cells containing piknotic nuclei. These effects were more extensive in β-HCH fed rats than in γ-HCH fed animals. Thus, liver histopathology has revealed hypertrophy of the hepatocytes which is consistent with the chemical data on liver DNA content. Similarly, cytoplasmic vacuolation is consistent with higher fat levels found in rats treated with HCH isomers. The focal necrosis observed in β- and γ-HCH treated rats in the present study, has also been reported in animals



Control /β-HCH

Fig.1 Sections of rat liver after /β-HCH treatment showing: (a) Hypertrophy of the hepatocytes; (b) Vacuolated hepatocytes and focal necrosis (Haematoxylin-eosin X 100)

poisoned with technical HCH (Tashkhodzhav et al.1973). The present study revealed that in terms of severeity, /β-HCH produced more liver pathology than the γ-isomer.

The serum protein profile of HCH isomer treated and control rats is given in Table 3. Significantly higher total serum protein content was observed in rats fed /β- and γ-HCH for two weeks. Serum albumin level was significantly lower in HCH treated rats, whereas serum globulins were significantly higher than the controls in /β- and γ-HCH treated groups. Thus, the albumin/globulin ratio was decreased in both the isomer groups as compared to controls. Quantitative data on albumin and individual globulin fractions after electrophoretic separation on polyacrylamide gels (Table 4) revealed that the increase in globulins in HCH treated animals was confined to the γ-fraction. γ-Globulins in /β- and γ-HCH treated animals accounted for about 30 and 25% of the total serum proteins in contrast to 13% in control rats. α_1-Fraction of globulins decreased in the two HCH isomer groups by about 41 and 21% respectively. α_2- and /β-globulins were unaffected in these rats.

Table 3. Serum protein profile under the influence of dietary HCH isomers.

Type	Control	β-HCH	γ-HCH
Total proteins	7.37 ± 0.18	8.24 ± 0.15*	8.18 ± 0.07*
Albumins	3.52 ± 0.11	2.79 ± 0.10*	3.20 ± 0.06*
Globulins	3.85 ± 0.13	5.45 ± 0.09*	4.98 ± 0.05*
Albumin/Globulin ratio	0.92 ± 0.04	0,51 ± 0.02*	0.64 ± 0.02*

Values (g/100ml) are mean ± SEM of five animals in the control group and of eight animals in HCH groups.
* Statistically significant compared to control value.

Table 4. Polyacrylamide gel electrophoretic fractions of serum proteins in HCH isomers treated rats.

Protein fraction	Control	β-HCH	γ-HCH
Albumins	48.4 ± 0.5	34.2 ± 3.3*	38.0 ± 2.1*
Globulin-Total	51.6 ± 0.5	65.8 ± 4.3*	62.0 ± 3.7*
α_1-	11.1 ± 0.3	6.6 ± 0.4*	8.8 ± 0.2*
α_2-	8.1 ± 0.1	8.6 ± 0.2	9.5 ± 0.6
β-	19.2 ± 0.6	20.6 ± 0.5	18.2 ± 0.6
γ-	13.2 ± 0.3	30.0 ± 3.5*	25.5 ± 2.1*
Albumin/Globulin ratio	0.94 ± 0.04	0.52 ± 0.09*	0.61 ± 0.08*

Values (% of total serum proteins) are mean ± SEM of 5 rats in the control group and of 8 rats in HCH groups.
* Statistically significant compared to control value.

Liver insufficiency is regarded as the most significant causative factor in the genesis of dysproteinemia. The observation in the present study of hypoalbuminemia in conjunction with hyperglobulinemia is regarded as a common feature of serum dysproteinemia evoked by toxic chemicals (De Bruin 1976). The striking serum protein changes observed in the current study, which resulted in a significant decrease in the albumin/globulin ratio in rats fed β- and γ-HCH, is a clear indication of hepatotoxicity. Decreases in serum albumins and increases in serum globulins in rats given a single dose of γ-HCH has been reported (Kulagin 1970). Increases in γ-globulins whether relative or absolute, are known to occur in conditions of disturbed liver function. The synthesis of γ- or the antibody fraction of serum globulins takes place in the reticuloendothelial cells, especially in plasma cells derived from small lympho-

cytes. The reduction of the α_1-globulin fraction here indicates insufficient synthesis of this fraction due to reduced liver function as the liver is the site of its synthesis. This phenomenon is somewhat similar to that of liver cirrhosis, which is characterised by a decrease in albumins, increase in γ-globulins, normal or subnormal α-globulin and normal or increased β-globulin (De Bruin 1976). An increase in γ-globulin has also been observed in DDT poisoning (De Bruin 1976).

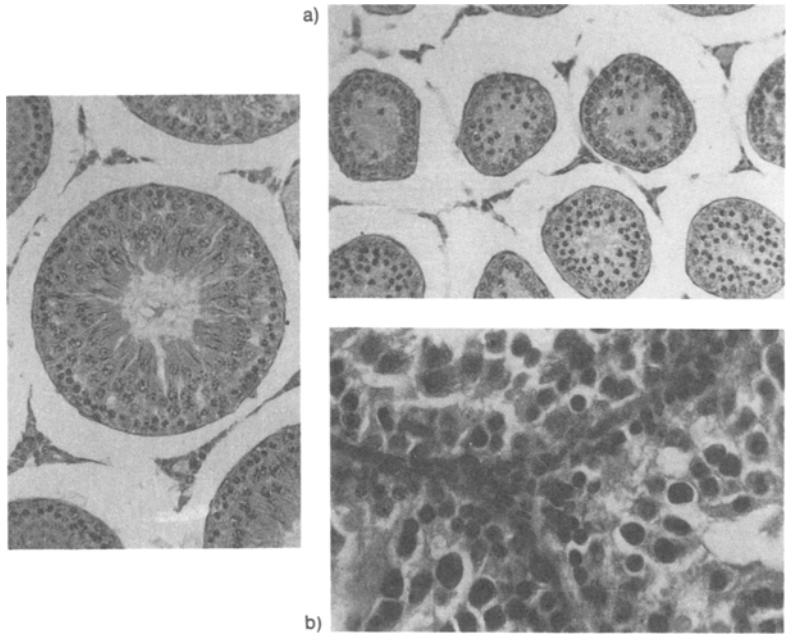
Dietary β- and γ-HCH did not affect testis weight but the protein content was higher in β- and γ-HCH fed rats (about 24 and 9% respectively) than the controls (Table 5). Testicular DNA content was significantly lower in β- and γ-HCH groups compared to control rats. Higher RNA (about 127% of control) content was detected in the testis of β-HCH treated rats. Total lipids and the individual fractions of lipid, viz., phospholipids, acylglycerides and cholesterol were unaffected by dietary HCH isomers.

Histological observation of the testis of β- and γ-HCH fed rats showed extensive pathological lesions. There was a reduction in the diameter of the seminiferous tubules and tubular atrophy was observed wherein the tubules carried necrosed spermatogenic cells and the lumen was devoid of spermatids(Fig.2). The interstitial space was increased and filled with oedematus fluid and debris cells in HCH fed rats (Fig.2). The above effects were greater in β-HCH treated rats compared to γ-HCH fed animals.

Table 5. Effect of dietary β- and γ-HCH on testicular constituents.

Constituent	Control	β-HCH	γ-HCH
Organ weight (g/100g body wt)	1.38 ± 0.03	1.42 ± 0.07	1.39 ± 0.03
Protein (mg)	55.8 ± 0.9	69.6 ± 1.8*	60.6 ± 1.9*
DNA (µg)	487 ± 24	409 ± 21*	363 ± 16*
RNA (µg)	29.1 ± 2.5	37.1 ± 2.0*	25.1 ± 1.3
Total lipids (mg)	21.3 ± 0.3	22.8 ± 0.6	22.7 ± 0.9
Phospholipid (mg)	9.80 ± 1.05	8.64 ± 0.54	8.62 ± 0.95
Cholesterol (mg)	5.32 ± 0.06	5.44 ± 0.06	5.38 ± 0.21
Acylglyceride(mg)	6.20 ± 1.12	8.75 ± 1.10	8.65 ± 1.77

Values(expressed per g tissue) are mean ± SEM of 5 rats in the control group and of 8 rats in HCH groups.
* Statistically significant compared to control value.



Control /β-HCH

Fig.2 Sections of testis from rats fed /β-HCH showing: (a)Severe reduction in tubular diameter, tubular atrophy and spermatogenic arrest(Haematoxylin-eosin X 100); (b) Atrophy of the interstial cells (Haematoxylin-eosin X 250)

The degenerative changes observed in the current study, in the testis of HCH isomers treated animals is deleterious for potential reproductive processes. Testicular atrophy and spermatogenic arrest has also been observed in inbred mice fed technical HCH over 3-6 month period (Nigam et al.1979) as well as in albino rats fed technical HCH for 3 months (Shivanandappa and Krishnakumari 1983). Observations made in the current study indicate that both /β- and γ-isomers contribute to the testicular pathology produced by technical HCH of earlier studies. Further, it is shown here that the degenerative changes in testicular tissue produced by /β-HCH are more severe than by γ-HCH. Because pathological changes were observed in the testis of HCH treated rats, the organ was analysed for some of its biochemical contituents. These data indicated that much of the change is largely confined to the proteins and nucleic acids of the testis the lipid content being unaltered. Increased protein content was accompanied by increased RNA levels in /β-HCH treated rats.

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