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Toxicity of β- and γ-Hexachlorocyclohexane in Rats of Different Ages

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Hexachlorocyclohexane (HCH) is the most widely used insecticide in India and other developing countries. The technical product is a mixture of several stereoisomers of which the γ-isomer is responsible for the insecticidal action. Technical HCH is persistent in biosphere chiefly because of its β-isomer and the latter is known to possess high chronic toxicity to mammals (Davidow and Frawley 1951). Both β- and γ-isomers of HCH have been shown to produce severe hepatotoxic and nephrotoxic effects in young experimental rats following chronic dietary intake (Srinivasan and Radhakrishnamurty 1988; Srinivasan et al 1984 & 1988; Ravinder et al 1989). It is probable that the age of the animals may play a role in the severity of toxicity or resistence following exposure to these HCH isomers. In the present study, we have exposed rats of varying ages to dietary β- and γ-isomers of HCH for 2 weeks and the toxic effects produced by these chemicals have been compared among animals of various age groups.

MATERIALS AND METHODS

Pure grade HCH isomers from K & K Labs, USA were used in this study. Male Wistar rats of five different age groups, viz., 5 weeks, 10 weeks, 16 weeks, 32 weeks and 16 months were maintained (ad libitum) for 2 weeks on diets containing 80 mg% β-HCH or 80 mg% γ-HCH. The basal diet consisted of 21% casein, 10% cane sugar, 10% peanut oil, 1% vitamin mixture, 2% salt mixture and 56% corn starch (Srinivasan et al 1988). Required quantities of HCH isomers were incorporated as solution in peanut oil. At the end of two weeks HCH feeding term, the animals were sacrificed by light ether anaesthesia. Liver, kidney and testis were quickly excised; washed with 0.9% saline and the organ weights determined.

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Approximately one gm portions of liver tissues were weighed and stored frozen pending lipid extraction and analysis. Extraction, purification, and quantitation of total lipids in liver samples were done according to Folch et al (1957). Cholesterol in lipid extracts was determined by the method of Searcy and Bergquist (1960). Statistical analysis of the data was performed by Student's t-test (1967).

RESULTS AND DISCUSSION

Effect of two weeks dietary HCH isomers treatment on body and organ weights is shown in Table 1. Body wts were not affected by β- or γ-HCH treatment in animals of any age group. Liver wts were increased by dietary HCH isomers in rats of all ages. This increase in liver wts produced by HCH isomers was higher in younger rats as compared to aged rats. The liver wt increase caused by β-HCH over the corresponding controls was 99, 67, 44, 41 and 24% respectively in rats of age groups: 5 weeks, 10 weeks, 16 weeks, 32 weeks and 16 months. Similarly, the liver wt increase caused by γ-HCH over the corresponding controls was 40, 40, 31, 18 and 13% respectively in the rats of age groups: 5 weeks, 10 weeks, 16 weeks, 32 weeks and 16 months. Significant increases in kidney wts (expressed as % body wt) were seen only in rats of 5 weeks and 10 weeks age. HCH isomers did not affect the kidney wts in the rats of higher ages. Testis wts were unaffected by either of the HCH isomers in rats of any age.

The total fat content in the enlarged livers of HCH isomers fed rats are presented in Table 2. Liver total fat expressed per g tissue was significantly higher in rats of 5 weeks and 10 weeks age groups consequent to either β- or γ-HCH treatment. The hepatic fat content was not altered by these HCH isomers in rats of higher ages. Hepatic cholesterol was also not altered by HCH isomers treatment in rats of any of the age groups.

We have earlier shown that a variety of morphological and biochemical lesions are produced in the liver during continued dietary intake of β- and γ-isomers of HCH by young rats (Srinivasan and Radhakrishnamurty 1988, 1989; Srinivasan et al 1988; Ravinder et al 1989). The hepatotoxic effects produced by these HCH isomers in experimental animals include hepatomegaly, fatty metamorphosis of the liver, hyperlipemia, elevated levels of serum aminotransferases and alkaline phosphatase with associated lowerings of hepatic cytoplasmic enzymes. The hepatomegaly resulting from HCH isomers treatment has been shown to be predominantly due to hypertrophy (Srinivasan et al 1988). In the present investigation, data on the organ wts indicate that the hepatomegaly

Table 1. Effect of two weeks dietary HCH isomers (80 mg%) on organ weights.

(g/100g body wt)

Organ	Control	β-HCH	γ-HCH
Age: 5 Weeks			
Body wt.(g)	104 ± 1.2	103 ± 1.3	99 ± 1.6
Liver	5.04 ± 0.13	10.0 ± 0.65*	7.06 ± 0.57*
Kidney	0.73 ± 0.04	0.89 ± 0.06*	0.84 ± 0.03*
Testis	1.38 ± 0.08	1.42 ± 0.09	1.39 ± 0.06
Age: 10 Weeks			
Body wt.(g)	186 ± 1.7	190 ± 2.5	187 ± 2.1
Liver	3.90 ± 0.13	6.50 ± 0.40*	5.46 ± 0.11*
Kidney	0.70 ± 0.03	0.80 ± 0.03*	0.79 ± 0.01*
Testis	1.40 ± 0.05	1.37 ± 0.04	1.40 ± 0.02
Age: 16 Weeks			
Body wt.(g)	245 ± 5.3	234 ± 2.5	235 ± 3.7
Liver	3.82 ± 0.13	5.50 ± 0.10*	5.02 ± 0.15*
Kidney	0.62 ± 0.05	0.69 ± 0.01	0.67 ± 0.02
Testis	1.24 ± 0.09	1.19 ± 0.02	1.25 ± 0.04
Age: 32 Weeks			
Body wt.(g)	297 ± 5.5	293 ± 4.5	284 ± 4.4
Liver	3.44 ± 0.17	4.84 ± 0.13*	4.06 ± 0.13*
Kidney	0.61 ± 0.04	0.65 ± 0.02	0.63 ± 0.02
Testis	1.12 ± 0.09	0.95 ± 0.08	1.15 ± 0.02
Age: 16 Months			
Body wt.(g)	325 ± 4.4	334 ± 3.1	314 ± 3.8
Liver	3.29 ± 0.06	4.10 ± 0.15*	3.72 ± 0.13*
Kidney	0.62 ± 0.02	0.63 ± 0.01	0.65 ± 0.01
Testis	0.93 ± 0.09	0.93 ± 0.03	1.02 ± 0.03

All values are mean ± SEM of 5 animals in the control group and of 8 animals in HCH isomer groups.

* Significant deviation from control ($P < 0.05$)

Table 2. Effect of two weeks dietary HCH isomers on liver total fat and cholesterol.

(mg/g liver)

Age group	Control	/3-HCH	Y-HCH
Total Fat			
5 Weeks	45.0 ± 1.1	62.8 ± 2.4*	53.0 ± 1.5*
10 Weeks	46.7 ± 4.4	57.4 ± 2.7*	62.0 ± 6.2*
16 Weeks	38.7 ± 1.8	43.0 ± 1.2	41.0 ± 2.2
32 Weeks	40.0 ± 2.2	44.7 ± 1.1	39.0 ± 1.8
16 Months	52.5 ± 1.3	52.7 ± 3.4	48.3 ± 1.8
Cholesterol			
5 Weeks	4.18 ± 0.08	3.51 ± 0.18	4.00 ± 0.09
10 Weeks	4.53 ± 0.41	5.09 ± 0.41	5.34 ± 0.58
16 Weeks	3.91 ± 0.14	3.53 ± 0.31	3.53 ± 0.22
32 Weeks	4.65 ± 0.38	4.23 ± 0.25	3.75 ± 0.28
16 Months	4.92 ± 0.67	5.66 ± 0.65	5.46 ± 0.47

All values are mean ± SEM of 5 animals in the control group and of 8 animals in HCH isomer groups.

* Significant deviation from control ($P < 0.05$)

produced by dietary HCH isomers is very much dependent on the age of the animals for a given dosage. Thus, increase in liver wts caused by HCH treatment is higher in younger animals as compared to older ones.

Fatty changes in liver is one of the several toxicological manifestations induced by HCH isomers in their spectrum of hepatotoxic effects (Srinivasan and Radhakrishnamurty 1989). The increase in liver fat content is seen here only in the rats of 5 weeks and 10 weeks age and possibly the dosage and duration of HCH treatment used in this study has produced hepatomegaly and hepatotoxicity in only these younger age groups.

Our earlier observation with young albino rats is that dietary /3- and Y-HCH affect kidney as well (Srinivasan et al 1984). The nephrotoxic effects are characterised by increased kidney wts, hypertrophy and degeneration of renal tubular epithelia affecting the reabsorption of low threshold metabolites causing their excessive excretion (Srinivasan et al 1984). The levels of

certain enzymes of renal tissue were altered as well in these HCH isomers fed animals (Srinivasan and Radhakrishnamurty 1977). The present study indicates that the increase in kidney wt caused by HCH isomers is limited to rats of 5 week and 10 week age groups. Thus, it appears that for a given dose and duration of HCH treatment, young animals are uniquely susceptible, whereas these chemicals are relatively safer at a later period of life. The difference in response during early life is supposedly a consequence of the relative insufficiency of various metabolic and excretory pathways, the greater susceptibility of certain organs, and immaturity of the blood-brain barrier.

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