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Bioaccumulation of HCH Isomers in Different Tissues of Young and Old Rats: A Comparison*

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Chlorinated hydrocarbon insecticides dichlorodiphenyl trichloroethane (DDT) and hexachlorocyclohexane (HCH) are still widely used in agriculture and vector control because of low cost and long lasting effects. An estimated 36,785 tons of HCH are used annually in India (Graham 1993). The lipophilicity of HCH and its relative resistance to biodegradation are reasons for getting into food chains and the environment (Siddiqui et al. 1982). The accumulation of HCH as reported in human tissues, fluid and even in mother's milk (Siddiqui et al. 1981, 1982) depends on the rate of their absorption, distribution, metabolism and excretion (Chand and Ramchandran 1980, Eichler et al. 1983, Srinivasan Radhakrishnamurty 1983). These pharmacokinetic parameters, influencing the degree of bioaccumulation, are also age dependent (Cassarett 1986). There are numerous reports of bioaccumulation of different HCH isomers in various tissues of experimental animals following oral treatment (Srinivasan et al. 1984). However, an evaluation of the age factor on the accumulation of different HCH isomers and their tissue-specific distribution might be significant in order to provide a better understanding of the pharmacokinetically influenced bioaccumulation of HCH isomers in a population of various ages from a polluted environment or contaminated food.

In the present study, organ distribution of HCH isomers from technical grade material was determined in young and old rats following oral treatment to elicit the impact of age, if any, on the bioaccumulation of this widely used insecticide. This study is one aspect of our long-term project to determine the bioaccumulation of pesticides in mother and neonates and neonatal health implications of pesticide accumulation.

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MATERIALS AND METHODS

Two age groups of albino rats of dukray strain (Industrial Toxicology Research Centre Colony), 6 weeks and 20 weeks, weighing 100 and 220 ± 10 g, respectively, were given a single dose of 40 mg/kg of HCH dissolved in peanut oil (EC50, γ-HCH 6.5%, β-HCH 35.0% and α-HCH 8.5% obtained from Hindustan Insecticide Ltd), by oral intubation. After 24 h animals were sacrificed and blood collected in pre-heparinized tubes. All the tissues viz. kidney, liver, brain, spleen, lungs, and heart were dissected out, blotted dry, and stored at 4°C until analysed (generally within 48 h). One g of these tissues except spleen and heart (whole tissue) was first weighed and then homogenized in formic acid for extraction of HCH as described by Siddiqui et al. (1981). Similarly one mL blood was extracted. The total hexane extract was concentrated, dried with Na_2SO_4 (Anhyd), and cleaned with concentrated H_2SO_4 (Dale et al. 1965, 1970).

Analysis of the samples were carried out on a Gas Chromatograph Varian Aerograph Series 6000 equipped with a ^{63}Ni electron capture detector.

Conditions of analysis were:

Column : Glass Column (6 m long; 4 mm (i..d.) packed with 1.5% OV-17 + 1.95% OV-210 on Chromosorb-W, (80/100).

Carrier Gas : IOLAR Grade-1 Nitrogen (99.9%); purified by passing through silica gel and molecular sieve to remove moisture and oxygen, respectively.

Flow rate : 60 mL/min.
Injector Temp : 250°C
Detector Temp : 250°C
Column Temp : 180°C
Chart Speed : 0.5 cm/min.

The peaks obtained for the different isomers of HCH were compared with those of analytical-grade standards of known concentration for quantitation.

Student 't' test was used to determine the significance of difference in HCH accumulation in different tissues of young and old rats.

RESULTS AND DISCUSSION

The tissue distribution of α-, β- and γ-isomers of HCH, after oral treatment of rats, is given in Tables 1,2 and

3, respectively. It is clear from Table 1 that there is a statistically significant ($p < 0.050$) difference of α-HCH accumulation only in kidneys of young and old rats with younger rats accumulating more than twice that of older rats. The differences in the α-HCH accumulation in the blood, liver, brain, lung, spleen, and heart in the two age groups of rats were not statistically significant.

Table 1. Distribution of α-HCH in different organs of rats treated with technical HCH.

Tissue	Young rats (6 weeks)	Old rats (20 weeks)	Bioconcentration Ratio[a]	
			Young rats	Old rats
Blood	0.044 ±0.038	0.032 ±0.009	1	1
Liver	3.653 ±1.317	3.616 ±1.555	83.02	113.0
Kidney	10.313 ± 7.185	4.626‡ ±1.412	234.39	144.56
Brain	6.092 ±1.594	6.127 ±0.716	138.45	191.47
Lung	4.576 ±0.836	5.185 ±3.056	104.0	162.03
Spleen	2.213 ±1.589	3.022 ±0.641	50.29	94.44
Heart	1.385 ±0.715	1.655 ±0.538	31.48	51.72

Values in ppm, are Mean ± S.D. of 6 rats.

‡Statistically significant difference between concentration in young and old rats ($P < 0.05$)

[a]Bioconcentration ratio is the concentration in the tissue/concentration in the blood.

Table 2 indicates that accumulation of β-HCH in the heart of young and old rats was statistically significant with older rats accumulating more than thrice that of younger rats. Like α-HCH, differential accumulation of β-HCH in blood, liver, kidney, brain, lung and spleen in the young and old groups of rats was not statistically significant.

Interestingly, accumulation of the insecticidally active isomer of HCH (i.e. γ-HCH) in liver, kidney, brain, and spleen in the two age groups of rats was significant (Table 3). However, concentrations in blood, lung, and heart showed no differences in the accumulation of γ-HCH.

Table 2. Distribution of β-HCH in different organs of rats treated with technical HCH.

Tissue	Young rats (6 weeks)	Old rats (20 weeks)	Bioconcentration Ratio	
			Young rats	Old rats
Blood	0.323 ±0.0035	0.074 ±0.271	1	1
Liver	16.745 ±11.208	10.968 ± 4.629	51.84	23.14
Kidney	16.61 ±12.691	16.589 ±11.572	51.42	35.0
Brain	7.95[a]	7.44 ± 3.40	24.61	15.70
Lung	14.96[a]	7.43[a]	46.31	15.67
Spleen	19.38 ±9.71	7.24[a]	60.0	15.27
Heart	0.515 ±0.349	1.635[‡] ±0.167	1.59	3.45

Values in ppm, are Mean ± S.D. of 6 rats.

[a] = β-HCH detected in one sample only out of six samples analysed for β-HCH.

[‡] Statistically significant difference between concentration in young and old rats ($P < 0.001$).

The bioconcentration ratio, an apparent measure of biomagnification of residues from blood to tissue, for a-, β – and γ-HCH as shown in Tables 1, 2 and 3, respectively, records the highest factor for α- and γ-HCH in kidneys of the younger age group. In the older age group this factor is highest for α-HCH in brain, for β-HCH in kidney, and for γ-HCH in spleen and lung.

Humans generally are exposed to γ-HCH or to technical grade HCH which contains the α-, β-, γ- and δ- isomers. Oral exposure is quite significant because of the large scale contamination of food chains with different isomers of HCH in India (Kaphalia et al. 1990, Siddiqui et al. 1980-81, Swarn Lata et al. 1984). The present study shows that the overall distribution of γ-HCH was greatest in the kidney followed by lung, liver, brain, spleen, heart, and blood in the younger age group whereas in the older age group, the order was spleen > lung > kidney > brain > liver > heart > blood, suggesting that age influences the distribution profile of γ-HCH (Table 3). Similarly, age influenced the distribution of α- and β-HCH in the present study (Table 1 and 2). This difference might be due to differential absorption, distribution, accumulation and metabolism of HCH in the two age groups of rats. Effects of aging on liver protein synthesis has been

Table 3. Distribution of γ-HCH in different organs of rats treated with technical HCH.

Tissue	Young rats (6 weeks)	Old rats (20 weeks)	Bioconcentration Ratio	
			Young rats	Old rats
Blood	0.101 ±0.018	0.113 ±0.019	1	1
Liver	6.828 ±0.805	4.582‡ ±1.579	67.60	40.55
Kidney	22.543 ± 9.88	10.325‡ ± 3.207	223.20	91.37
Brain	6.526 ± 1.451	4.669‡ ±1.079	64.61	41.32
Lung	21.823 ±10.053	12.707 ± 5.057	216.07	112.45
Spleen	6.189 ±3.713	12.862‡ ± 4.530	61.28	113.82
Heart	0.569 ±0.232	1.664 ±0.966	5.63	14.72

Values in ppm, are Mean ± S.D. of 6 rats.

‡Statistically significant difference between concentration in young and old rats ($P < 0.05$).

described (Cassarett 1986,) which suggests that a difference in xenobiotic metabolising enzyme activities might, in part, be responsible for the differential accumulation of different HCH isomers in the two age groups of the present study. Srinivasan and Radhakrishnamurty (1983) have also reported similar distribution profiles of HCH in rats exposed for 5, 10 and 15 days, but lacked the information on the impact of age on the overall residue levels in different organs as shown in this study. Other investigators have demonstrated the importance of hepatic microsomal enzymes in the detoxification of HCH (Baker et al. 1985, Chadwick et al, 1981). Thus, an evaluation of the age factor on the accumulatory pattern of different HCH isomers and their tissue specific distributionmight be extrapolated to the exposure of humans, of different ages, to HCH isomers from food-chain contamination resulting from environmental pollution.

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REFERENCES

Baker MT, Nelson RM and Van Dyke R (1985) The formation of chlorobenzene and benzene by the reductive metabolism of lindane in rat liver microsomes. Arch. Biochem. Biophys. 236: 506-514.

Casarett LT et al. (1986) Toxicology - The Basic Science of Poisons. 3rd edn edited by CD Klassen, MO Amdur, J Doull, Macmillan Press, p 90.

Chadwick R, Copeland M, Mole M et al. (1981) Comparative effect of pretreatment with phenobarbital, Aroclor 1254, and beta -nephthoflavone on the metabolism of lindane. Pest. Biochem. Physiol. 15: 120-136.

Chand B and Ramachandran M (1980) Effect of dietary hexachlorocyclohexane on certain biochemical changes in Albino rats. Indian J. Exp. Biol. 18: 735-736.

Dale WE, Miles JW and Gaines TB (1970) Quantitative method for determination of DOT and DDT metabolities in blood serum. J. Assoc. Off. Anal. Chem. 53: 1287-1292.

Dale WE, Copeland MF and Hayes WJ (1965) Chlorinated insecticides in the bodies of people in India. Bull. Wrd. Hlth. Orgn. 33: 471-477.

Eichler D, Heupt W and Paul W (1983) Comparative study on the distribution of alpha- and gamma- hexachlorocyclohexane in the rat with particular reference to the problem of isomerization. Xenobiotics 13: 639-647.

Graham A (1993) Pesticide Registration, formulation and application in India. Pesticide Information XIX (II): 1-4.

Kaphalia BS, Takroo R, Mehrotra S, Nigam U and Seth TD (1990) Organochlorine pesticide residues in different Indian cereals, pulses, spices, vegetables, fruits, milk, butter, deshi ghee and edible oils. J. Assoc. Off. Anal. Chem. 73: 509-512.

Kaphalia BS and Seth TD (1981) DDT and BHC residues in some body tissues of goats, buffalo and chickens, Lucknow, India. Pest. Monitor. J. 15: 103-106.

Siddiqui MKJ, Saxena MC, Seth TD and Krishna Murti CR (1980-81) Organochlorine pesticides in edible oils. Pest. Ann. 108-10.

Siddiqui MKJ, Saxena MC, Bhargava AK et al. (1981) Agrochemicals in the maternal blood, milk and cord blood: A source of toxicants for prenates and neonates. Environ. Res. 24: 24-32,

Siddiqui MKJ (1982) Biochemical studies on tissues accumulating pesticides. Ph.D. Thesis (179+iii).

Srinivasan K, Radhakrishnamurty R (1983) Studies on the distribution of beta- and gamma- isomers of hexachlorocyclohexane in rat tissues. J. Environ. Sci. Hlth. 818: 401-418.

Srinivasan K, Ramesh HP and Radhakrishnamurty R (1984) Renal tubular dysfunction caused by dietary hexachlorocyclohexane (HCH) isomers. J. Environ. Sci. Health B19 (4&5): 453-466.

Swarn Lata, Siddiqui MKJ and Seth TD (1984) Chlorinated pesticide residues in deshi ghee. J. Food. Sci. Technol . 21: 94-95.