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# Evaluation of Technical HCH Residues in Differentiating Rat Intestinal Epithelial Cells

S. Shukla, R K. Upreti, R. B. Raizada, M. K. Srivastava, A. M. Kidwai

Industrial Toxicology Research Centre, Post Box No. 80, M. G. Marg, Lucknow, 226001, India

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Technical HCH (Hexachlorocyclohexane) is a mixture of different isomers and its major insecticidal activity is due to the gamma-isomer. HCH have been shown to accumulate in fat depots after ingestion (Dikshith et al, 1989, 1991). The accumulation of HCH causes the disturbances of lipid metabolism (Carlson and Kolmodin-Hidman, 1972; Barros et al, 1991). Membranes are target sites for immediate and delayed action of HCH (Antunes-Medeira and Medeira. 1985) and are known to play a significant role in the mobilization of lipids from tissues to plasma. Gamma HCH alters the activity of some membrane bound proteins and the membrane permeability (Magour et al, 1984; Carrero et al, 1989; Tandon et al. 1989).

HCH is absorbed from GI-tract, skin and lungs. A large amount of HCH isomers have been shown to be absorbed through GI-tract (Oshiba and Kawakita, 1973). However, not much literature is available on the effect of technical HCH and its metabolites on rat intestinal epithelial cell lining. Although HCH is stored in fatty tissues but the intestinal cell lining is the first target site of its contact following oral administration. Present investigation deals with the analysis of HCH-isomers in differentiating intestinal epithelial cells. The residual levels of HCH isomers in the intestinal epithelial cells would be important in understanding their residual effect on function and differentiation of epithelial cells.

## MATERIALS AND METHODS

Male albino rats weighing 100 to 120 g were procured from Industrial Toxicology Research Centre, Lucknow,

*Correspondence to:* A. M. Kidwai

animal breeding centre and housed under normal husbandary conditions. Technical hexachlorocyclohexane (HCH) was procured from Tata Chemicals, Bombay. The compositional analysis of the technical HCH for various stereo isomers is as described earlier (Dikshith et al, 1989). Rats were orally administered with 50 mg HCH/kg body weight (1/40 of $LD_{50}$ value) in refined oil and sacrificed by cervical doslocation after 24, 48, 72 and 120 hr.

Over night fasted rats were sacrificed. Small intestines were removed and flushed gently with normal saline containing 1.0 mM dithiothretol. Intestinal epithelial cells were prepared along crypt to villus axis on gradient of differentiation according to Weiser (1973). Epithelial cells in their sequence of dissociation from the intestine were pooled into four fractions on the basis of their protein content and alkaline phosphatase activity as described by Panini et al (1979). These fractions were designated as Upper villi (UV); Middle villi (MV); Lower villi (LV) and Crypt (C), containing approximately 20, 30, 30 and 20 per cent of the total protein, respectively. These four groups of cells were suspended in appropriate volume of 2.5 mM EDTA-NaOH buffer (pH 7.4) and homogenized. The homogenate was then centrifuged at 900 g for 15 min and sediment discarded.

Preparations containing 10 mg protein/ml were extracted with n-hexane three times in a ratio of 1:3. The combined extract was cleaned with two step procedure prior to analysis. Samples were cleaned through liquid-liquid partition method with acetonitrile and passed through prewashed mixed phase chromatography column embeded with sodium sulphate (1X5 cm) activated floresil (1X3 cm) and celite (600 mg). The extract obtained was concentrated in rotary evaporator to about 2.0 ml and treated with equal volume of concentrated sulfuric acid. After removing the acid phase, the final extract was washed thoroughly with distilled water and analysed on GLC (CHEMTECH-3865) equipped with a 63 Ni-electron capture detector. A glass column (1X5 m X 2 mm I.d) packed with 1.5 % OV-17+1.95% Q F-1 on 100-200 mesh chromasorb WHP was used. Operation temperature were 195, 200 and 220°C for column, injector and detector, respectively, Purified nitrogen gas passing through silica gel ($8X10^{-9}$ A°) and molecular sieves ($22X10^{-9}$A°) was used as a carrier gas at a flow rate of 60 ml per min.

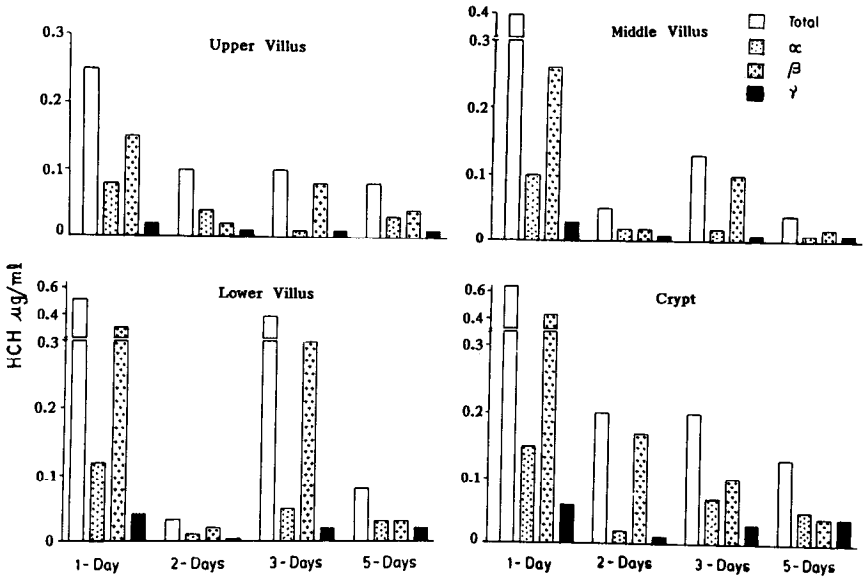


Figure 1. HCH-residual content of differentiating intestinal epithelial cell fractions following single oral dose of HCH (50 mg/kg body weight).

The over all recovery of HCH-isomers in different epithelial cell fractions were found to be more than 95 per cent, therefore a recovery correction factor has not been applied for the calculation of results. All the values were calculated and compared with respective reference standards obtained from Environmental Protection Agency, USA.

## RESULTS AND DISCUSSION

Gastrointestinal tract is one of the major route of absorption for environmental chemicals including pesticides. After entry into the GI-tract through food and water, majority of HCH is absorbed. In rats after oral dose of HCH, the intestinal absorption percentages are generally in the range of 80-95s (Oshiba and Kawakita, 1973; Albro and Thomas, 1974). Rats intestinal enterocytes residual analysis from different zones following single dose of HCH administration (Figure 1) indicated a maximum retention of different HCH isomers after one-day with a gradual increase from upper villus region to crypt base. The second days analysis revealed gradual decrease in accumulation of HCH isomer levels in the upper villus, middle villus and lower villus because of

their metabolism and also probably due to the shedding of these proliferating cells. After third day, residual analysis showed high affinity of residual retention in middle and lower villus. This is possibly due to the progressive upward migration of immature crypt cells covering the denuded area, caused by the fast shedding of the upper mature cells. Further, the crypt cells were observed to have higher residual levels for longer duration. As the crypt zone contains immature cells, it is possible that they are not in a position to properly metabolize the HCH and/or its isomers. Therefore, with the upward movement of these immature cells, the residual content would remain in comparatively higher levels in the lower as well as middle villus cells.

The presence of gamma-isomer in the enterocytes during the entire period of study suggest that being the most effective isomer, it could alter important functional aspects of these cells. However, the low levels of gamma isomer in the enterocytes as compared to the other HCH isomers indicates its rapid metabolism and elimination from the intestine. Presence of higher Levels of alpha-isomer in the enterocytes might be due to its high amount present in the technical HCH. Presence of comparatively higher levels of alpha-isomer further indicates that its possible toxicological effect on the functional aspects of intestinal cell lining can not totally be ruled out. Alpha-isomer is also knownto cause liver tumor(Nagasaki et a1,1975).The presence of higher levels of beta HCH residue in almost all the cells of different regions following single exposure of technical HCH could very well be linked with its stable nature and/or due to its slow rate of metabolism (Macholz et al, 1982). Further studies on the toxicological implications imposed by technical HCH and lindane on intestinal epithelial cell functions in long term exposure under various dietry conditions are in progress.

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