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DNA Binding and Mutagenicity of Lindane and Its Metabolites

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HCH (1,2,3,4,5,6-Hexachlorocyclohexane) is one of the extensively used organochlorine pesticides in India and other developing countries. The wide spread use of HCH is one of the primary problems to the environment and consequently the human food chain. This organic molecule has various structural isomers, with the gamma-isomer (lindane) being the most insecticidally active component of the technical product (Metcalf 1955). Lindane has been shown to bind to macromolecules in mouse liver (Sagelsdorff et al 1983; Iverson et al 1984). Lindane undergoes several types of biotransformations in mammals to yield several metabolites such as chlorinated benzenes and phenols, the latter being excreted either in the free form or as conjugates (Grover and Sims 1965; Chadwick et al 1983; Macholz and Kujawa 1985). Mammalian liver microsomal cytochrome P 450 mediated mixed function monooxygenase activity has been shown to play a role in the metabolism of lindane (Oesch et al 1982). It has been shown that hexachlorobenzene (HCB) and pentachlorophenol (PCP) are metabolites of lindane and these are formed by aromatization and oxidative dechlorination (Macholz and Kujawa 1985; Gopalaswamy and Aiyar 1986).

In this paper, DNA binding and mutagenicity of lindane and metabolites HCB and PCP were studied with a view to understanding their genotoxicity.

MATERIALS AND METHODS

Male Wistar rats 100 - 125 g were maintained on a nutritionally adequate laboratory stock diet. ^{14}C-labelled lindane (specific activity 54 mCi per mmole) and ^{14}C-hexachlorobenzene (specific activity 106 mCi per mmole) were obtained from M/s. Amersham International Ltd., Amersham, U.K. Calf thymus DNA and NADPH were from M/s. Sigma Chemical Co., St. Louis, U.S.A. Lindane, PCP and HCB were from the Environmental Protection Agency, Research Triangle Park, North Carolina,

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U.S.A. Arochlor 1254 was obtained from Monsanto Chemical Co., St. Louis, U.S.A. All other chemicals were of analytical grade.

For in vitro binding study, rats were killed by decapitation and liver microsomal fractions from control rats and rats maintained on drinking water containing 0.1% sodium phenobarbital for two weeks were isolated (Shah and Bhattacharya 1982). Microsomal protein concentration was estimated by the method of Lowry et al (1951).

Binding of labelled lindane and HCB to DNA was studied by incubating 0.5 mg Calf thymus DNA with 0.1 mCi ^{14}C-lindane (specific activity 54 mCi per mmole) or ^{14}C-HCB (specific activity 106 mCi per mmole) and 1.0 mg microsomal protein in 0.1 M Tris-HCl, pH 7.2 containing 0.65 mM NADPH and 5.0 mM $MgCl_2$ in a total volume of 1.0 ml at 37°C for 1.0 hr. After the incubation, the reaction was stopped by rapid cooling on ice. From the reaction mixture the DNA was precipitated with alcohol after two phenol extractions. The DNA was dissolved in 0.1 X standard saline citrate (SSC, 1 X = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and reprecipitated and washed three times with alcohol. The DNA was finally dissolved in 0.5 ml of 0.1 X SSC. Aliquots were taken for DNA estimation by diphenylamine reaction or by measuring absorbance at 260.0 nm. For measuring radioactivity, 1.0 mg DNA was counted in an LKB Rack Beta Liquid Scintillation Spectrometer. The specific activity is expressed as picomoles of compounds bound per mg DNA. Each experiment was done in triplicate. The amount of ^{14}C-lindane or ^{14}C-HCB bound to DNA due to nonenzymatic reaction was substracted from experimental values.

For in vivo studies, rats untreated or pretreated with phenobarbital as before, were administered ^{14}C-lindane or ^{14}C-HCB (25 mg/kg, i.p., specific activity 14.0 mCi per mmole) in 0.1 ml refined peanut oil. The animals were sacrificed 24 hr post-treatment and the livers were removed. The liver tissue was homogenized in ten volumes of 0.15 M NaCl-0.01 M EDTA, pH 8.0 containing 1.0% sodium dodecylsulphate. The DNA was isolated by the procedure described by Marmur (1961) and further purified free of traces of proteins with proteinase K treatment. The ratio of absorbencies of the DNA solution in 0.1 X SSC at 260 nm and 280 nm was = 1.9. Estimations of DNA and radioactivity were carried out as described earlier.

The mutagenicity of lindane, HCB and PCP was examined using the microbial/liver microsomal test system of Ames et al (1975). Metabolic activation was provided by liver post-mitochondrial supernatant (S9) obtained from the Arochlor 1254-induced rats. The compounds were dissolved in acetone (500 µg per ml) and added at different concentrations to molten top

agar along with 0.1 ml 1 X 10^7 cells of the tester strain of Salmonella typhimurium TA98 and 0.4 ml S9 reaction mixture containing the microsomes and cofactors. The plates were incubated at 37°C for 48 hrs and the number of revertant colonies were scored.

RESULTS AND DISCUSSION

In the present studies, the genotoxic potential of lindane and its metabolites HCB and PCP was investigated by determining the capacity of these compounds to bind DNA in vitro and in vivo. The results of the in vitro studies on binding of lindane and HCB to DNA are presented in Table 1. The level of binding was higher with lindane than the metabolite HCB in both control and phenobarbital induced microsomes.

Table 1. In vitro binding of ^{14}C-lindane and ^{14}C-hexachlorobenzene to calf thymuys DNA - effect of phenobarbital induced microsomes.

Pretreatment of animals	Compound	Binding to DNA p moles/mg DNA
None	Lindane	22.43 ± 0.84
None	HCB	8.88 ± 0.49
Phenobarbital	Lindane	32.21 ± 1.18
Phenobarbital	HCB	12.00 ± 0.66

^{14}C-lindane or ^{14}C-HCB bound activity to DNA in vitro is expressed as pmoles of the radioactive compound bound per mg exogenously added DNA. Values are means ± S.E. n = 8.

The results of the in vivo studies are listed in Table 2. This data also shows that the binding to DNA was lower in the case of HCB as compared to that for lindane. The in vivo DNA binding values of HCB and lindane, 2.23-6.90 pmoles per mg DNA, are in agreement with the values reported for lindane binding to DNA in mouse liver system (Sagelsdorff et al 1983; Iverson et al 1984). The low order of binding observed in the present studies is also similar to those reported for other organochlorine pesticides such as dieldrin and aldrin which also have produced hepatocellular carcinoma (Decloitre et al

Table 2. In vivo binding of ^{14}C-lindane and ^{14}C-hexachlorobenzene to rat liver DNA - effect of phenobarbital pretreatment of animal

Pretreatment of animals	Compound	Binding to DNA pmoles/mg DNA
None	Lindane	5.82 ± 0.31
None	HCB	2.23 ± 0.27
Phenobarbital	Lindane	6.90 ± 0.14
Phenobarbital	HCB	3.56 ± 0.18

DNA. Values are means ± S.E., n = 8.

1975; Wright et al 1977). Lindane was highly carcinogenic in rats and mice and has been shown to induce benign and malignant neoplasms (Reuber 1979). The carcinogenicity could be due to the action of the metabolites of lindane in the mammalian organism or lindane per se. HCB and PCP have been reported to arise from lindane in rats (Gopalaswamy and Aiyar 1986; Macholz and Kujawa 1985). HCB has been reported to cause teratogenicity and carcinogenicity in animals (Khera 1974; Cabral et al 1977).

The results of several experiments on the mutagenic activity of lindane, HCB and PCP in S. typhimurium TA98 are presented in Table 3. These compounds exhibited weak mutagenic response, and in all cases activation by rat liver microsomal fraction was necessary.

An enormouse body of literature exists to indicate some degree of correlation between the carcinogenicity of a chemical and its covalent binding to DNA following in vivo administration (Lutz 1986). The present studies have provided evidence that lindane and its metabolites bind covalently to DNA and possess the characteristics of a genotoxic agent. The metabolism of lindane in mammals involves the formation of olefins and a subsequent epoxidation. The toxicity of halogenated hydrocarbons could arise from irreversible binding of the epoxide intermediates to cellular constituents such as DNA or membranes.

Lindane and its metabolites cause uncoupling of oxidative phosphorylation in rat liver mitochondria (Gopalaswamy and

Table 3. Mutagenicity of lindane and metabolites in Salmonella typhimurium TA 98

Compound	Concentration µg per plate	Number of revertants (per plate)* -S9	+S9
Lindane	50	11 ± 2	135 ± 11
	100	9 ± 1	158 ± 18
HCB	50	10 ± 2	151 ± 15
	100	7 ± 1	178 ± 19
PCP	50	12 ± 1	186 ± 13
	100	14 ± 2	280 ± 9

The values are mean ± S.E., n = 6
*Spontaneous revertants = 11-16 per plate.

Aiyar 1984; Masini et al 1985). Also, a statistically significant increase in chromosomal abnormalities has been reported in cytogenetic studies on pesticide-workers (Bhatt 1977). In view of the mutagenic, carcinogenic and teratogenic potential and DNA binding ability of lindane and metabolites due caution should be taken while using these pesticides.

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