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Influence of Phenobarbital and Carbon tetrachloride on the Modulation of Tissue Retention Profile of Hexachlorocyclohexane in Rats

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The study was designed to investigate the role of hepatic metabolic activity on body burden of HCH residue. Male albino rats were orally administered 0, 5, and 10 mg/kg HCH for 90 days, followed by either sodium phenobarbital or carbon tetrachloride treatment for 0, 15 and 30 days after withdrawal of their respective HCH administration. The liver weight was significantly increased at 30 days after the administration of phenobarbital and carbon tetrachloride in both 5 mg and 10 mg/kg HCH withdrawal groups when compared to control. HCH residue was maximun in fat followed by adrenal>thymus>liver>kidney>spleen>testes>brain>plasma. Carbon tetrachloride caused an accumulation of HCH residues in the liver 15 and 30 days after administration of both doses of HCH. Phenobarbital did not show significant variation in HCH residues in hepatic tissue. Phenobarbital treatment caused significant induction of hepatic RED, APD, AHH, GST and QR activities. Significant decreases in activities were observed by carbon tetrachloride when compared to animals treated with HCH alone. The overall results clearly suggest the role of P450 protein on the body burden of HCH residues.

Key words: Hepatic metabolic activity; Residue; Sodium phenobarbital; Carbon tetrachloride; Hexachlorocyclohexane; Accumulation

INTRODUCTION

Hexachlorocyclohexane (HCH) is one of the chlorinated hydrocarbon insecticides widely used in agriculture and public health in developing countries. The pesticide has high chronic toxicity mainly due to the accumulation of its ß-isomer in tissues[1-4]. A number of studies have demonstrated that certain xenobiotics that induce microsomal enzymes may also increase the rate of depletion of body burdens of organochlorine compounds[5-7]. Conversely, reports on the levels of pesticide residues in human adipose tissues have shown that organochlorine levels were often higher in patients with abnormal liver[8,9]. In experimental animals higher levels of DDT were observed in the liver and fat following hepatic damage by CCl_4[10,11]. Pretreatment with CCl_4 has also been known to increase tissue storage of PCB' s in the rat[12].

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One of the most important properties of a toxicant is the degree of bioavailability in various tissues of the body. Further, it has also been suggested that the rates of decline or increase of such toxicants can be modulated by certain stress conditions and drugs[13,14] . In the present investigation, an attempt was made to study the influence of phenobarbital and carbon tetrachloride on the modulation of tissue burden of HCH with a possibility of involvement of hepatic xenobiotic metabolizing enzymes.

MATERIAL AND METHODS

Chemicals

Glucose-6-phosphate dehydrogenase, 1-chloro-2,4-dinitrobenzene (CDNB), flavin adenine dinucleotide, 2,6-dichlorophenol-indophenol and bovines serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Benzo(a)pyrene, aminopyrine hydrochloride, aniline hydrochloride and resorufin were obtained from Aldrich Chemical Co. (Milwaukee, USA). 7-Ethoxyresorufin was a product of the Pierce Chemical Co. (Rockford, USA). NADPH, NADP and NADH were procured from Boehringer Mannheim GmbH (Mannheim, Germany). PB was a product of the Mallinckrodet Chemical Co. (St Louis, MO, USA). Commercial HCH was a product from Hindustan Insecticide Ltd. (New Delhi, India). All other chemicals used were of the highest purity commercially available.

Animals and Treatment

Male Druckery albino rats (120±10g), derived from the Industrial Toxicology Research Centre, Lucknow animal breeding colony raised on commercial pellet diet (Hindustan Lever, Bombay, India) and water ad *libitum* under standard laboratory conditions, were used in the present study. The animals were divided into three groups. The first group was given oral intubation of HCH (5 mg/kg) in 0.2 mL of peanut oil. The second group was administered 10 mg HCH/kg in similar fashion. The third group received the same amount of peanut oil and served as controls. The treatment schedule was continued for 90 days. All the three groups of animals were further subdivided into 17 groups and treated with either sodium phenobarbital (PB) or carbon tetrachloride according to the schedule shown in Table 1.

TABLE 1

Phenobarbital and Carbon tetrachloride Dosing Schemes for Rats

Treatment	Groups[a]																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Dose of HCH(mg/kg)	0	0	0	5	5	5	10	10	10	5	5	10	10	5	5	10	10
Treatment Received After Removal from HCH Administration[b,c]	0	0	0	0	0	0	0	0	0	PB	PB	PB	PB	CCl_4	CCl_4	CCl_4	CCl_4
Day Animals Were Killed After Removal from Their Respective HCH Treatment	0	15	30	0	15	30	0	15	30	15	30	15	30	15	30	15	30

[a]six animals per group were taken.

[b]sodium phenobarbital was administered i.p. at a dose of 25 mg/kg body weight 5 days/week.

[c]carbon tetrachloride was administered orally at a dose of 0.5 cc/kg body weight in corn oil on the 1st, 4th, 8th, 12th days when animals were killed after 15th day. In animals killed on the 30th day, CCl_4 was also given on the 16th and 24th day.

The animals of different groups were sacrificed 0, 15 and 30 days after withdrawal of their respective HCH administration as mentioned in Table 1. Blood was taken in a heparinized tube and plasma separated. Tissues were removed, rinsed with cold physiological saline and kept at -20℃ for HCH residue analysis. A portion of fresh liver was utilized for measurement of xenobiotic metabolizing enzymes.

Enzyme Assay Methodologies

Livers were homogenized in 4 vol of ice cold 0.1mol/L phosphate buffer (pH 7.4) containing 0.15mol/L KCl. Post mitochondrial supernatant was prepared as described previously[15]. N-demethylation of aminopyrine (APD) was determined according to the method of Cochine and Axelrod[16] by measuring formaldehyde formed as described by Nash[17]. The activity of APD was expressed as n moles formaldehyde formed/min/mg protein. Aryl hydrocarbon hydroxylase (AHH) activity was assayed by using benzo(a)pyrene as substrate according to the modified method of Dehnen *et al*. [18], the details of which were described earlier[15]. The activity of AHH was represented as p moles 3-hydroxybenzo(a)pyrene formed/min/mg protein. Deethylation of 7-ethoxyresorufin (ERD) was determined by the method of Pohl and Fouts[19]. The activity of ERD was represented as p moles resorufin formed/min/mg protein. Glutathione S-transferase (GST) was assayed in cytosol using CDNB as a substrate[20]. The activity of GST was calculated in the n moles conjugate formed/min/mg protein. Quinone reductase (QR) was monitored as cytosol fraction by using NADH as an electron donor employing the modified method of Ernster[21], the details of which were described by Khan *et al*.[22]. The activity of QR was represented as n moles dichlorophenol indophenol disappeared/min/mg protein. The protein content was estimated by the conventional method of Lowry *et al*.[23] by using BSA as standard.

Analysis of HCH Residues in Tissues

The analysis of HCH was carried out by the method of Khanna *et al*.[2]. The tissues were homogenized in 4 volumes of 25% methanol in water. The plasma samples were diluted with 7 volumes of 50% methanol in water. The tissues and plasma samples were extracted successively with 10, 10, 10, 5 and 5 mL of hexane. The hexane extracts were pooled and passed through a florisil column. Solvents were concentrated at reduced pressure, dried with anhydrous Na_2SO_4 and transferred to 10 mL volumetric flasks and made upto the mark with hexane.

The analysis of HCH residue was carried out by using a Varian Vista 6000 GC equipped with an electron capture detector (Ni63). A glass column (183 cm×0.64 cm) packed with 1.5% OV-17+1.95% QF-1 on a 80-100 mesh chromosorb GH/P was used. The carrier gas was N_2 at a flow rate of 60 mL min. The injector, detector and column temperatures were maintained at 210℃, 210℃ and 190℃, respectively. The chart speed was 0.5 cm/min and the attenuation was fixed at 4×10^{-10}. The recorder response of elution of every compound was a single sharp peak, and the height was found to be proportional to the concentrations. The identification of the peak for compounds in tissues was based on its retention time matched with the reference standards and the absence of these peaks in samples of control animals. The recovery of HCH in different spiked tissues and plasma samples following the procedure outlined above was found to be 87.5±6.5%.

Statistical Analysis

The analysis of variance (ANOVA) with rank ordering[24] was employed to calculate the

significance of difference between control and experimental values. *P* value less than 0.05 was considered to be significant.

RESULTS

The effect of HCH, phenobarbital and carbon tetrachloride treatments on liver weights is shown in Table 2. The liver weight was found to be significantly ($P<0.05$) increased in animals treated with 10 mg/kg dose of HCH for 90 days when compared to control. Phenobarbital treatment to both 5 mg/kg and 10 mg/kg HCH withdrawal groups after 30 days significantly ($P<0.05$) increased the liver weights. Similarly, carbon tetrachloride treatment to both 5 mg and 10 mg/kg HCH groups significantly increased liver weights after 30 days when compared to control. Further, CCl_4 treatment to 5 mg/kg HCH withdrawal group after 15 days resulted in increased liver weight.

TABLE 2

Liver Weights of Rats After Withdrawal of HCH

Treatment	Liver Weight (g) Days After Withdrawal of HCH		
	0	15	30
Control	3.85 ± 0.10	3.93 ± 0.07	3.35 ± .09
HCH(5 mg/kg)x90 days	3.64 ± 0.11	3.88 ± 0.13	3.53 ± 0.08
HCH(5 mg/kg)+Phenobarbital		4.47 ± 0.15	4.25 ± 0.09[a]
HCH(5 mg/kg)+CCl_4		4.83 ± 0.11[a]	5.08 ± 0.14[a]
HCH(10 mg/kg)x90 days	4.39 ± 0.08[a]	3.99 ± 0.10	3.65 ± 0.09[a]
HCH(10 mg/kg)+Phenobarbital		4.16 ± 0.19	4.67 ± 0.08[a]
HCH(10 mg/kg)+CCl_4		4.07 ± 0.20	5.25 ± 0.15[a]

Note. Values represent $\bar{x} \pm s$ of 6 animals. Liver weights are given as g of % body weight.

[a] Significantly different when compared to control ($P<0.05$).

The distribution of total HCH level in plasma and different tissues of rats administered 5 mg and 10 mg/kg HCH for 90 days is shown in Table 3. The HCH residue was maximum in fat followed by adrenal>thymus>liver>kidney>spleen>testes>heart>brain>plasma. The decay profile for HCH in plasma is shown in Fig. 1. The significant difference was obtained in 15 days withdrawal group of 5 mg/kg of HCH. Phenobarbital treatment to 15 days withdrawal group of 5 mg/kg HCH caused a decreased level of HCH residues in the plasma; while carbon tetrachloride showed a significant ($P<0.05$) increased level of residues. These effects were not observed after 30 days. The decay profile for HCH in fat is shown in Fig. 2. In animals administered 5 mg/kg of HCH, carbon tetrachloride caused an increase in the tissue level of HCH at both time intervals. However, animals receiving 10 mg/kg of HCH, carbon tetrachloride caused an increase in the level of HCH only at the 30 day interval. The decay profile for HCH in liver is shown in Fig.3. Carbon tetrachloride caused an accumulation of HCH residues in the liver 15 and 30 days after administration of both doses of HCH. Phenobarbital did not show significant variation in HCH residues in hepatic tissue.

In vivo dose dependent effect of HCH on hepatic drug metabolizing enzymes of rats is shown in Table 4. HCH caused significant induction of hepatic ERD (77%-177%) and AHH (39%-323%) activities. However, catalytic activity of APD was not significant altered at both doses of HCH. A dose dependent decrease of GST (28%-34%) and OR (41%-46%)

was also observed (Table 4).

TABLE 3

Distribution of Total HCH Level in Plasma and Different Tissues of Rats Fed HCH

Tissues	Concentration[a] of HCH (μg/gm or μg/mL)	
	5 mg/kg	10 mg/kg
Plasma	0.65 ± 0.01	1.95 ± 0.09
Liver	13.20 ± 0.94	28.25 ± 0.80
Kidney	9.45 ± 1.30	14.64 ± 0.65
Spleen	7.77 ± 1.34	10.59 ± 1.45
Heart	4.85 ± 0.18	8.43 ± 0.74
Brain	3.31 ± 0.90	5.91 ± 0.43
Adipose	86.36 ± 18.00	150.00 ± 20.00
Thymus	18.68 ± 1.40	25.00 ± 1.20
Adrenal	52.25 ± 4.08	76.00 ± 5.75
Testes	6.03 ± 0.30	9.55 ± 0.54

[a]Values represent $\bar{x} \pm s$ of 6 animals.

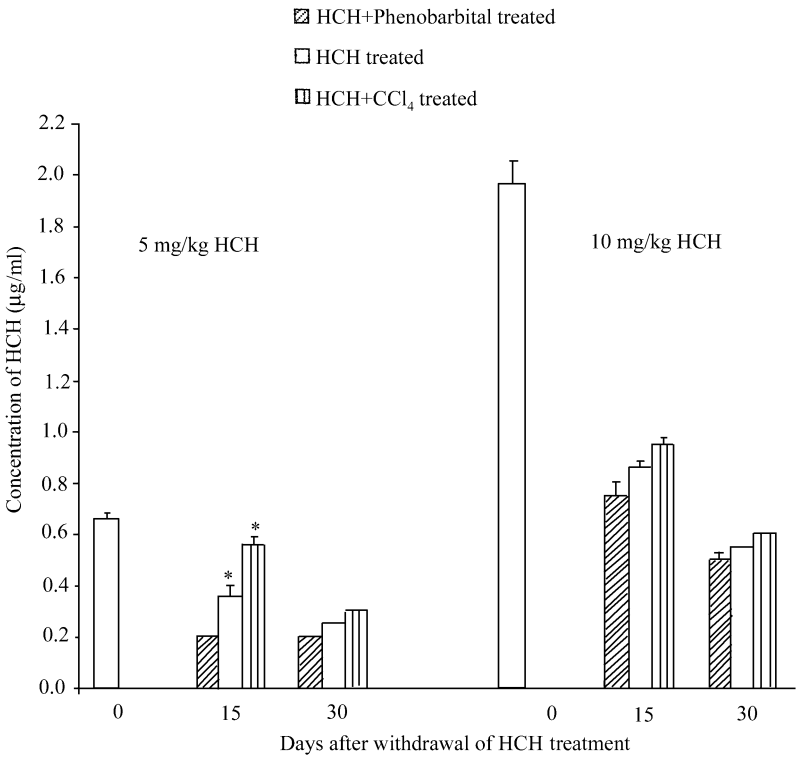

FIG.1. Hexachlorocyclohexane decay profile in plasma.
*Significantly different from HCH treated groups (P<0.05).

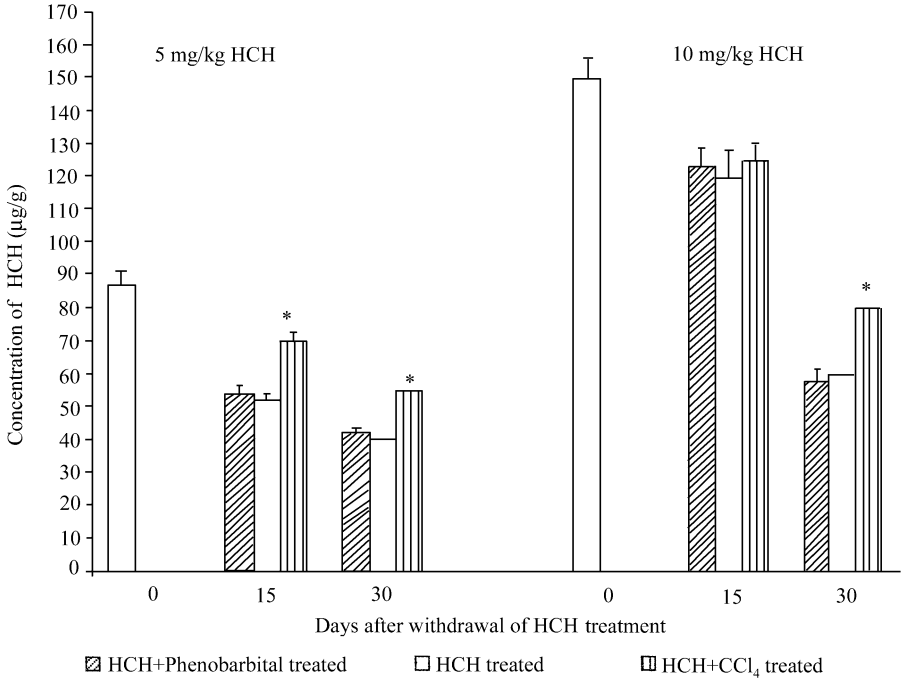

FIG.2. Hexachlorocyclohexane decay profile in adipose.
*Significantly different from HCH treated groups ($P<0.05$).

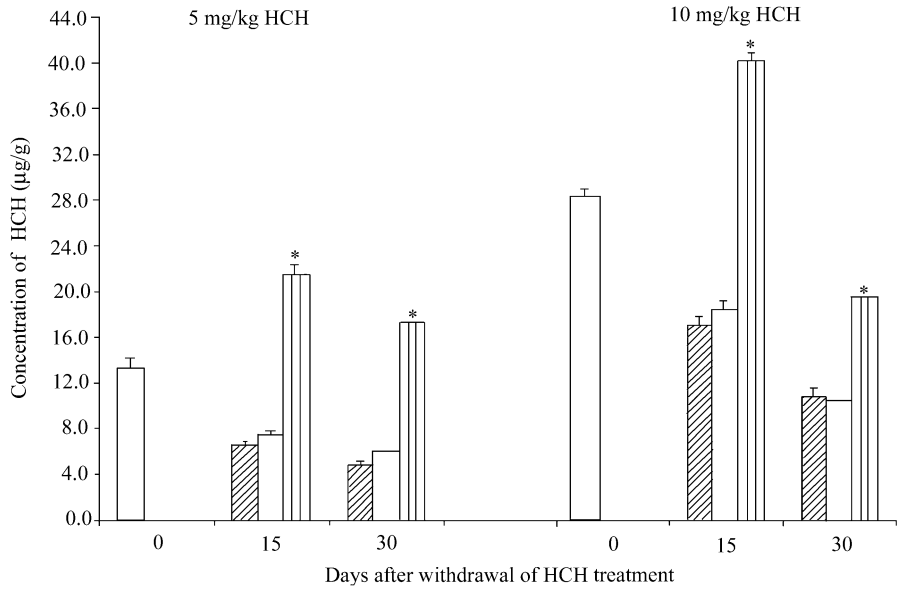

FIG.3. Hexachlorocyclohexane decay profile in liver.
*Significantly different from HCH treated groups ($P<0.05$).

TABLE 4

Effect of HCH on Hepatic Drug Metabolizing Enzymes in Rats

Parameters	Control	HCH(5mg)	HCH(10 mg)
ERD	35.2±5.0	62.3±5.5* (77%)	97.6±10.8* (177%)
AHH	33.7±4.5	54.8±5.9* (39%)	142.6±18.6* (323%)
APD	2.85±0.40	2.75±0.50	2.45±0.21 (14%)
GST	1448±182	1048±105* (28%)	951±131* (34%)
QR	217±31	127±10* (41%)	117±10* (46%)

Note. Values represent $\bar{x} \pm s$ of 6 animals.

*$P<0.05$ when compared to controls.

The effect of PB on hepatic drug metabolizing enzymes after withdrawal of HCH is shown in Table 5. PB treatment to animals 15 days after withdrawal of HCH caused 100%-160% induction of APD activity, while ERD and AHH activities were not changed as compared to respective HCH induced animals. Further, PB treatment to HCH (5 mg/kg) induced animals showed an increase of 123% in GST activity while no significant change was observed in 10 mg/kg HCH induced animals (Table 5).

TABLE 5

Effect of Phenobarbital on Hepatic Drug Metabolizing Enzymes After Withdrawal of HCH in Rats

Parameters	HCH(5 mg/kg)	HCH(5 mg/kg)+PB	HCH(10 mg/kg)	HCH(10 mg/kg)+PB
	15 Days After Withdrawal of HCH			
ERD	41.9±4.6	45.1±6.5	183.8±24.5	192.6±38.1
AHH	43.5±4.2	47.3±5.3	49.5±3.9	43.2±8.6
APD	2.78±0.28	5.56±0.54* (100%)	2.66±0.23	6.92±0.93* (160%)
GST	465±12	1035±111* (123%)	586±53	543±33
QR	130±12	118±27	137±7	152±27
		30 Days After Withdrawal of HCH		
ERD	38.6±8.5	58.9±9.8* (53%)	33.3±3.5	52.3±8.8* (57%)
AHH	20.7±2.7	41.4±9.0* (100%)	41.7±1.5	52.5±7.5
APD	2.70±0.40	6.20±0.26* (130%)	3.60±0.40	9.80±0.80* (172%)
GST	501±86	1069±47* (113%)	357±48	335±37
QR	205±20	358±28* (75%)	193±22	397±44* (106%)

Note. Values represent $\bar{x} \pm s$ of 6 animals.

*$P<0.05$, when compared to respective HCH treated animals.

Treatment of HCH induced animals with PB also led to 53%-57% increase of ERD activity 30 days after withdrawal of HCH when compared to respective doses of HCH treated animals (Table 5). An induction of 130%-172% in APD activity was observed by PB treatment to both groups of HCH induced animals. However, an increase of 100% AHH activity was observed following PB treatment to 5 mg/kg HCH treated rats but no significant effect was observed in 10 mg/kg HCH induced animals. GST activity was found to be increased (113%) by PB treatment to 5 mg/kg HCH induced animals while it showed no change in 10 mg/kg HCH treated rats. Interestingly, QR reductase activity showed 75%-106% increase following PB treatment to both groups of HCH induced animals (Table 5).

The effect of CCl_4 treatment on hepatic drug metabolizing enzymes after withdrawal of HCH CCl_4 is shown in Table 6. CCl_4 treatment to both groups of HCH induced animals after 15 days withdrawal of HCH showed a significant decrease in ERD (37%-65%), AHH (73%-81%), APD (50%-59%), and GST (31%) activity when compared to animals treated with 5 mg/kg and 10 mg/kg HCH. However, no change was noticed in QR activity (Table 6).

TABLE 6

Effect of Carbon tetrachloride on Hepatic Drug Metabolizing Enzymes After Withdrawal of HCH in Rats

Parameters	HCH(5 mg/kg)	HCH(5 mg/kg)+CCl_4	HCH(10 mg/kg)	HCH(10 mg/kg)+CCl_4
	15 Days After Withdrawal of HCH			
ERD	41.9±4.6	13.2±1.2* (65%)	183.8±24.5	116.6±14.0* (37%)
AHH	43.5±4.2	8.1±1.8* (81%)	49.5±3.9	14.1±3.5* (72%)
APD	2.78±0.28	1.39±0.14* (50%)	2.66±0.23	1.09±0.19* (59%)
GST	465±12	323±29* (31%)	586±53	406±59* (31%)
QR	130±12	100±10	137±7	109±10
		30 Days After Withdrawal of HCH		
ERD	38.6±8.5	14.7±1.5* (62%)	33.3±3.5	23.4±2.0* (30%)
AHH	20.7±2.7	8.6±2.3* (59%)	41.7±1.5	15.2±2.1* (64%)
APD	2.70±0.40	1.34±0.14 (50%)	3.60±0.40	0.72±0.10* (80%)
GST	501±86	472±37	357±48	346±36
QR	205±20	160±12* (22%)	193±22	149±14

Note. Values represent $\bar{x} \pm s$ of 6 animals.

*$P<0.05$, when compared to respective HCH treated animals.

Treatment of HCH induced animals with CCl_4 also caused a decrease of ERD (30%-62%), AHH (59%-64%) and APD (50%-80%) activities 30 days after withdrawal of HCH when compared to respective doses of HCH treated animals (Table 6). A decrease of 22% in QR activity was noted following CCl_4 treatment to HCH (5mg/kg) induced animals. CCl_4 treatment to HCH induced animals showed no effect on hepatic GST activity (Table 6).

DISCUSSION

The present study was designed primarily to investigate the role of hepatic metabolic activity on body burden of HCH residue. Phenobarbital, a known inducer of microsomal enzymes, was used to stimulate liver function[25] while carbon tetrachloride, a known hepatotoxin to decrease microsomal enzyme activity[26]. Liver weights, expressed as percent body weight, indicated hypertrophy at higher dose of HCH administration. Earlier studies have shown that lindane treatment to CF1 strain of mice led to a great increase in absolute and relative liver weight[27]. Interestingly, withdrawal of HCH after 90 days exposure showed no signs of hypertrophy. Further, both phenobarbital and carbon tetrachloride treatment to animals in 30 day withdrawal group of HCH caused increased liver weights at both doses of HCH.

Metabolism of lindane involves dechlorination, dehydrochlorination, dehydrogenation and conjugation with glucuronide, sulphate and glutathione, while the intial step is the formation of an intermediate pentachlorocyclohexenone-gem-chlorohydrin[28-32]. Most of the steps of biotransformation involved various xenobiotic metabolizing enzymes present in hepatic tissue[33-35]. The authors present study suggests that HCH induces monooxygenases (ERD, AHH) related to cytochrome P450 IA1/2 while very slight increase is evident in catalytic activity of P450 2B isozyme (APD). However, previous studies showed that lindane could cause induction of aniline hydroxylase, APD and ethoxycoumarin-O-dealkylase[27,36]. The results of the present study also indicate that HCH caused significant induction of cytosolic GST and QR activities which may be a phenomenon of defence mechanism leading to the formation of mercapturic acids and hydroxy derivatives[37]. This is supported by the evidence that lindane has been shown to increase GST and glucoronyl transferase enzymes[27,36]. The results also suggest that the dose dependent induction of phase I and II enzymes by HCH is directly linked with the increase of HCH residues in different tissues including plasma.

Studies indicate that P450 inducers like PB and Aroclor substantially alter the metabolism of lindane[37]. The authors' results suggest that in spite of induction of hepatic P450 monooxygenase, APD by PB in HCH treated animals, there was no change in the levels of HCH residues either in liver or in adipose tissues. Nonetheless, decrease in plasma levels of HCH residues in 5 mg/kg HCH dose following PB treatment for 15 days suggests the possibility of beneficial effect in decreasing the body burden of HCH. Interestingly, using the other modulatory compound, CCl_4 in HCH treated animals, the inhibition of all the monooxygenases *viz* ERD, AHH and ADP was evident along with the increased levels of HCH residues in liver and adipose tissue. Further, no clear correlation could be established for phase II enzymes in relation to retention of HCH residues in CCl_4 treated HCH induced animals.

The overall results clearly suggest the role of P450 protein in the body burden of HCH residues. However, further studies are required to investigate the involvement of specific isozymes of P450 in relation to tissue retention and/or bio-clearance of HCH residues.

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REFERENCES

1. Baumann, K., Angerer, J., Heinrich, P., and Lehnert, G. (1980). Occupational exposure to hexachlorocyclohexane, body burden of HCH-isomers. *Int. Arch. Occup. Enviorn. Health* **47** 119-127.
2. Khanna, R.N., Gupta, R., Gupta, G.S.D., Gopal, K., Agarwal, D., Anand, M., and Ray, P.K.(1990). Effects of the level of dietary protein on the toxicity of hexachlorocyclohexane in rats. *Toxicol. Environ. Chem.* **25**, 91-103.
3. Siddiqui, M.K.J., Nigam, U., Kaul, P.P., and Seth, T.D. (1996). Bioaccumulation of hexachlorocyclohexane isomers in different tissues of young and old rats. *Bull. Environ. Contam. Toxicol.* **56**, 896-902.
4. Jung, D., Becker, H., Edler, L., Janys Flesh, D., Gurn, P., Konietzko, J., Manz, and A., Papke, O. (1997). Elimination of β-hexachlorocyclohexane in occupationally exposed persons. *J. Toxicol. Environ. Health* **51**, 23-34.
5. Lambert, G. and Brodeur, J. (1976). Influence of starvation and hepatic microsomal enzyme induction on the mobilization of DDT residues in rats. *Toxicol. Appl. Pharmacol.* **36**, 111-120.
6. Brodeur, J. and Lambert, G. (1973). Further studies on the effect of starvation and microsomal enzyme induction of the mobilization of DDT residues in rats. *Toxicol. Appl. Pharmacol.* **25**, 488-496。
7. Young, R.A. and Mehendale, H.M. (1986). Effect of endrin and endrin derivatives on hepatobiliary function and carbon tetrachloride-induced hepatotoxicity in male and female rats. *Food Chem. Toxicol.* **24**, 863-868.
8. Radomski, J.L., Deichman, W.B., and Clizer, E.E. (1968). Retention of pestcides in human adipose tissue: Preliminary report. *Ind. Med. Surg.* **37**, 21-28.
9. Casarett, L.J., Fryer, G.C., Yaunger, W.L., and Klemmer, H.W. (1968). Organochlorine pesticide residues in human tissue. *Arch. Environ. Health* **17**, 306-311.
10. Grant, D.L. and Phillips, W.E.J. (1972). The effect of liver damage on the storage of p,p-DDT in the rat. *Bull. Environ. Contam. Toxicol.* **7**, 284-287.
11. Mehendale, H.M.(1989). Mechanism of the lethal interaction of chlordecone and CCl_4 at non-toxic doses. *Toxicol. Lett.* **49**, 215-241.
12. Grant, D.L., Phillips, W.E.J., and Villeneure, D.C. (1971). Metabolism of a polychlorinated biphenyl (Aroclor 1254) mixture in the rat. *Bull. Environ. Contam. Toxicol.* **6**, 102-112.
13. Davies, J.E., Edmundson, W.F., Carter, C.H., and Barquet, A. (1969). Effect of anticonvulsant drugs on DDT residues in man. *Lancet* **2**, 7-9.
14. Alary, J.G., Guay, P., and Brodeur, J. (1971). Effect of phenobarbital pre-treatment on the metabolism of DDT in the rat and the bovine. *Toxicol. Appl. Pharmacol.* **18**, 457-468.
15. Das, M., Seth, P.K., and Mukhtar, H. (1981). Characterization of microsomal aryl hydrocarbon hydroxylase of rat brain. *J. Pharma. Exp. Thera.* **216**, 156-161.
16. Cochine, J. and Axelrod, J. (1959). Biochemical and pharmacological changes in rats following chronic administration of morphine, nalomorphine and normorphine. *J. Pharma. Exp. Thera.* **125**, 105-110.
17. Nash, T. (1953). The colorimetric estimation of formaldehyde by means of Hantsch reaction. *Biochem. J.* **55**, 416-421.
18. Dehnen, W., Tomingas, R., and Ross, J. (1973). A modified method for the assay of benzo(a) pyrene hydroxylase. *Anal. Biochem.* **53**, 373-383.
19. Pohl, R.J. and Fouts. J.R. (1980). A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Anal. Biochem.* **107**, 150-155.
20. Habig, W.H., Pabst, M.J., and Jakoby, W.B.(1974). Glutathione-S-tansferase the first step in mercapturic acid formation. *J. Biol. Chem.* **249**, 7130-7139.
21. Ernster, L. (1967). DT-Diaphorase. In. Methods in Enzymology, Edited by R.W. Estrabook and M.E. Pullman, Academic Press, New York **10**, 309-317.
22. Khan, W. A., Das, M., Stick, S., Javed, S., Bickers, D.R., and Mukhtar, H. (1987). Induction of epidermal NAD (P)H: quinone reductase by chemical carcinogens: A possible mechanism for the detoxification. *Biochem. Biophys. Res. Commun.* **146**, 126-131.
23. Lowry, O. H., Rosebrough, M.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 165-175.
24. Snedecor, G.W. and Cochran, W.G. (1967). *Statistical Methods*. The Iowa State University, Press, Ames, Iowa, USA.
25. Orrenius, S., Ericsson, J., and Ernster, L. (1965). Phenobarbital induced systhesis of the microsomal drug metabolizing enzyme system and its relationship to the proliferation of endoplasmic membranes. *J. Cell. Biol.* **25**, 627-639.
26. Glende, E.A. (1972). On the mechanism of carbontetrachloride: Coincidence of loss of drug metabolizing activity with peroxidation of microsomal lipid. *Biochem. Pharmacol.* **21**, 2131-2138.
27. Oesch, F., Friedberg, T., Herbst, M., Paul. W., Wilhelm, N., and Bentley, P. (1982). Effects of lindane treatment

on drug metabolizing enzymes and liver weight of CFI mice in which it evoked hepatomas and in non-susceptible rodents. *Chem. Biol. Interact.* **40**, 1-14.
28. Grover, P.L. and Sims, P. (1965). The metabolism of γ-2,3,4,5,6-pentachlorocyclohex-1-ene and γ-hexachlorocyclohexane in rats. *Biochem. J.* **96**, 521-525.
29. Kurihara, N., Tanaka, K., and Nakajima, M. (1977). Pathways of chlorophenyl-mercapturic acids formation in biodegradation of lindane. *Agr. Biol. Chem.* **41**, 1317-1324.
30. Tanaka, K., Kurihara, N., and Nakajima, M. (1977). Pathways of chlorophenol formation in oxidative biodegradation of BHC. *Agr. Biol. Chem.* **41**, 723-730.
31. Chadwick, R.W., Chuang, L.T., and Williams, K. (1975). Dehydrogenation: A previously unreported pathway of lindane metabolism in mammals. *Pest. Biochem. Physiol.* **5**, 575-586.
32. Chadwick, R.W., Freal, J.J., Sovocool, G.W., Bayden, C.C., and Copeland, M.F. (1978). The identification of three previously unreported lindane metabolites from mammals. *Chemosphere* **7,** 633-640**.**
33. Soucek, P. and Gut, I. (1992). Cytochrome P450 in rats: structures, functions, properties and relevant human forms. *Xenobiotica* **22**, 83-103.
34. Goeptar, A.R., Scheerens, H., and Vermeulen, N.P.E. (1995). Oxygen and xenobiotic reductase activities of cytochrome P450. *Crit. Rev. Toxicol.* **25**, 25-65.
35. Omura, T. (1999). Forty years of cytochrome P450. *Biochem. Biophys. Res. Commun.* **266**, 690-698.
36. Pelissier, M.A., Faudemay, F., Manchon, Ph., and Albrecht, R. (1978). Induction par le lindane des monooxygenases microsomales du foie chez le rat: effects d' un regime hypocalcique. *Food Cosmet. Toxicol.* **16**, 27-31.
37. Chadwick, R.W., Copeland, M.F., Mole, M.L., Nesnow, S., and Cooke, N. (1981). Comparative effect of pretreatment with phenobarbital, Aroclor-1254 and β-Naphthoflavone on the metabolism of lindane. *Pest. Biochem. Physiol.* **15**, 120-136.

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