Effects of Chlorinated Hydrocarbons on Plasma α-Lipoprotein Cholesterol in Rats

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Ten chlorinated hydrocarbons (tetradifon, Chlorobenzilate, Kepone, lindane, Kelthane, toxaphene, dieldrin, hexachlorobenzene, dienochlor, and Aroclor 1254; see below) were evaluated in rats to assess agents which might selectively elevate high-density lipoprotein cholesterol (C-HDL). Single, nontoxic doses of Aroclor, dieldrin, and Kepone elevated C-HDL at day 7 (31%, 26%, and 24%, respectively, $p < 0.05$). The C-HDL elevations were maintained at days 21 and 60 (28% and 31%, $p < 0.01$) in the Aroclor group, while C-HDL returned to baseline in the dieldrin and Kepone groups. Liver function tests were unchanged from control, suggesting that the changes in C-HDL were not the consequence of hepatotoxicity. The hepatic microsomal cytochrome P-450 content of animals receiving Aroclor, dieldrin, and Kepone was 67%, 56%, and 44% higher than control values ($p < 0.02$), indicating probable hepatic enzyme induction. If HDL (an antiatherogenic lipoprotein) is also selectively elevated by chlorinated hydrocarbons in man, then analogues might potentially be developed as antiatherogenic pharmacologic agents. Alternatively, the implications of man's exposure to halogenated hydrocarbons may be better understood.

IN EPIDEMIOLOGIC STUDIES[1] and in subjects with familial hyperalipoproteinemia[2,3] a strong inverse correlation between the level of high-density lipoprotein cholesterol (C-HDL) and coronary artery disease has been demonstrated. Pharmacologic attempts to selectively elevate C-HDL might have promise as an alternative approach to atherosclerosis prevention or therapy.[4]

Carlson and Kolmodin-Hedman reported that chlorinated hydrocarbon pesticides elevated C-HDL in man.[5] These compounds are ubiquitous in natural food chains[6] and are discernible in breast milk from women with incidental pesticide exposure.[7] This study focused on 10 chlorinated hydrocarbons*

*Chlorinated hydrocarbons:

1. tetradifon (p-chlorophenyl-2,4,5-trichlorophenylsulfone)
2. Chlorobenzilate (ethyl-4,4'-dichlorobenzilate)
3. Kepone (decachlorooctahydro-1,3,4-methano-2H-cyclobuta [cd] pentalen-2-one)
4. lindane (1,2,3,4 5,6-hexachlorocyclohexane); γ-isomer
5. Kelthane (4,4'-dichloro-α-(trichloromethyl) benzhydrol)
6. toxaphene (polychlorobicyclic terpenes)
7. dieldrin (1,2,3,4, 10,10-hexachloro-6,7-epoxy-1,4,4a, 5,6,7,8,8a-octa hydro-1,4-endoexo-5,8-dimethanonaphthalene)
8. hexachlorobenzene
9. dienochlor (decachloro-*bis* (2,4-cyclopentadiene-1-yl) (2,4-cyclopentadiene-1-yl))
10. Aroclor 1254 (pentachlorobiphenyl).

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Received for publication March 24, 1977.

Supported by General Clinical Research Center Grant RR 00068-15. A portion of this work was done during Dr. Glueck's tenure as an Established Investigator of the American Heart Association.

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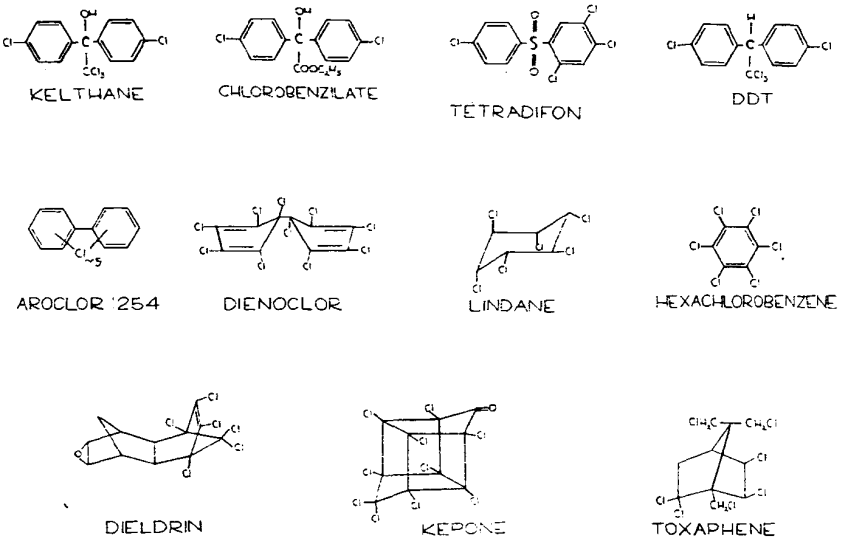

Fig. 1. Structures of the 10 chlorinated hydrocarbons and DDT.

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Table 1. Effects of Chlorinated Hydrocarbons on High-Density Lipoprotein Cholesterol (mg/dl, Mean ± SD)

Compound	Day 0	Day 7	Day 21	Day 60
Control	36 ± 4	35 ± 9	38 ± 10	41 ± 7
Aroclor	32 ± 6	42 ± 11*	41 ± 6†	42 ± 5†
Dieldrin	35 ± 5	44 ± 9*	37 ± 11	36 ± 5
Kepone	33 ± 4	41 ± 5*	43 ± 14	34 ± 4

*$p < 0.05$, days 7, 21, and 60 relative to day 0, eight rats per compound, paired t test, mixed analysis of variance with nesting. There was no significant change $p > 0.1$, in C-HDL in animals receiving dienochlor, hexachlorobenzene, toxaphene, Kelthane, lindane, Chlorobenzilate, and tetradifon.

†$p < 0.01$

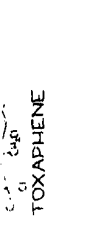

Fig. 1. Structures of the 10 chlorinated hydrocarbons and DDT.

to identify agents which might selectively elevate C-HDL and to increase our understanding of the effects of these compounds on lipid metabolism.

MATERIALS AND METHODS

Chlorinated Hydrocarbons

Ten chlorinated hydrocarbons tetradifon, Chlorobenzilate (Ciba-Geigy, McIntosh, Ala.), Kepone (Allied Chemical, Hopewell, Va.), lindane, Kelthane (Rohm and Haas, Philadelphia, Pa.), toxaphene, dieldrin, hexachlorobenzene, dienochlor, and Aroclor 1254 (Monsanto, St. Louis, Mo.), were evaluated (Fig. 1, Tables 1–4). Kelthane, Chlorobenzilate, and tetradifon, analogues of DDT, were studied because they are structurally analogous to compounds affecting lipoproteins in man.[5] Dienochlor, dieldrin, toxaphene, Kepone, and Aroclor 1254 have widespread distribution in the environment[6] and in commercial use.

The chlorinated hydrocarbons were suspended in 200 μl of diethyl ether with subsequent addition of corn oil to give an approximate concentration of 24 mg/ml, with the exception of lindane, which was made up to 3.6 mg/ml because of known toxicity in rats at higher dose levels. A 40-mg/kg dose of each compound in 0.5 ml of vegetable oil (lindane 12 mg/kg) was given intraperitoneally to each animal on day 0 following the collection of baseline blood samples. The control animals received 0.5 ml of the vegetable oil vehicle.

Animals

Male albino Sprague Dawley strain rats (Kalamazoo, Mich.) weighing 300–350 g were used. They were allowed to acclimate to their environment for 14 days prior to treatment. Purina Laboratory Chow and water were provided ad libitum throughout the study. Eight animals were randomly placed into each of 11 groups; 10 groups received chlorinated hydrocarbons and one served as a control (Table 1).

Table 2. Effects of Chlorinated Hydrocarbons on Total Plasma Cholesterol (mg/dl, Mean ± SD)

Compound	Day 0	Day 7	Day 21	Day 60
Control	69 ± 13	73 ± 8	72 ± 12	69 ± 12
Aroclor	69 ± 7	80 ± 8*	80 ± 16*	68 ± 7
Dienochlor	73 ± 7	78 ± 9	71 ± 8	61 ± 7†
Dieldrin	73 ± 10	81 ± 7†	70 ± 13	57 ± 5†
Toxaphene	70 ± 6	74 ± 6	67 ± 8	57 ± 6†
Kepone	72 ± 13	77 ± 9	72 ± 12	57 ± 8†
Tetradifon	68 ± 6	77 ± 8*	73 ± 10	60 ± 5

*$p < 0.05$, days 7, 21, and 60 relative to day 0, eight rats per compound, paired t test, mixed analysis of variance with nesting. There was no significant change, $p > 0.1$, in total cholesterol in animals receiving hexachlorobenzene, Kelthane, lindane, and Chlorobenzilate.

†$p < 0.01$.

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Table 3. Effects of Chlorinated Hydrocarbons on Plasma Triglycerides (mg/dl, Mean ± SD)

Compound	Day 21	Day 60
Control	117 ± 32	76 ± 25
Aroclor	83 ± 23*	76 ± 22
Dieldrin	72 ± 22†	94 ± 19
Kelthane	82 ± 20*	80 ± 15
Lindane	74 ± 22†	73 ± 10
Kepone	64 ± 21†	61 ± 15

*$p < 0.05$, experimental groups versus controls. There were no significant differences, $p > 0.1$, in plasma triglycerides comparing controls and animals receiving dienochlor, hexachlorobenzene, toxaphene, Chlorobenzilate, and tetradifon.

†$p < 0.01$.

Lipid and Lipoprotein Quantitation

Blood samples from individual restrained animals were collected following 1.2-mm terminal amputation of the tail. Plasma C-HDL was quantitated by precipitation gas liquid chromatography methods[8,9] with one modification. 5 μl of a 1:1 mixture containing 2.0 *M* $MnCl_2$ and 5000 USP units of heparin was added to 50 μl of rat plasma. In human plasma, preparation of C-HDL is carried out with a final Mn^{++} concentration of 0.046 *M*, although a somewhat increased final Mn^{++} concentration of 0.06 *M* has been suggested to improve accuracy and precision.[8] In 6 rats, the Mn^{++} concentration was derived by titration of rat plasma, with $MnCl_2$ concentrations of 1.25, 1.50, and 2.0 *M*, and heparin at 5000 USP units,[8] to ascertain the most reproducible C-HDL levels. The 2.0 *M* $MnCl_2$ gave a final Mn^{++} concentration of 0.091 *M*, which provided the most reproducible C-HDL levels. The accuracy of the C-HDL determination using a final Mn^{++} concentration of 0.091 *M* was assessed by comparison in 4 Sprague Dawley rats of C-HDL levels in total plasma, C-HDL levels in the $d > 1.063$ fraction cut after ultracentrifugation,[10] and C-HDL levels in a heparin $MnCl_2$ treated aliquot of the $d > 1.063$ fraction cut. C-HDL levels determined by any of these three approaches (mean ± SE: 38 ± 4, 38 ± 3, and 38 ± 3 mg/dl, respectively) did not differ when compared by two-way analysis of variance, $p > 0.1$.

Twenty μl of the resultant supernatant after heparin Mn^{++} precipitation and 20 μl of a separate aliquot of plasma were analyzed for C-HDL and total cholesterol using gas liquid chromatography.[8,9] Blood samples were collected at day 0 and at days 7, 21, and 60 after adminis-

Table 4. Effects of Chlorinated Hydrocarbons at Days 7 and 60 on Rat Hepatic Weight, Microsomal Protein, and Microsomal Cytochrome P-450 Activity (Mean ± SE)

	Day 7			Day 60	
	Microsomal Protein (mg/ml)	Liver Weight (Percent of Total Body Weight)	Cytochrome P-450 (nmoles/mg)	Microsomal Protein (mg/ml)	Liver Weight (Percent of Total Body Weight)
Control	22.6 ± 0.9	5.4 ± 0.2	0.18 ± 0.03	27 ± 2	4.4 ± 0.2
Aroclor	23.2 ± 0.7	5.8 ± 0.2	0.30 ± 0.05*	30 ± 4†	5.1 ± 0.1*
Dienochlor				35 ± 1*	4.5 ± 0.3
Hexachlorobenzene				42 ± 2*	4.2 ± 0.3
Dieldrin	23.3 ± 0.9	5.8 ± 0.1	0.28 ± 0.04*	27 ± 2	4.6 ± 0.1
Kepone	23.1 ± 1.1	6.1 ± 0.5†	0.76 ± 0.04†	26 ± 2	4.8 ± 0.1†
Chlorobenzilate				26 ± 2	4.9 ± 0.1†
Tetradifon				33 ± 1*	4.4 ± 0.1

*$p < 0.01$, comparison of experimental group with control. There were no significant differences, $p > 0.1$, in microsomal protein or liver weight as a percent of total body weight comparing animals receiving toxaphene, Kelthane, and lindane to controls.

†$p < 0.05$.

‡$p < 0.02$.

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tration of the chlorinated hydrocarbons (Tables 1, 2). Microquantitation of total cholesterol and C-HDL in a capillary blood vein allowed paired longitudinal analyses with each animal serving as its own control. Plasma triglycerides, which required more plasma, were quantitated[11] only at days 21 and 60, with comparisons of the treatment to the control rats.

Effects of Chlorinated Hydrocarbon on the Liver

Sixty days after injection of the test compounds, the 11 groups of rats were exsanguinated. The livers were weighed and the microsomal protein was determined.[12]

Liver function, hepatotoxicity, and liver induction were studied in a separate group of 16 rats, 4 each receiving Aroclor, dieldrin, Kepone, and corn oil (control). SGOT, SGPT, bilirubin, albumin, globulin, and the ratio of albumin to globulin were measured. Liver induction was examined by quantitation of the liver as a percent of body weight, microsomal protein per gram of liver, and microsomal cytochrome P-450 content.[13] Liver microsomes were prepared[12] by homogenizing the liver in 3 volumes of 0.15 *M* cold KCl. After cold centrifugation, the S-9 fraction[12] was rapidly frozen and stored at - 70°C until needed. Protein was determined by the biuret reaction using albumin as the standard. Cytochrome P-450 was measured using reduced carbon monoxide versus oxidized carbon monoxide microsomes to eliminate hemoglobin interferences.[13]

Statistical Methods

Paired *t* tests[14] were used to compare levels of C-HDL and total cholesterol pre- and post-injection (Tables 1, 2). In addition, mixed analyses of variance[15] were used to allow paired comparison of C-HDL (and total cholesterol) of each animal across treatment groups. Changes in plasma triglycerides, weight, microsomal protein, and microsomal enzyme activity were evaluated by comparison of the treatment groups to the controls using the standard *t* test[14] (Tables 3, 4).

RESULTS

Effects of Chlorinated Hydrocarbons on α-Lipoprotein Cholesterol

Two-factor mixed design analysis of variance[15] revealed that Kepone, dieldrin, and Aroclor increased C-HDL levels in the rats (Table 1). C-HDL increased in the animals receiving Aroclor on days 7, 21, and 60 ($F = 4.76$, $df = 3,21$, $p = 0.011$). Dieldrin produced an increase in C-HDL on day 7 ($F = 4.91$, $df = 3,21$, $p = 0.01$), with mean levels returning to baseline on day 21 and remaining at baseline on day 60 (Table 1). No significant changes were observed within the control group, or with the other seven compounds, over the total test period. For all 11 groups, the C-HDL values were not different from each other on day 0 ($F = 1.58$, $df = 10,77$, $p = 0.128$).

Effects of Chlorinated Hydrocarbons on Total Cholesterol

The total cholesterol values for the 11 groups on day 0 did not differ ($F = 0.866$, $df = 10,77$, $p = 0.568$). In animals receiving Aroclor there was an increase in total cholesterol at 7 days postinjection, which remained elevated on day 21 ($F = 5.29$, $df = 2,14$, $p = 0.019$), and then returned to baseline on day 60 (Table 2). Dieldrin produced an increase of total cholesterol on day 7 ($F = 4.84$, $df = 2,14$, $p = 0.025$) with return to baseline on day 21. For the Aroclor and dieldrin groups, the mean increase in total cholesterol at day 7 (11 and 8 mg/dl) was essentially accounted for by mean increments in C-HDL (10 and 9 mg/dl, respectively; Tables 1, 2). Tetradifon also elevated total cholesterol on day 7 ($F = 5.42$, $df = 2,14$, $p = 0.018$), with a return to threshold levels by day 21. At 60 days postinjection the total cholesterol values fell, as compared to baseline,

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for the animals which received dienochlor, dieldrin, toxaphene, and Kepone ($p < 0.01$). No significant changes in total cholesterol were observed for the controls, hexachlorobenzene, lindane, or chlorobenzilate.

Effects of Chlorinated Hydrocarbons on Plasma Triglycerides

Twenty-one days after injection of the compounds, plasma triglycerides in animals receiving Aroclor, dieldrin, Kelthane, lindane, and Kepone were lower than in corn oil controls ($p < 0.05$; Table 3). Sixty days postinjection, most group mean triglyceride levels (including the control rats') were generally lower than at 21 days and there were no significant differences between triglycerides in control or treatment groups (Table 3).

Effects of Chlorinated Hydrocarbons on Weight Gain

Mean increments in weight between days 0, 7, 21, and 60 did not differ significantly in a comparison of control and treatment groups of rats ($p < 0.1$).

Effects of Chlorinated Hydrocarbons on the Liver

As shown in Table 4, at 60 days postinjection, the amount of microsomal protein per milliliter microsomal fluid[12] was generally similar to control values, with slightly higher protein content in the Aroclor, dienochlor, hexachlorobenzene, and tetradifon groups.

The liver weight as a percentage of total body weight at 60 days was similar to that of the control animals with the exception of slight increases in the liver weight ratio for Kepone and chlorobenzilate and significant increases in the weight ratio for Aroclor. At day 7, in the three groups of rats with elevated C-HDL (Aroclor, dieldrin, Kepone), plasma levels of SGOT, SGPT, bilirubin, albumin, globulin, and the ratio of albumin to globulin did not differ from the control values. At day 7, liver microsomal protein content and liver weight as a percent of the total body weight were generally similar to those of the controls (Table 4). At day 7, the Aroclor, dieldrin, and Kepone groups had higher cytochrome P-450 content than the controls ($p < 0.02$, Table 4).

DISCUSSION

Selective elevations in C-HDL were obtained using a single, nontoxic[16] dose of chlorinated hydrocarbons. Of the three compounds which elevated C-HDL at day 7 (Aroclor, +31%; dieldrin, +26%; and Kepone, +24%) only Aroclor maintained significant C-HDL elevations at days 21 (28%) and 60 (31%) postinjection. Insufficient blood was available to determine whether any anomaly existed in the actual lipid or apoprotein composition of HDL. There was no apparent hepatotoxicity.

Polychlorinated biphenyls (PCB) and polychlorinated terphenyls (PCT) enhance the formation of lipid droplets[17] and alter rat liver triglycerides and phospholipids.[18,19] Membrane-bound enzymes and cytochrome P-450 are affected by PCB.[20] Holub et al. found no significant effect of PCB in vivo on the activity of "four key microsomal enzymes responsible for lipid biosynthesis,"[21] and postulated that catabolism or transport of hepatic lipids was altered.

Most of the chlorinated hydrocarbons used in this study have been demon-

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strated to induce hepatic microsomal enzyme activity.[16,22,24] The single dose of Aroclor 1254 used here was about 8%, 10%, of that given for acute induction of microsomal enzymes in metabolic studies of mutagenicity with a bacterial reversion test.[25] Microsomal cytochrome P-450 content was significantly increased for Aroclor, dieldrin, and Kepone as compared with controls. Aroclor's C-HDL elevating effect does not appear to be a consequence of hepatotoxicity, but may represent some aspect of hepatic microsomal enzyme induction.[16,22,24] Although enzyme induction has been linked to steroid metabolism,[16] the relationship between hepatic microsomal induction and plasma C-HDL remains to be elucidated. Further studies could determine if other potent liver inducers such as phenobarbital or Dilantin (Park, Davis, Detroit, Mich.) increase plasma C-HDL in rats or men.

Since Aroclor, dieldrin, and Kepone elevated C-HDL, it might be useful to study lipoproteins in humans with considerable and generally inescapable[5,16,23,24] environmental exposure. We have initiated studies in industrial workers with high occupational Aroclor exposure, in collaboration with National Institute for Occupational Safety and Health and the Center for Disease Control with results forthcoming. If C-HDL is also selectively elevated by chlorinated hydrocarbons in man, then pharmacologically useful new anti-atherosclerosis compounds may be developed. The implications of man's environmental exposure to chlorinated hydrocarbons may also be better understood.

ACKNOWLEDGMENT

The authors would like to acknowledge the excellent technical assistance of Bruce Johnson, Lori Mains, Grace Evans, Edward Rooch, Cynthia Yeakle, Jeffrey Seebohm, George Kokenakis, and Joseph Eukaeh.

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