REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	30 May 90	FINAL 2 Mar 87 to 1 Mar 90

4. TITLE AND SUBTITLE

PHARMACOKINETICS OF LIPOPHILIC AGENTS FOLLOWING PREEXPOSURE NON-CYTOCHROME P-450 MEDIATED MECHANICS

5. FUNDING NUMBERS

G - AFOSR-87-0185
PE - 61102F
PR - 2312
TA - A5

6. AUTHOR(S)

Dr Lawrence R Curtis, Dr Hilary M. Carpenter

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Oregon State University
P.O. Box 1086
Corvallis, OR 97339-1086

8. PERFORMING ORGANIZATION REPORT NUMBER

AFOSR-TR- 90 0812

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

T. JAN CERVENY, Lt Col, USAF
AFOSR/NL
Building 410
Bolling AFB, DC 20332

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release:
distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Low levels of lipophilic organochlorine compounds (OCs) are present in teh environment Despite the fact that there is no question regarding the toxicity of many of these compounds on an acute high dose basis, the chronic effecta of low levels of these materials has not been adequately examined. Since the chemical properties of these materials make them incompatible with water, the cell must use specialized means for handling them. These include xenobiotic metabolizing enzymes, cytosolic binding proteins and lipid storage depots. The studies performed during the period of this grant were an attempt to characterize a pretreatment disposition response (PDR) system which is a portion of the cellular response to low levels of OCs. It is apparent from our studies that PDR is not due to changes in the total lipid content of cells but may be due to an alteration in cytosolic binding proteins.

14. SUBJECT TERMS			15. NUMBER OF PAGES
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(U)	(U)	(U)	(U)

PHARMACOKINETICS OF LIPOPHILIC AGENTS FOLLOWING PREEXPOSURE: NON-CYTOCHROME P-450 MEDIATED MECHANISMS

Air Force Grant No. 87-0185

Hillary M. Carpenter
Lawrence R. Curtis
Oak Creek Laboratory of Biology
Oregon State University
Corvallis, Oregon 97331

May 30, 1990

Final Report for grant period 1 March 1987 - 28 February 1990

Prepared for Jan Cerveny, Lt. Colonel, USAF
Air Force Office of Scientific Research
Building 410, Bolling AFB, D.C. 20332

Summary

Low levels of lipophilic organochlorine compounds (OCs) are present in the environment. Despite the fact that there is no question regarding the toxicity of many of these compounds on an acute high dose basis, the chronic effects of low levels of these materials has not been adequately examined. Since the chemical properties of these materials make them incompatible with water, the cell must use specialized means for handling them. These include xenobiotic metabolizing enzymes, cytosolic binding proteins and lipid storage depots. The studies performed during the period of this grant were an attempt to characterize a pretreatment disposition response (PDR) system which is a portion of the cellular response to low levels of OCs. It is apparent from our studies that PDR is not due to a simple redistribution phenomenon, not dependent on metabolism, not due to changes in the total lipid content of cells but may be due to an alteration in cytosolic binding proteins.

Research Progress

Previous work has repeatedly shown that pretreatment of mice, rats or rainbow trout with small doses of chlordecone (CD), TCDD or dieldrin results in an altered distribution of a subsequent dose of the same [^{14}C]labeled OC. We refer to this response as a pretreatment disposition response or PDR (Shubat and Curtis, 1986; Carpenter and Curtis, 1989; 1990; Curtis et al., 1990). OC-induced PDR appears to have some degree of specificity (Carpenter and Curtis, 1989) and the type of tissues which respond differs depending on the compound being examined (Carpenter and Curtis, 1989; Curtis et al., 1990). For instance CD-induced PDR in mice consists of two components. The first is a reduction in the amount of label remaining in the liver following a pretreatment and the second is an increase in the amount of residual label in other tissues such as kidney, fat and muscle (Carpenter and Curtis, 1989). However, with TCDD the response is different (Curtis et al., 1990). Here, following pretreatment there is an increased retention of label by the liver at the expense of the amounts located in non-hepatic tissues. These studies showed that, despite the differences in PDR, the systems involved respond in a dose related manner and are saturable. These results are consistent with a high affinity low capacity system and suggest the mechanisms of cell response for different OCs involves different proteins or protein systems.

An assessment of the biochemical mechanisms relating to PDR showed that OC-induced PDR is not related to altered rates of metabolism following pretreatment. There were no PDR-related changes in the hepatic xenobiotic metabolizing system (Carpenter and Curtis, 1990, Table 1; Table 2). Induction experiments showed that, as expected, B-naphthoflavone (BNF) was an effective inducer of cytochrome P-450 in the B6 but not the D2 strain (Table 1). Chlordecone also induced cytochrome P-450 in the B6 but not the D2 strain. The level of induction was similar for

Table 1

The effect of CD and BNF on EROD activity, cytochrome P-450 specific content and amount of microsomal protein

CD was administered as a single ip treatment of 40 mg/kg four days prior to determinations. BNF was administered daily (80 mg/kg ip) for four days and the animals were killed 24 hours following the final treatment. Control animals received ip injections of corn oil. Values are expressed as mean ± SE, N=4 for each group.

Treatment	Microsomal Protein (mg/ g liver)	Cytochrome P-450 (nmol/ mg microsomal protein)	EROD Activity (nmol/min/mg microsomal protein)
		C57BL/6N	
Control	13.6 ± 0.9	0.18 ± 0.04	0.02 ± 0.01
CD	15.9 ± 1.0	0.63 ± 0.08[a]	0.10 ± 0.01[a]
BNF	12.0 ± 0.8	0.62 ± 0.04[a]	2.93 ± 0.15[a]
		DBA/2N	
Control	8.1 ± 1.2	0.31 ± 0.05	0.04 ± 0.00
CD	11.3 ± 2.7	0.50 ± 0.07	0.08 ± 0.01
BNF	10.0 ± 0.6	0.21 ± 0.02	0.03 ± 0.00

[a] Values were significantly different from controls at $P \leq 0.05$.

Table 2

Dose response for hepatic monoxygenases after a CD-induced treatment regimen in male B6 mice

CD (5 mg/kg) or corn oil was administered three days after a single pretreatment of CD (5 or 40 mg/kg) or corn oil. Animals were killed 16 hr following treatment. Values are expressed as mean ± SE, N=4.

Treatment[a]	Liver weight (% body weight)	Microsomal protein (mg/g liver)	Cytochrome P-450 (nmol/mg microsomal protein)	/ Max	EROD (nmol/min/mg microsomal protein)	ECOD (nmol/min/mg microsomal protein)
0/0	6.4 ± 0.2	9.7 ± 1.3	0.262 ± 0.021	450 ± 0	0.034 ± 0.002	0.067 ± 0.013
0/5	5.8 ± 0.2	8.4 ± 0.9	0.211 ± 0.032	450 ± 0	0.046 ± 0.007	0.048 ± 0.015
5/5	5.8 ± 0.6	9.2 ± 1.4	0.232 ± 0.009	450 ± 0	0.084 ± 0.020	0.064 ± 0.008
40/5	6.1 ± 0.1	11.2 ± 0.9	0.374 ± 0.020[b]	450 ± 0	0.201 ± 0.008[b]	0.110 ± 0.005[b]

a Pretreatment (mg/kg)/Treatment (mg/kg).
b $P \leq 0.05$ compared to control (0/0).

both BNF and CD and occurred despite the fact that there is no evidence that CD is metabolized by this enzyme system in mice (Guzelian, 1982). BNF also caused marked elevations in the activity of EROD in the B6 but not the D2 strain. Here, CD caused a 5-fold induction in EROD activity in the B6 but not the D2 strain. This a much smaller increase in EROD than that which occurred with BNF, indicating that CD is at best a weak agonist for the cytosolic Ah receptor. A CD-treatment regimen which previously had been shown to cause a PDR (Carpenter and Curtis, 1989) caused no changes in either the liver to body weight ratios or the microsomal protein content (Table 2). The 40 mg/kg CD pretreatment 5 mg/kg CD treatment dose did cause elevations in the specific content of cytochrome P-450 and in the activities of EROD and ECOD. These results indicate that despite the fact that CD is, at best, only poorly metabolized in mice CD is an inducer of the hepatic drug metabolizing enzyme system of the liver. However, since a marked PDR was induced with much lower doses of CD than it took to elicit a cytochrome P-450 response, CD-PDR appears to occur independently of induction of the cytochrome P-450 system. These results do not preclude the possibility that cytochrome P-450 isozymes are capable of acting as non-enzymic binding proteins. Such an action might explain the inductive ability of CD despite the fact that it is a poor substrate for these isozymes.

An additional argument against the involvement of metabolism in OC-induced PDR is provided by the observation that there were no detectible differences in the levels of metabolites between the OC-pretreated animals and the controls (Carpenter and Curtis, 1989; Curtis et al., 1990).

An examination of the total lipid contents of the livers and kidneys of mice following pretreatment with CD showed that CD pretreatment did not cause any changes in the total lipid contents of these tissues (Carpenter et al., 1990, in preparation; Table 3). The values for liver averaged 5.0% lipid/g and those for kidney averaged 3.5%. There were, however, dose related differences in the concentrations of several fatty acids in livers (Table 3). The relative amounts of 16:0, 16:1, 18:3n9 and 18:1n9 decreased in a dose related fashion while the amounts of 18:0 and 20:4n-6 were increased. These results indicate that CD-induced PDR is not due to changes in total cellular lipid, but that CD does cause as yet unexplained differences in the relative amounts of several fatty acids. The alteration in amounts of specific fatty acids may be analogous to the process of homeoviscous adaptation during the process of thermal acclimation. These results suggest that, if an interaction between CD and tissue lipids explains PDR, more than simple hydrophobic partitioning is involved.

An examination of the effect of CD-pretreatment on the subcellular distribution of a subsequent dose of [^{14}C]CD showed that there were CD-induced changes in the amounts of residual label associated with various subcellular fractions (Table 4).

Table 3. The effect of chlordecone pretreatment on total lipid and fatty acid content of liver and kidney.

	Chlordecone treatment (pretreatment/tracer)			
	0/0	0/5	5/5	40/5
Liver				
Fatty acid				
14:0	0.2 ± 0.1	0.3 ± 0.0	0.1 ± 0.1	0.1 ± 0.0
16:0				
16:1	23.9 ± 0.6	26.2 ± 0.4	23.5 ± 0.4	22.2 ± 0.5
18:0	7.9 ± 0.7	5.9 ± 0.3	8.6 ± 0.3	10.7 ± 0.6*
18:1n9	19.0 ± 1.0	20.1 ± 0.2	18.4 ± 0.3	16.2 ± 0.5*
18:2n6	25.6 ± 1.0	26.8 ± 1.0	26.0 ± 0.2	26.1 ± 0.8
18:3n3	1.2 ± 0.1	1.1 ± 0.0	0.6 ± 0.2	0.3 ± 0.2*
20:3n6	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.0	0.5 ± 0.1
20:4n6	14.3 ± 1.2	12.2 ± 0.4	14.8 ± 0.5	16.6 ± 0.8*
22:5n6	2.0 ± 0.3	1.6 ± 0.4	1.4 ± 0.1	1.5 ± 0.1
22:6n3	5.3 ± 0.5	4.7 ± 0.1	5.2 ± 0.2	5.2 ± 0.3
Unknown	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.0	0.6 ± 0.0
Recovery (% lipid/g)	5.3 ± 0.5	5.7 ± 0.4	4.7 ± 0.2	4.5 ± 0.3
Kidney				
Fatty acid				
14:0	0.6 ± 0.1	0.7 ± 0.0	0.5 ± 0.1	0.4 ± 0.2
16:0				
16:1	19.8 ± 0.5	21.2 ± 0.6	19.7 ± 0.6	21.0 ± 0.4
18:0	9.7 ± 0.4	8.8 ± 0.3	9.7 ± 0.3	10.4 ± 0.5
18:1n9	15.6 ± 0.9	17.1 ± 1.3	16.1 ± 0.3	14.6 ± 0.6
18:2n6	15.3 ± 1.2	17.2 ± 0.3	16.8 ± 0.9	15.7 ± 0.8
20:3n6	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 1.1	0.6 ± 0.1
20:4n6	16.8 ± 0.9	15.3 ± 0.7	16.5 ± 0.6	18.4 ± 0.9
22:5n6	5.2 ± 0.4	4.0 ± 0.2	4.3 ± 0.3	4.7 ± 0.4
22:6n3	14.0 ± 0.9	11.9 ± 0.5	12.7 ± 0.6	12.4 ± 0.5
Unknown	0.9 ± 0.1	1.0 ± 0.0	0.9 ± 0.0	0.7 ± 0.2
Recovery (% lipid/g)	3.4 ± 0.0	3.9 ± 0.2	3.1 ± 0.1	3.0 ± 0.2

Values are mean ± S.E.M., n = 4.
* P <0.05 compared to 0/0 control.

Table 4
The effect of CD pretreatment on the subcellular distribution of [^{14}C]CD in liver

[^{14}C]CD (5 or 40 mg/kg) was administered 3 days following the pretreatment of mice with corn oil or CD (5 or 40 mg/kg). Animals were killed 16 hr following the tracer and their livers were examined for residual label. Details for cell fractionation tecniques are presented in Materials and Methods. Values are expressed as mean ± SE; N is in parenthesis.

	Tracer/Pretreatment				
	5 mg/kg [^{14}C]CD tracer			40 mg/kg [^{14}C]CD tracer	
	Control (8)	5 mg/kg CD (8)	40 mg/kg CD (7)	control (3)	5 mg/kg CD (4)
	nmol/ g liver				
Homogenate	80.5 ± 6.9	58.1 ± 2.5[a]	38.1 ± 3.0[b]	368.3 ± 8.5	396.7 ± 18.5
Cell Fraction					
Nuclear/Debris	35.1 ± 1.1	29.5 ± 0.4[a]	18.1 ± 0.9[b]	188.0 ± 10.1	196.5 ± 5.6
Mitochondrial	16.7 ± 0.8	15.4 ± 0.9	9.3 ± 0.6[b]	95.4 ± 1.9	106.1 ± 6.0
Microsomal	16.2 ± 0.4	12.3 ± 0.5	9.0 ± 0.7[b]	65.4 ± 4.4	88.4 ± 4.1[a]
Cytosol	0.7 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	8.7 ± 0.3	9.8 ± 0.3[a]
	Percent of recovered dose				
Cell Fraction					
Nuclear/Debris	51.7 ± 1.0	51.2 ± 0.6	49.4 ± 0.6	51.2 ± 1.4	49.1 ± 0.8
Mitochondrial	24.3 ± 1.2	26.6 ± 1.3	24.4 ± 1.2	28.0 ± 1.4	26.5 ± 1.3
Microsomal	23.5 ± 0.5	21.3 ± 0.8	24.7 ± 0.9	18.3 ± 0.5	22.0 ± 0.7[a]
Cytosol	1.1 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	2.5 ± 0.1	2.5 ± 0.3

[a] $P < 0.05$ compared to respective control.
[b] $P < 0.05$ compared top respective control and the 5 mg/kg pretreatment group.

Consistent with previous results (Carpenter and Curtis, 1989) the livers and kidneys of mice treated with various doses of CD exhibited a PDR when a 5 mg/kg [^{14}C]CD tracer was administered. The amounts of label in liver homogenates decreased in a dose related fashion while the amounts in the kidney homogenates were increased following treatment. The distribution of [^{14}C]CD (total nmol recovered) in subcellular fractions of the liver varied in manner consistent with the whole homogenates (Table 4). However, when calculated as percent of total radiolabel recovered the differences between the subcellular fractions disappear. When a 40 mg/kg [^{14}C]CD tracer was given there was no apparent PDR in the homogenates. This was again consistent with previously reported results (Carpenter and Curtis, 1989) and probably due to the fact that this high dose of CD overwhelmed the CD-induced changes in the cell. When the subcellular fractions from these animals were examined it was apparent that the microsomes of the pretreated mice had a higher affinity for the label. These results are consistent those of Poland et al. with TCDD (1989a; 1989b). These researchers showed that following pretreatment, TCDD congeners were found in higher concentrations in liver than in controls, a PDR. Subsequent analysis showed that percent of recovered label was higher in the microsomal fraction from pretreated animals. This was shown to be due to binding to a specific cytochrome P-450 (P_3-450) which did not result in increased metabolism of TCDD. Interpretation of these results in regard to those we obtained for CD would seem to suggest that CD-PDR might be partially explained by changes in the cytochrome P-450 system, perhaps with cytochrome P-450s acting as binding proteins rather than performing a catalytic function.

There were no apparent changes in the distribution of label (either as total nmol or as per cent recovery) in subcellular fractions of the kidney with the lower tracer dose, but there was a slight decrease in the percent of label recovered in the mitochondrial fraction (Table 5).

We determined the effect of a dose of CD (5 mg/kg), previously shown to cause a PDR, on the distribution of a subsequent dose of [^{14}C]cholesterol (Figure 1). CD caused a significant reduction in the amount of [^{14}C]cholesterol retained in the liver and the kidney and increased the amount of label seen in the fat following a tracer dose of 10 mg/kg [^{14}C]cholesterol. These changes were not apparent in the 1 or 100 mg/kg [^{14}C]cholesterol tracer groups, perhaps indicating a threshold and a saturation for this phenomenon. When the time after administration of 10 mg/kg dose of ^{14}C-cholesterol tracer prior to killing of the mice was varied from 1, 2, 4, 8 and 16 hr, it was apparent that the hepatic concentration of cholesterol peaked at about 8 hours while that in the fat continued to increase until 16 hours (Figure 2). This was different from the previous observations with CD, where pretreatment resulted in decreased levels of material in the livers and increased amounts of label in non-hepatic tissues.

Table 5

The effect of CD pretreatment on the subcellular distribution of [^{14}C]CD in kidney

[^{14}C]CD (5 or 40 mg/kg) was administered 3 days following the pretreatment of mice with corn oil or CD (5 or 40 mg/kg). Animals were killed 16 hr following the tracer and their kidneys were examined for residual label. Details for cell fractionation techniques are presented in Materials and Methods. Values are expressed as mean ± SE; N is in parenthesis.

	Tracer/Pretreatment				
	5 mg/kg [^{14}C]CD tracer			40 mg/kg [^{14}C]CD tracer	
	Control (8)	5 mg/kg CD (8)	40 mg/kg CD (7)	control (3)	5 mg/kg CD (4)
	nmol/ g liver				
Homogenate	10.3 ± 1.2	11.4 ± 0.3	14.2 ± 0.7[a]	162.1 ± 7.8	167.6 ± 5.4
Cell Fraction					
Nuclear/Debris	5.6 ± 0.4	6.3 ± 0.4	8.1 ± 0.2	69.7 ± 6.0	85.6 ± 4.3
Mitochondrial	2.7 ± 1.6	2.9 ± 0.3	3.2 ± 0.3	65.4 ± 3.0	69.0 ± 3.0
Microsomal	1.4 ± 0.2	1.2 ± 0.1	1.5 ± 0.1	16.2 ± 3.3	20.3 ± 2.3
Cytosol	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	4.9 ± 0.3	5.6 ± 0.3
	Percent of recovered dose				
Cell Fraction					
Nuclear/Debris	57.2 ± 2.3	59.3 ± 1.1	61.6 ± 1.6	42.0 ± 3.3	47.5 ± 3.3
Mitochondrial	27.0 ± 1.6	26.9 ± 1.6	24.4 ± 1.6	42.8 ± 1.4	38.2 ± 1.2[a]
Microsomal	13.7 ± 1.4	11.3 ± 1.4	11.7 ± 0.3	11.8 ± 1.8	11.2 ± 1.1
Cytosol	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	4.0 ± 0.8	3.1 ± 0.2

[a] $P < 0.05$ compared to respective control.

Figure 1.

The effect of CD pretreatment on the distribution of [^{14}C]Cholesterol. [^{14}C]Cholesterol was administered 3 days after a single pretreatment of CD (5 mg/kg ip) or corn oil. Animals were killed 16 hr following tracer and their tissues examined for residual label. Values are mean ± S.E.M., n = 4 (error bars not visible are hidden by the symbols). *P ≤ 0.05 compared to corn oil treated controls.

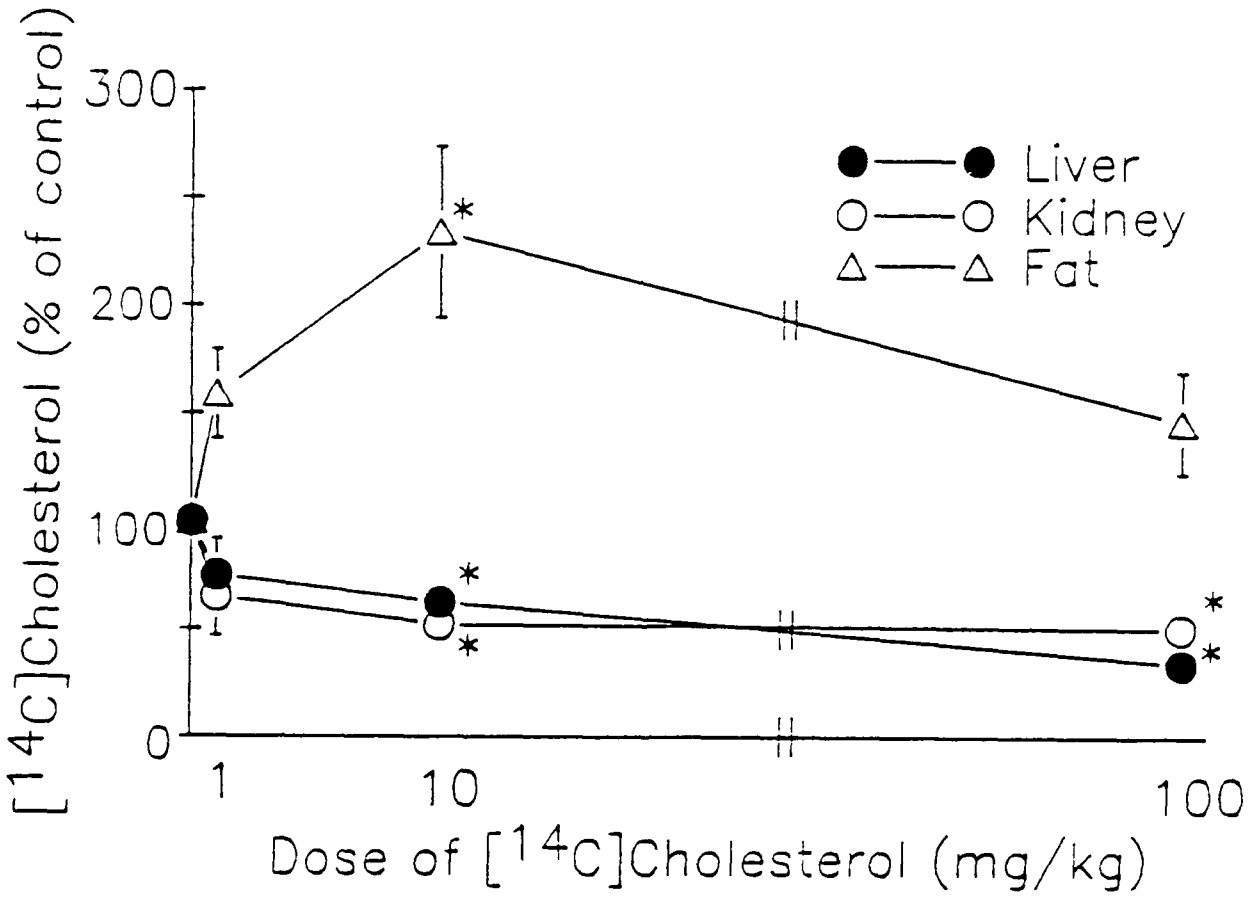

Figure 2.

Time response of cholesterol to a CD pretreatment disposition response. [^{14}C]Cholesterol (10 mg/kg) was administered 3 days following pretreatment with CD (5 mg/kg). Animals were killed at various times following the tracer and their tissues were examined for residual label. Values are means ± S.E.M, n = 4 (error bars not visible are hidden by the symbols). *P < 0.05 compared to corn oil treated controls.

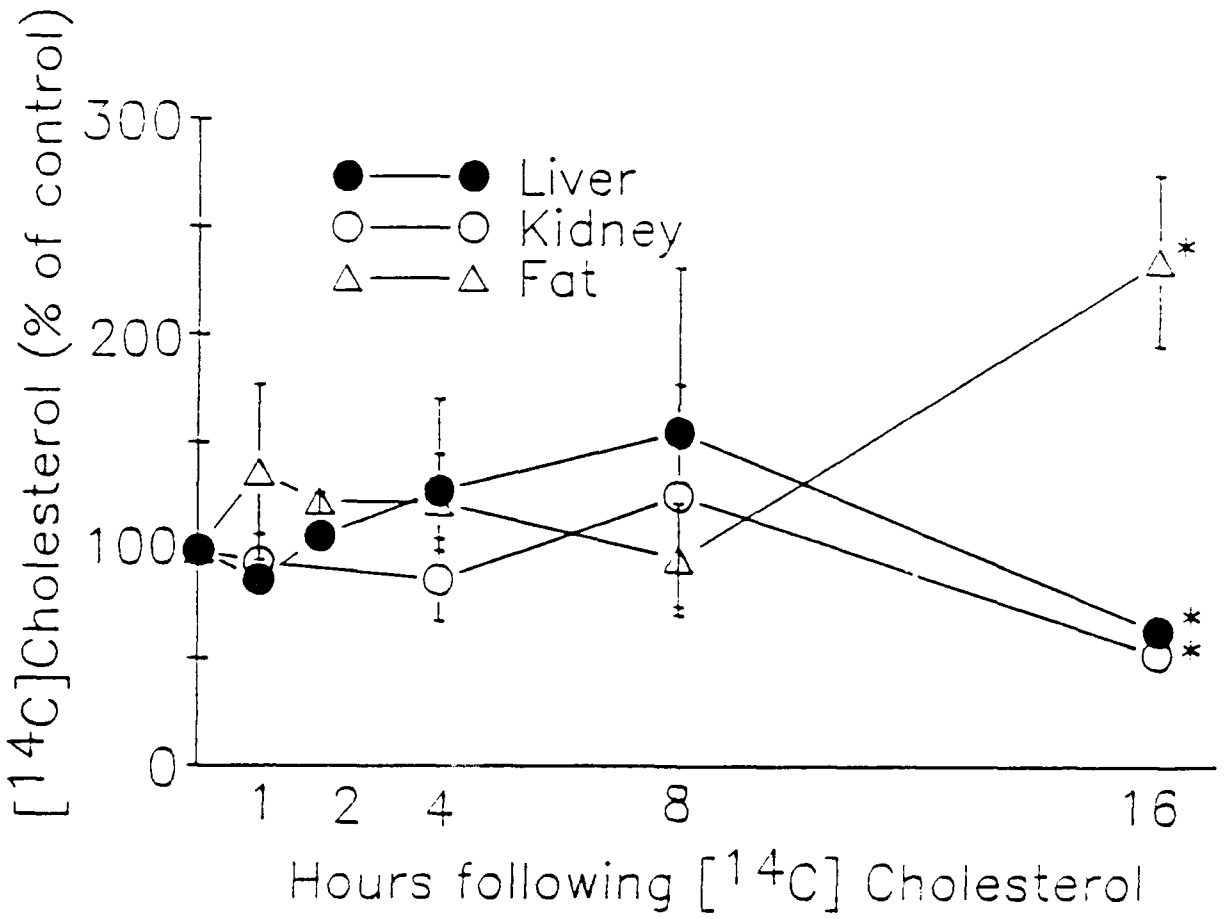

Table 6

The effect of phenobarbital pretreatment on the distribution of [^{14}C]Chlordecone and [^{14}C]Cholesterol

Phenobarbital (80mg/kg ip) was administered daily for 4 days prior to the administration of a [^{14}C]CD (5 mg/kg) or [^{14}C]Cholesterol tracer. A single dose of corn oil or CD was administered 3 days prior to [^{14}C]CD and served as controls. Animals were killed 16 hr following the tracer and their tissues were examined for residual label. Values are expressed as mean ± SE, N = 4 except were noted.

	nmol/g of tissue					
	[^{14}C]CD Treatment			[^{14}C]Cholesterol Treatment		
Pretreatment:	Corn Oil	CD	Phenobarbital	Corn oil	CD	Phenobarbital
Liver	82.9 ± 1.1	69.6 ± 3.1[b]	99.9 ± 1.2[b]	22.9 ± 1.3	9.8 ± 0.5[b]	11.1 ± 1.1[b]
Kidney	11.8 ± 0.4	13.5 ± 0.8	10.5 ± 0.3[b]	5.9 ± 1.4	1.9 ± 0.2	1.8 ± 0.1
Fat	8.7 ± 0.5	10.9 ± 1.1	7.6 ± 0.5	27.0 ± 3.7	65.5 ± 4.3[b]	44.4 ± 9.7
Plasma	5.7 ± 1.0	9.2 ± 3.3	9.9 ± 3.6	139.7 ± 7.9	90.9 ± 9.3[b]	6.5 ± 0.4[b]
Gall bladder/ bile	0.1 ± 0.0	0.2 ± 0.0	0.2 _ 0.0	7.9 ± 1.8	5.4 ± 0.7	5.2 ± 0.6

[a] N = 3 for this group
[b] P < 0.05 compared to corn oil treatment group.

Cytosolic proteins bind to lipophilic compounds including xenobiotics, perhaps transporting these materials within the cell or to tissues with greater metabolic and/or storage capacity (Clarke and Armstrong, 1989). Some of the proteins which have been suggested for possible action in this role are the glutathione transferases (Kaplowitz, 1982; Tipping et al., 1976; Listowsky et al., 1988), sterol carrier proteins (Clarke and Armstrong, 1989) and the fatty acid binding proteins (Bass, 1988), and most recently specific cytochrome P-450 isozymes (Poland et al., 1989a; 1989b, Voorman and Aust, 1987; 1989). It seems possible therefore, that our results with CD might be explained by a similar mechanism. Several proteins involved in the maintenance and handling of cholesterol appear to have a role in the intracellular processing of CD (Soine et al., 1982; 1984). This includes several hepatic proteins which have a high affinity for both CD and cholesterol (Soine et al., 1984). Recent work has indicated that the non-specific lipid transfer protein (sterol carrier protein 2) is particularly important in the intracellular processing of cholesterol (van Amerogen et al., 1989), and that the cellular content of this and other lipid binding proteins are regulated such factors as hormones and the dietary content of fats (Clarke and Armstrong, 1989). It seems likely that if these proteins are inducible by CD pretreatment these cholesterol-CD binding proteins could be responsible for the observed CD-induced changes in [^{14}C]CD concentrations in the liver as well as the CD-induced increased disposition of [^{14}C]cholesterol to the fat, ie., an increased hepatic processing of cholesterol such that more material is placed in the fat for storage.

An additional cellular mechanism for movement of lipophilic compounds such as TCDD and benzo[a]pyrene involves intracellular lipoprotein complexes as transport vehicles (Lesca et al., 1987; Soues et al., 1989a; 1989b). These lipoprotein complexes have been shown to be markedly elevated by pretreatment with phenobarbital (Lesca et al., 1987). Since CD is a phenobarbital type inducer in mice, it seemed possible that CD-induced PDR might be explained by the induction of lipoproteins by CD. We therefore pretreated mice with phenobarbital or CD and treated them with [^{14}C]CD To see if the effects were similar (Table 6). Phenobarbital pretreatment caused an increase in the amount of [^{14}C]CD retained by the liver and a decrease in the amount found in the kidney, the reverse of what is observed with CD pretreatment (Table 6). Both CD and phenobarbital decreased the amount of residual [^{14}C]cholesterol in the liver. CD pretreatment again caused an increase in the amount of [^{14}C]cholesterol that ended up in the fat, but there was no change following phenobarbital pretreatment.

Additional studies examined whether or not CD-induced PDR could be attributed to changes in the ultrastructure of the cell. Mice received a treatment regimen which resulted in a PDR. Following treatment the animals were anesthetized and their livers and kidneys prepared for microscopy (Carpenter et al.,

1990). Thick sections of control liver showed abundant lipid droplets (containing neutral triglycerides) that were greatly variable in size and located throughout the cell without apparent organization. Ito cells also appeared to be storing abundant lipids. Following pretreatment with 5 mg/kg CD there was a decrease in the numbers of these hepatocellular fat droplets compared to control animals (Figure 3a). The droplets that remained were smaller in size and of a more uniform shape than those seen in the controls. There also appeared to be abundant Ito cell fat. In the 40 mg/kg pretreatment group there was still abundant Ito cell fat despite the fact that the numbers of fat droplets were greatly reduced, and those that remained were very small and had either moved to the surface or were located in the microvilli protruding into the sinusoids. There were also many other changes which were indicative of increased synthetic activity of the cell. Control animals also had abundant lipoprotein secretory vesicles (LPSV) which were oriented toward the bile canaliculi. Following treatment of the mice with 5 mg/kg CD there appeared to be more LPSV present than in control animals, and the golgi aparati were distended with LPSV associated with the forming face. Following the 40 mg/kg CD dose the golgi (when present) were greatly expanded, and the cells exhibited abundant LPSV throughout the cytoplasm. CD treatment of animals also caused marked changes in the glycogen aggregates located in the cell and caused an increased number of phagolysosomes in the cytoplasm. The 40 mg/kg pretreatment group had increased numbers of lysosomes and peroxisomes and they had an increased electron density. CD treatment also caused marked increases in the amounts of smooth endoplasmic reticulum (SER). and in the abundance of free cytosolic polyribosomes. These results suggest that CD can increase mobilization of lipids from their tissue storage sites and that these lipids are being used in the synthesis of new membrane structures in the cell. Ultrastructurally, treated mice exhibited mitochondria that were swollen and irregular (Figure 3b). They also have decreased amounts of glycogen and increased amounts of smooth endoplasmic reticulum. In the high dose animals there also appeared to be an increase in the numbers of electron dense cytoplasmic lipoprotein vesicles. These may be cytosolic lipoproteins pinching off the golgi apparatus indicative of elevated synthesis and transport of cellular materials. CD treatment causes marked changes in the ultrastructure of cells. These changes are consistent with a markedly stimulated production of membrane structures such as SER and LPSV. Whether or not these changes are responsible for CD-induced PDR remains to be determined.

The role of cellular proteins functioning to sequester and transport OCs rather than to metabolize them has, as yet, received little attention. This is probably a result of the fact that most studies to date have used acute pretreatments with maximally tolerated doses. High dose acute exposure, in addition to having arguable relevance to environmental or occupational exposures (which tend to be chronic and occur at low levels), would tend to mask the types of response presented here and

Figure 3: Liver, mouse, controls (A,C) and treated with chlordecone (B,D) at 40 mg/kg followed in 3 days with 5 mg/kg tracer dose of C^{14} chlordecone and necropsied 16 hours later. Numerous lipid droplets (arrowheads) in controls compared to few lipid droplets in treated mice. In treated mice (D), electron micrographs show irregular swollen mitochondria, increased profiles of smooth endoplasmic reticulum (arrow) and election dense cytosol containing numerous ribosomes. A,B methylene blue, post fixed in osmium tetroxide, thick sections (1-2 μm. Bar = 10 μm. C,D electron micrographs, Bar = 10 μm.

A
B
C
D

previously reported (Shubat and Curtis, 1986; Carpenter and Curtis, 1989; Curtis et al., 1990).

These data, in addition to the results presented here, indicate that tissue distribution and accumulation of OCs is modulated at low doses in a manner which is consistent with an inducible, high affinity, low capacity system. OC-induced PDR may be part of an as yet poorly defined process which gives cells the ability to deal with stable lipophilic compounds such as OCs.

Literature Cited

Bass, N.M. (1988). The cellular fatty acid binding proteins: Aspects of structure, regulation and function. Int Rev. Cytology **111**; 143-184 (1988).

Carpenter, H.M. and L.R. Curtis (1989). A characterization of pretreatment altered distribution of chlordecone in mice. Drug Metabol. Dispos. **17**; 131-138 (1989).

Carpenter, H.M. and L.R. Curtis (1990). Biochemical correlates of chlordecone pretreatment disposition response in mice. Submitted to Drug. Met. Disp.

Carpenter, H.M. ,Z.-W. Cai, O.R. Hedstrom, J. Duimstra and L.R. Curtis (1990). Cellular ultrastructural and lipid changes associated with chlordecone-induced pretreatment disposition response. In preparation for Tox. Appl. Pharmacol.

Clarke, S.D. and M.K. Armstrong (1989). Cellular lipid binding proteins: expression, function, and nutritional regulation. FASEB J. **3**; 2480-2487.

Curtis, L.R., L. Steppen, N. Kerkvliet and H.M. Carpenter (1990). Pretreatment altered pharmacokinetics of TCDD in mice. Fund. Appl. Tox. **14**; 523-531.

Kaplowitz, N. (1980). Physiological significance of glutathione S-transferases. Am. J. Physiol. **239**; G439-G444.

Lesca. P., N. Fernandez and M. Roy (1987). The binding components for 2,3,7,8-tetrachlorodibenzo-p-dioxin and polycyclic aromatic hydrocarbons. J. Biol. Chem. **262**; 4827-4835.

Lewandowski, M., P. Levi and E. Hodgson (19898). Induction of cytochrome P-450 isozymes by mirex and chlordecone. J. Biochem. Toxicol. **4**; 195-199 (1989).

Listowsky, I., M. Abramovitz, H. Homma, and Y. Niitsu (1988). Intracellular binding and transport of hormones and xenobiotics by glutathione-S-transferases. Drug Metabol. Rev. **19**; 305-318.

Poland, A., P. Teitelbaum and E. Glover (1989a). [^{125}I]2-iodo-3,7,8-trichlorodibenzo-p-dioxin binding species in mouse liver induced by agonists for the Ah receptor: Characterization and identification. Molec.Pharmacol. **36**; 113-120.

Poland, A., P. Teitelbaum, E. Glover and A. Kende (1989b). Stimulation of in vivo hepatic uptake and in vitro hepatic binding of [^{125}I]2-iodo-3,7,8-tetrachlorodibenzo-p-dioxin by the administration of agonists for the Ah receptor. Molec. Pharmacol. **36**; 121-127.

Shubat, P.J. and L.R. Curtis (1986). Ration and toxicant preexposure influence dieldrin accumulation by Rainbow trout. Environ. Toxicol. Chem. **5**; 69-77.

Soine, P.J., R.V. Blanke, P.S. Guzelian and C.C. Schwartz (1982). Preferential binding of chlordecone to the protein and high density lipoprotein fractions of plasma from humans and other species. J. Toxicol. Environ. Health. **9**; 107-118.

Soine, P.J., R.V. Blanke and C.C. Schwartz (1984). Isolation of chlordecone binding proteins from pig liver cytosol. J. Toxicol. Env. Health. **14**; 305-317.

Soues, S., N. Fernandez, P. Souverain and P. Lesca (1989a). Separation of different classes of intrahepatic lipoproteins from various animal species. Biochem. Pharmacol. **38**; 2833-2839.

Soues, S., N. Fernandez, P. Souverain and P. Lesca (1989b). Intracellular lipoproteins as carriers for 2,3,7,8-tetrachlorodibenzo-p-dioxin and benzo[a]pyrene in rat and mouse liver. Biochem. Pharmacol. **38**; 2841-2847.

Tipping, E., B. Ketterer, L. Christodoulides and G. Enderby (1976). The non-covalent binding of small molecules by ligandin. Eur. J. Biochem. **67**; 583-590.

van Amerogen, A., M. van Noort, J.R.C.M. van Beckhoven, F.F.G. Rommerts, J. Orly and K.W.A. Wirtz (1989). The subcellular distribution of the nonspecific lipid transfer protein (sterol carrier protein 2) in rat liver and adrenal gland. Biochim. Biophys. Acta. **1001**; 243-248.

Voorman, R. and S. D. Aust (1987). Specific binding of polyhalogenated aromatic hydrocarbon inducers of cytochrome P-450d to the cytochrome and its inhibition of estradiol 2-hydroxylase activity. Toxicol. Appl. Pharmacol. **90**; 69-78.

Voorman, R. and S.D. Aust (1989). TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a tight binding inhibitor of cytochrome P-450d. J. Biochem. Toxicol. **4**; 105-109.

Publications

1. H. M. Carpenter and L. R. Curtis. 1989. A characterization of chlordecone pretreatment altered disposition in mice. Drug Metabolism and Disposition 17:131-138.

2. L. R. Curtis, L. Baecher-Steppan, N. I. Kerkvliet and H. M. Carpenter. 1990. 2,3,7,8-Tetrachlorodibenzodioxin pretreatment of female mice altered tissue distribution but not hepatic metabolism of a subsequent dose. Fund. Appl. Tox. 14:523-531.

3. L. G. Rochelle, T. L. Miller and L. R. Curtis. 1990. Chlordecone impairs Na^+-stimulated [^{3}H]L-glutamate transport and mobility of 16-doxl stearate in rat bile canalicular enriched vesicles. Tox. Appl. Pharmacol. In press.

4. H. M. Carpenter and L. R. Curtis. 1990. Biochemical correlates of chlordecone pretreatment disposition response in mice. Submitted to Drug Metab. Dispos.

5. H. M. Carpenter, Z.-W. Cai, O. R. Hedstrom, J. R. Duimstra and L. R. Curtis. 1990. Changes in cellular ultrastructure and lipids associated with chlordecone-induced pretreatment disposition response. In preparation Tox. Appl. Pharmacol.

6. L. G. Rochelle and L. R. Curtis. 1990. Evidence for the bile canaliculi as a site of action in chlordecone impaired organic anion transport. In preparation for J. Biochem. Tox.

Participating professionals

Hillary M. Carpenter, Ph.D., Assistant Professor, Dept. of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon 97331.

Lawrence R. Curtis, Ph.D., Associate Professor, Dept of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon 97331.

Abstracts

1. H. M. Carpenter and L. R. Curtis 1986. Chlordecone (CD) pretreatment altered disposition of ^{14}C-CD in mice. The Pharmacologist. 28: 136.

2. L. R. Curtis and H. M. Carpenter 1988. Chlordecone (CD) preexposure-altered disposition (PAD) of a subsequent dose exhibits threshold and saturation in mice. The Toxicologist. 8: 181.

3. H. M. Carpenter, Z.-W. Cai and L. R. Curtis 1989. Biochemical correlates of chlordecone (CD)-induced pretreatment disposition response (PDR) in mice. The Toxicologist 9: 25.

4. L. R. Curtis, H. M. Carpenter, L. Baecher-Steppan and N. I. Kerkvliet 1989. 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) pretreatment of C57BL mice altered disposition but not hepatic metabolism of a subsequent dose of [^{14}C] TCDD. The Toxicologist 9: 24.

5. H. M. Carpenter and L. R. Curtis 1989. A comparison of pretreatment disposition responses (PDRs) to organochlorine compounds (OCs) of diverse structure. Presented at the annual meeting of the Pacific Northwest Association of Toxicologists, September 17, 1989, Vancouver, B.C.

6. H. M. Carpenter, O.R. Hedstrom, J. R. Duimstra and L. R. Curtis. 1990. Chlordecone induced pretreatment dispsition response may not be related to changes in cytosolic lipoprotein complexes. To be presented at the International Society for the Study of Xenobiotics annual meeting, Oct. 21-25, 1990, San Diego, CA.

New discovery/patent discussion

None