Chapter XVI-6

ENDOCRINE FUNCTION

1. Introduction

TCDD is known to produce a broad spectrum of metabolic phenomena in animal experimental subjects treated with sufficiently large doses. The pattern of effects is quite complex. Hypothyroxinemia has been produced in rats (Potter et al, 1983), and this may be associated with increased biliary elimination of thyroxine (Bastomsky, 1977). Hypoglycemia has been produced in rats (Gasiewicz et al, 1980, Potter et al, 1983) at the same time that serum and pancreatic insulin levels fell (Potter et al, 1983). TCDD has been observed to reduce hepatic catabolism of testosterone in the rat (Nienstedt et al, 1979).

Based on animal data, the physical examination in this study obtained data for thyroid function (T3 uptake, serum T4 and the free thyroxine index or FTI), glucose metabolism (blood glucose level taken 2 hours after a standard carbohydrate load) and serum testosterone level. These 5 variables are listed in Table XVI-6-1 together with a description of normal and abnormal levels provided by the Kelsey-Seybold contract effort.

Table XVI-6-1

FIVE ENDOCRINOLOGICAL VARIABLES
AND THEIR NORMAL AND ABNORMAL LEVELS

Variable Name	Variable Abbreviation	Abnormal (Low)	Normal Range	Abnormal (High)
T3 Uptake	T3	<27%	27%-37%	>37%
Serum T4	T4	<4.7 µg/dl	4.7-12.5 µg/dl	>12.5 µg/dl
Free Thyroxine Index	FTI	<1.3	1.3-4.6	>4.6
2 Hour Post-prandial Glucose	GLU 2 HR	NA	<120 mg/dl	≥120 mg/dl
Serum Testosterone	TEST	<400 ng/dl	400-1200 ng/dl	>1200 ng/dl

Each study subject was asked to follow a standardized diet prior to arrival at the examination site. Not all participants complied with the diet. Table XVI-6-2 shows dietary compliance by group.

Table XVI-6-2

DIETARY COMPLIANCE BY GROUP

Group	Complied with Diet	Did Not Comply With Diet	Dietary Compliance Unknown
Ranch Hand	896 (86%)	96	53
Comparison	676 (87%)	70	27

The groups are not different as regards dietary compliance (P = 0.262). Also dietary compliance was not found to be associated with the likelihood of being in the high abnormal GLU 2 HR category. Thus, in Tables XVI-6-3 and XVI-6-5 participants were used irrespective of dietary compliance status.

2. Data Analysis

Table XVI-6-3 shows unadjusted percentages of the 5 endocrinological variables by variable level and group. (For this table and all other analyses in this chapter, all Ranch Hand participants (N = 1045) and all original controls (N = 773) were used as the basic data set). In the analysis of thyroid hormones, data from individuals with thyroidectomies were removed (7 Ranch Handers and 3 original comparisons), and in the analysis of testosterone, data from individuals with orchiectomies (5 Ranch Handers and 1 original comparison) were removed. Other denominator variations occurred due to missing covariates.

A group difference in T3 uptake is noted in Table XVI-6-3. The Ranch Hand group has fewer individuals in the low category and more individuals in the high category than does the comparison group. The same directionality is noted with the T4 and FTI variables. No group differences are found in GLU 2 HR or TEST.

Since hormone levels can be correlated with age and physical habitus, an analysis of the 5 endocrinological variables was attempted adjusting for age in years (dichotomized as less than or equal to 40 years and greater than 40 years) and for percent body fat (trichotomized as less than 10%, 10-25%, greater than 25%). There are too few abnormalities for a full analysis of any of the 5 endocrinological variables. However, for T3 and TEST, analyses could be performed on those individuals with 10% body fat or greater and having low abnormal or normal dependent variable values. Similarly, an analysis of GLU 2 HR values was possible on those individuals with 10% body fat or greater. The data for these 3 adjusted analyses are presented in Tables XVI-6-4, XVI-6-5 and XVI-6-6. Log-linear models were used in these analyses.

Table XVI-6-3

UNADJUSTED PERCENTAGES FOR FIVE ENDOCRINOLOGICAL VARIABLES BY VARIABLE LEVEL AND GROUP

			Variable Level			
Variable	Group	N	Low	Normal	High	P Value For Group Difference
T3	RH	1032	5.72%	93.41%	0.87%	0.020
	COM	767	8.47%	91.26%	0.26%	
T4	RH	1033	0.10%	99.13%	0.77%	0.250
	COM	767	0.39%	99.22%	0.39%	
FTI	RH	1033	0.00%	99.71%	0.29%	0.085
	COM	767	0.26%	99.74%	0.00%	
GLU 2 HR	RH	1040	NA	84.81%	15.19%	0.234
	COM	770	NA	82.73%	17.27%	
TEST	RH	1034	4.93%	94.58%	0.48%	0.414
	COM	769	6.37%	93.11%	0.52%	

Table XVI-6-4 shows a group difference in T3 uptake which is age specific ($P = 0.005$). There are more low T3 values in the comparison group than in the Ranch Hand group in the 40 and under-40 age group, but the groups are similar above 40 years of age. A highly statistically significant association of T3 hypothyroxinemia with body fat is noted within the groups ($P = 0.004$).

Table XVI-6-5 shows no group difference in the observed proportions of hyperglycemia ($\geq$ 120 mg/dl). Age and body fat are seen to influence these proportions ($P < 0.001$ in both instances), and the effect is about the same in both groups.

Table XVI-6-6 shows no group difference in the observed proportions of low testosterone. Age and body fat both influence these proportions ($P = 0.022$ for age and $P < 0.001$ for body fat), and the effect is approximately the same in both groups.

Using the categories for normal and abnormal levels shown in Table XVI-6-1, it was not possible to meaningfully carry out an exposure index analysis of the 5 endocrinological variables, due to sample size limitations.

Table XVI-6-4

PERCENT OF ABNORMALLY LOW T3 VALUES
BY GROUP, AGE AND BODY FAT CATEGORY*

Age	Group	% T3 Low Abnormal in 10-25% Body Fat Subgroup		% T3 Low Abnormal in > 25% Body Fat Subgroup	
≤40	RH	2.59	(9/347)	6.58	(5/76)
≤40	COM	7.89	(18/228)	19.15	(9/47)
>40	RH	6.49	(30/462)	10.94	(14/128
>40	COM	7.43	(28/377)	9.26	(10/108)

* Abnormally high individuals and lean individuals (less than 10% body fat) were removed from the analysis due to sample size limitations.

Table XVI-6-5

PERCENT ABNORMAL GLU 2 HR VALUES
BY GROUP, AGE AND BODY FAT CATEGORY*

Age	Group	% GLU 2 HR in Abnormal Category in 10-25% Body Fat Subgroup		% GLU 2 HR in Abnormal Category in >25% Body Fat Subgroup	
≤40	RH	6.25	(22/352)	17.11	(13/76)
≤40	COM	6.55	(15/229)	17.02	(8/47)
>40	RH	18.01	(85/472)	28.46	(37/130)
>40	COM	18.25	(69/378)	36.36	(40/110)

* Lean individuals (less than 10% body fat) were removed from the analysis due to sample size limitations.

Table XVI-6-6

PERCENT ABNORMAL LOW TESTOSTERONE VALUES
BY GROUP, AGE AND BODY FAT CATEGORY*

Age	Group	% Testosterone Low Abnormal in 10-25% Body Fat Subgroup		% Testosterone Low Abnormal in > 25% Body Fat Subgroup	
≤40	RH	2.00	(7/350)	7.89	(6/76)
≤40	COM	3.52	(8/227)	10.64	(5/47)
>40	RH	3.46	(16/463)	16.15	(21/130
>40	COM	4.00	(15/375)	19.09	(21/110)

* Abnormally high individuals and lean individuals (less than 10% body fat) were removed from the analysis due to sample size limitations.

Analysis of covariance is less vulnerable to the data limitations of sparse or empty cells than are log-linear models. Thus, the Ranch Hand group was contrasted with the comparison group in terms of the 5 endocrinological variables using analysis of covariance adjusting for age and percent body fat. In these analyses, all variables except group indicators were used as continuous variables. In the analysis of thyroid hormones, data from individuals with thyroidectomies were removed, and in the analysis of testosterone levels, individuals with orchiectomies were removed. In the analysis of glucose levels, all participant data were used irrespective of dietary compliance as compliance was not found to influence glucose levels.

Table XVI-6-7 provides unadjusted and adjusted means. When a group-by-age or group-by-body fat interaction was observed with $P < 0.10$, adjusted means, and age and body fat main effects are not reported.

One overall group difference is noted in Table XVI-6-7. Specifically, the Ranch Handers show a higher testosterone level than do comparison participants ($P = 0.02$ unadjusted, 0.06 adjusted). Both increasing age and increasing body fat were found to be associated with decreasing testosterone level with slopes being -3.8 ng/dl per year of life and -12.6 ng/dl per % body fat.

Table XVI-6-7

RANCH HAND - COMPARISON GROUP MEANS OF ENDOCRINE VARIABLES

Variable	Group	N	Unadj'd Mean	P Value for Unadj'd Means	Adj'd Mean	P Value for Adj'd Means	Remarks about Adjusting Covariates
T3	Com	770	30.14	0.21	*	*	Group-by-age interaction
Uptake (%)	RH	1037	30.28				(P = 0.026)
T4	Com	770	8.39	0.31	8.39	0.38	None significant at P<.05
(µg/dl)	RH	1038	8.46		8.45		
FTI	Com	770	2.51	0.07	2.51	0.13	Age (P<.001)
	RH	1038	2.54		2.54		% Body fat (P<.001)
GLU 2HR	Com	773	102	0.37	*	*	Group-by-age interaction
(mg/dl)	RH	1045	104				(P=.006)
TEST	Com	772	634	0.02	637	0.06	Age (P<.001)
(ng/dl)	RH	1039	654		652		% Body fat (P<.001)

Two other group differences are noted in Table XVI-6-7; however, these are associated with group-by-age interactions. In both the Ranch Hand and comparison groups, decreasing T3 uptake is observed associated with advancing age, but the slope was found to be -0.0068% per year in the comparison group while it is -0.0495% per year in the Ranch Hand group. Glucose levels, measured 2 hours into the glucose tolerance test, were observed to increase with age in both the comparison and Ranch Hand group; however, the rate of increase is 0.77 mg/dl per year in the comparison group and 1.53 mg/dl per year in the Ranch Hand group.

Dose-response data within the Ranch Hand group are provided in Tables XVI-6-8, XVI-6-9 and XVI-6-10. No overall statistically significant dose-response relationships were detected; however, 5 exposure group by covariate interactions were noted. These interactions are summarized in Table XVI-6-11. No interactions are seen with respect to the variables T3 or T4.

Table XVI-6-8

RANCH HAND OFFICERS
ENDOCRINE DOSE-RESPONSE DATA

Variable	Group	N	Unadj'd Mean	P Value for Unadj'd Mean	Adj'd Mean	P Value for Adj'd Mean	Remarks about Adjusting Covariates
T3	L	110	30.9	0.39	30.8	0.88	Age (P=0.033)
	M	126	30.6		30.7		% Body fat (P=0.039)
	H	125	30.6		30.6		
T4	L	110	8.21	0.12	8.23	0.89	None
	M	126	8.15		8.15		
	H	125	8.22		8.22		
FTI	L	110	2.51	0.59	*	*	Age-exposure interaction (P=0.042)
	M	126	2.47				
	H	125	2.49				
GLU 2 HR	L	111	106.7	0.90	*	*	% Body fat-exposure interaction (P=0.041)
	M	128	104.2				
	H	125	106.8				
TEST	L	111	614.8	0.85	*	*	% Body fat-exposure interaction (P=0.011)
	M	127	614.2				
	H	123	604.5				

Table XVI-6-9

RANCH HAND - FLYING ENLISTED PERSONNEL
ENDOCRINE DOSE-RESPONSE DATA

Variable	Group	N	Unadj'd Mean	P Value for Unadj'd Mean	Adj'd Mean	P Value for Adj'd Mean	Remarks about Adjusting Covariates
T3	L	59	29.6	0.57	29.6	0.59	None
	M	59	30.0		30.0		
	H	64	30.0		30.1		
T4	L	59	8.85	0.32	8.85	0.32	None
	M	59	8.48		8.49		
	H	64	8.48		8.50		
FTI	L	59	2.60	0.45	*	*	% Body fat-exposure interaction (P=0.03)
	M	59	2.51				
	H	64	2.60				
GLU 2 HR	L	59	102.3	0.88	102.3	0.78	Age (P=0.01)
	M	59	105.9		108.0		
	H	66	105.6		103.8		
TEST	L	59	663.5	0.98	659.8	0.90	% Body fat (P<0.001)
	M	58	657.8		653.5		
	H	66	658.5		666.7		

Table XVI-6-10

RANCH HAND - GROUND ENLISTED PERSONNEL
ENDOCRINE DOSE-RESPONSE DATA

Variable	Group	N	Unadj'd Mean	P Value for Unadj'd Mean	Adj'd Mean	P Value for Adj'd Mean	Remarks about Adjusting Covariates
T3	L	151	29.8	0.30	29.9	0.18	Age (P<0.001)
	M	176	30.2		30.1		% Body fat (P<0.003)
	H	148	30.3		30.4		
T4	L	151	8.58	0.89	8.59	0.89	None
	M	177	8.67		8.67		
	H	148	8.59		8.58		
FTI	L	151	2.55	0.69	2.55	0.53	Age (P=0.01)
	M	177	2.58		2.56		% Body fat (P=0.03)
	H	148	2.60		2.61		
GLU 2 HR	L	151	99.9	0.60	*	*	% Body fat-exposure interaction (P=0.09)
	M	179	104.8				
	H	148	103.7				
TEST	L	151	686.4	0.97	685.6	0.93	Age (P=0.02)
	M	179	680.5		678.2		% Body fat (P<0.001)
	H	146	683.0		684.4		

Table XVI-6-11

ENDOCRINE DOSE - COVARIATE INTERACTIONS

	T3	T4	FTI	GLU 2 Hr	TEST
Ranch Hand Officers	No interactions	No interactions	Age-exposure interaction (P=0.042)	% Body fat-exposure interaction (P=0.041)	% Body fat-exposure interaction (P=0.011)
Ranch Hand Flying Enlisted	No interactions	No interactions	% Body fat-exposure interaction (P=0.03)	No interaction	No interactions
Ranch Hand Ground Enlisted	No interactions	No interactions	No interactions	% Body fat-exposure interaction (P=0.09)	No interactions

The FTI shows an age-exposure interaction among the officers and a % body fat-exposure interaction in the flying enlisted Ranch Hand group. Among the officers, FTI increased by 0.0041 per year of life in the low exposure group but decreased by 0.0127 and 0.0079 per year in the medium and high exposure groups respectively. No effect of body fat was suggested by the officer data. Among the flying enlisted, FTI did not appear affected by age, but increased with increasing % body fat in the low and medium exposure groups (0.00295 and 0.00378 per % body fat respectively) while it decreased with body fat (-0.0241 per % body fat) in the high exposure group. These FTI effects are interesting; however, the lack of consistency between occupational and exposure categories leads to doubt that an actual herbicide effect exists.

Both Ranch Hand officers and ground enlisted personnel show comparable body fat-exposure interactions affecting glucose levels. The glucose level-body fat slopes are given in Table XVI-6-12. In both the officer and ground enlisted categories, the low exposed individuals show a decreasing blood glucose with increasing % body fat, but this relationship changes to a positive correlation in the medium and high exposure categories.

Table XVI-6-12

CHANGE IN GLUCOSE LEVEL PER % BODY FAT
(mg/dl PER % BODY FAT)
BY HERBICIDE EXPOSURE LEVEL IN TWO RANCH HAND GROUPS

Exposure Category	Ranch Hand Officers	Ranch Hand Ground Enlisted
Low	+1.18	-0.30
Medium	+2.94	+1.75
High	+1.26	+1.36

A % body fat by exposure interaction is also observed to affect testosterone levels in Ranch Hand officers with a very low probability that the effect could be due to chance ($P = 0.011$). Low exposed officers show a decrease in serum testosterone levels of 4.5 ng/dl per % body fat while medium and high exposed officers show decreases of 16.6 ng/dl and 15.3 ng/dl per % body fat respectively.

3. Summary

The Ranch Hand group was found to differ from the comparison group with respect to proportions of individuals in normal and abnormal thyroid hormone categories. The difference is a tendency toward hyperthyroxinemia which is directionally opposite to what would be expected on the basis of subacute animal studies. On the other hand, decreasing T3 uptakes are associated with advancing age in both groups with the slope being much steeper in the Ranch Hand group. Finally, no meaningful association of thyroid hormone levels with the exposure index were found. Thus, in sum, no definite herbicide effect on thyroid function can be considered demonstrated; however, it also cannot be confidently asserted that a herbicide effect on thyroid function has not occurred. As a group, Ranch Hand personnel have higher testosterone levels than comparison individuals and Ranch Hand officers evidence a decrease in testosterone level with increasing body fat that is related to herbicide exposure category (higher exposures are associated with greater decreases in testosterone with body fat). Since subacute animal studies have shown decreased catabolism of testosterone, higher serum levels could be expected. Thus, this finding in the present study may reflect an herbicide effect, whose long-term impact will require further clinical evaluation.

Overall, Ranch Hand blood glucose levels are not statistically significantly different from those of comparison individuals. However, positive associations of glucose levels with age are greater in the Ranch Hand group than in the comparison group, and in both the Ranch Hand officer and ground enlisted groups significant exposure - body fat interactions exist on glucose levels. Thus, a subtle toxicological effect of herbicide on glucose metabolism may have been detected. It will be important and interesting to follow these groups in time with respect to the incidence of diabetes.