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ANALYSIS OF ORGAN AND BODY WEIGHT
DATA FOR MICE INGESTING TCDD

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FOREWORD

This report was prepared by Colorado State University, Fort Collins, Colorado, under Contract No. F056117490182.

Dr. George M. Angleton, Associate Professor of Radiation Biology and Biostatistics, Colorado State University (CSU) was program manager at CSU for this research program.

Dr. Alvin L. Young was senior scientist and final program manager for the United States Air Force (USAF) for this contract. Dr. John W. Watters was the original program manager for the USAF. Dr. Louis F. Wailly was responsible for initiating the collaborative effort between CSU and the USAF.

ABSTRACT

Randomly trapped mice were divided into two groups of mixed sexes. A control group of 10 mice was dusted with plain alumina gel every three days for 28 days. A test group of 12 mice was treated in a similar manner except the alumina gel contained 2.24 ppb of TCDD. All animals were necropsied on the 29th day. No effects attributable to TCDD were detected in the body weights or in the principal organ weights.

Introduction

The mouse is known for its habit of cleaning itself frequently by licking its fur. Hence, if the coat of the mouse in the field were to become contaminated with a toxic substance, such as TCDD, then the mouse could be expected to ingest this toxic substance as a result of its licking habits.

The short term effects of such a toxic substance ingested in this manner can be studied using an end point such as body weight and organ weights. This report deals with such a study, a study designed to test the null hypothesis that there is no short term effects on body weight or principal organ weights due to the ingestion of trace amounts of TCDD.

Procedures

Mice trapped in a random manner from a field test area were transported to the laboratory, placed in Iso-cages, and maintained on standard laboratory chow. Mice were randomly assigned to one of two treatment groups. Group-I was given the designation of Control Group. Group-II was given the designation of Test Group. Each control animal was dusted with 100 mg. of alumina gel every third day for a period of 28 days for a total of 10 applications. Each test animal was treated in a similar manner except for the fact that the alumina gel contained 2.24 ppb (parts per billion) of TCDD.

All mice were necropsied on the 29th day and weighed. The internal organs were segregated and weighed. The data so obtained are listed in Table 1.

Analyses

Analyses of the data for the final body weights were performed with regard to a linear model using the equation:

$$W = \alpha_1 + \alpha_2 S + \alpha_3 T.$$

where

W = Body Weight

S = Sex (S=1 for males; S=2 for females)

T = Treatment (T=1 for control subjects;
T=2 for test subjects)

The results of these analyses are given in the analysis of variance (ANOVA) table given in Table 2.

Analyses of the data for the organ weights were performed with regard to the linear model

$$W = \alpha_1 + \alpha_2 B + \alpha_3 S + \alpha_4 T + \alpha_5 BT$$

where

W = Organ Weight

B = Body Weight

S = Sex

T = Treatment

BT = Interaction between B and T terms

The results of these analyses are summarized in the ANOVA tables given in Tables 3 through 10.

Summary

In regard to organ weights no significant effects due to interaction between treatment and total body weights were detected. This is equivalent to saying that there was no indication that organ weights were affected differently than total body weights. In regard to both total body weights and organ weights there was in turn no detectable effects attributable to the treatment.

The analyses were performed in such a manner that the data for both sexes were analyzed simultaneously but with differences attributable to sex being removed.

In the analyses of the organ weight data, total body weight was treated as an independent variable. Accordingly differences in organ weight as correlated with differences in body weight were removed from the analysis. Such procedures as the removal of differences due to sex and to total body weight are designed to facilitate the detection of differences attributable to treatment effects.

TABLE 1

BODY WEIGHTS AND ORGAN WEIGHTS FOR EXPERIMENTAL SUBJECTS DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL GROUP) OR ALUMINA GEL CONTAINING 2.24 PPB OF TCDD (TEST GROUP).

Treatment	Sex	Body Weight -gms-	Organ Weights (mg)							
			Heart	Lung	Liver	Spleen	Kidney	Gonads	Thymus	Adrenals
C	F	17.55	156	112	951	26	258	---	15	41
C	F	16.80	112	106	980	24	255	---	54	49
C	F	11.43	92	80	606	14	201	---	19	46
C	M	12.60	115	95	577	10	199	88	19	26
C	F	14.23	132	95	825	20	230	---	11	28
C	M	12.72	75	95	610	10	186	93	18	22
C	M	14.38	81	125	686	13	207	127	12	26
C	M	13.10	130	79	645	20	197	96	12	30
C	M	13.26	100	101	698	19	118	100	18	43
C	M	12.97	158	118	718	14	190	87	18	27
T	F	12.07	98	84	714	17	195	---	11	30
T	M	15.72	144	107	953	37	226	556	33	25
T	M	12.77	122	90	542	20	189	93	20	32
T	M	18.02	156	123	790	25	225	109	21	42
T	M	13.65	105	88	805	19	246	105	15	34
T	M	13.20	119	92	713	24	202	99	13	39
T	M	15.57	127	90	723	33	214	83	27	59
T	F	11.78	117	80	593	17	196	---	14	20

TABLE I, Pg. 2

Treat- ment	Sex	Body Weight -gms-	Heart	Lung	Liver	Spleen	Kidney	Gonads	Thymus	Adrenals
T	F	12.61	101	112	751	14	219	---	15	28
T	F	14.99	126	113	912	14	279	---	10	35
T	F	13.77	123	88	832	9	243	---	13	31
T	M	14.12	116	109	779	22	226	118	11	27

TABLE 2

ANOVA TABLE FOR THE ANALYSIS OF FINAL BODY WEIGHT DATA FOR MICE DUSTED WITH ALUMINA GEL CONTAINING 2.24 PPB OF TCDD

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Test Statistic -F _s -	Level of Significance P{F>F _s }
Observations	22	4,361.4335			
Model	3	4,292.8250			
Intercept	1	4,292.7016			
Sex (S)	1	0.0447	0.0447	0.012	0.914
Body Weight (B)	1	0.0786	0.0782	0.022	0.884
Error	19				

$$\text{Model } B = \alpha_1 + \alpha_2 S + \alpha_3 T$$

Sequential Conditional Hypotheses

$H_{01}: \alpha_3 = 0$, no effect due to treatment, accepted at the 0.05 level of significance

$H_{02}: \alpha_2 = 0$, no effect due to sex, accepted at the 0.0t level of significance.

TABLE 3

ANOVA TABLE FOR THE ANALYSIS OF HEART WEIGHT FOR MICE DUSTED WITH ALUMINA GEL CONTAINING 2.24 PPB OF TCDD

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Test Statistic -F _s -	Level of Significance P{F>F _s }
Observations	22	319,269.00			
Model	5	311,988.42			
Intercept	1	308,455.68			
Body Weight (B)	1	3,301.26	3.301.26	7.708	0.013
Sex (S)	1	5.29	5.29	0.012	0.914
Treatment (T)	1	151.30	151.30	0.353	0.560
Interaction (BT)	1	74.89	74.89	0.174	0.681
Error	17	7,280.58	428.20		

Model: $H = \alpha_1 + \alpha_2 B + \alpha_3 S + \alpha_4 T + \alpha_5 BT$

Sequential Conditional Hypotheses

$H_{01}: \alpha_5 = 0$; no effect due to body-weight with treatment interaction, accepted at the 0.05 level of significance

$H_{02}: \alpha_4 = 0$; no effect due to treatment, accepted at the 0.05 level of significance

$H_{03}: \alpha_3 = 0$; no effect due to sex, accepted at the 0.05 level of significance

$H_{04}: \alpha_2 = 0$; no effect due to body weight, rejected at the 0.013 level of significance

TABLE 4

ANOVA TABLE FOR THE ANALYSIS OF LUNG WEIGHT DATA FOR MICE DUSTED WITH ALUMINA GEL CONTAINING 2.24 PPB OF TCDD

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Test Statistic -F _s -	Level of Significance P{F>F _s }
Observation	22	220,626.0000			
Model	5	218,023.2542			
Intercept	1	216,414.7273			
Body Weight (B)	1	1,476.9833	1,476.9833	9.65	0.006
Sex (S)	1	78.1148	78.1148	0.51	0.485
Treatment (T)	1	51.8922	51.8922	0.34	0.567
Interaction (BT)	1	1.5366	1.5366	0.01	0.922
Error	17	2,602.7458	153.1027		

Model: $L = \alpha_1 + \alpha_2 B + \alpha_3 S + \alpha_4 T + \alpha_5 BT$

Sequential Conditional Hypotheses

$H_{01}: \alpha_5 = 0$; there exists no significant effect due to body-weight and treatment interaction: Accepted, at the 0.05 level of significance

$H_{02}: \alpha_4 = 0$; there exists no significant effect due to treatment: Accepted, at the 0.05 level of significance

$H_{03}: \alpha_3 = 0$; there exists no significant effect due to sex differences: Accepted, at the 0.05 level of significance

$H_{04}: \alpha_2 = 0$; there exists no significant effect due to body weight: Rejected at the 0.01 level of significance

TABLE 5

ANOVA TABLE FOR THE ANALYSIS OF LIVER WEIGHT DATA FOR MICE DUSTED WITH ALUMINA GEL CONTAINING 2.24 PPB OF TCDD

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Test Statistic -F _s -	Level of Significance P{F>F _s }
Observations	22	12,568,491.			
Model	5	12,465,795.			
Intercept	1	12,229,927.			
Body Weight (B)	1	188,584.	188,584	31.22	<0.001
Sex (S)	1	43,212.	43,212	7.15	0.016
Treatment (T)	1	2,532.	2,532	0.42	0.526
Interaction (BT)	1	1,538.	1,538	0.25	0.623
Error	17	102,695.	6,040		

Model: $L = \alpha_1 + \alpha_2 B + \alpha_3 S + \alpha_4 T + \alpha_5 BT$

Sequential Conditional Hypotheses

$H_{01}: \alpha_5 = 0$; no effect due to body-weight with treatment interaction, accepted at the 0.05 level of significance

$H_{02}: \alpha_4 = 0$; no effect due to treatment, accepted at the 0.05 level of significance

$H_{03}: \alpha_3 = 0$; no effect due to sex, rejected at the 0.016 level of significance

$H_{04}: \alpha_4 = 0$; no effect due to body weight, rejected at the 0.001 level of significance

TABLE 6

ANOVA TABLE FOR THE ANALYSIS OF SPLEEN WEIGHT DATA FOR MICE DUSTED WITH ALUMINA GEL CONTAINING 2.24 PPB OF TCDD

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Test Statistic -F _s -	Level of Significance P{F>F _s }
Observations	22	9,129.00			
Model	5	8,578.22			
Intercept	1	8,056.41			
Body Weight (B)	1	386.11	386.11	11.92	0.003
Sex (S)	1	48.60	48.60	1.50	0.237
Treatment (T)	1	74.33	74.33	2.29	0.149
Interaction (BT)	1	12.79	12.79	0.39	0.541
Error	17	550.78	32.40		

$$\text{Model: } S = \alpha_1 + \alpha_2 B + \alpha_3 S + \alpha_4 T + \alpha_5 BT$$

Sequential Conditional Hypotheses

- H₀₁: $\alpha_5 = 0$; no effect due to body-weight with treatment interaction, accepted at the 0.05 level of significance
- H₀₂: $\alpha_4 = 0$; no effect due to treatment, accepted at the 0.05 level of significance
- H₀₃: $\alpha_3 = 0$; no effect due to sex, accepted at the 0.05 level of significance
- H₀₄: $\alpha_2 = 0$; no effect due to body weight, rejected at the 0.003 level of significance

TABLE 7

ANOVA TABLE FOR THE ANALYSIS OF KIDNEY WEIGHT DATA FOR MICE DUSTED WITH ALUMINA GEL CONTAINING 2.24 PPB OF TCDD

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Test Statistic -F _s -	Level of Significance P{F>F _s }
Observations	22	1,027,675.00			
Model	5	1,017,622.43			
Intercept	1	1,004,518.23			
Body Weight (B)	1	7,046.30	7,046.30	11.916	0.003
Sex (S)	1	4,685.37	4,685.37	7.923	0.012
Treatment (T)	1	1,371.75	1,371.75	2.320	0.146
Interaction (BT)	1	0.78	0.78	0.001	0.975
Error	17	10,052.57	591.33		

Model: $K = \alpha_1 + \alpha_2 B + \alpha_3 S + \alpha_4 T + \alpha_5 BT$

Sequential Conditional Hypotheses

H₀₁: $\alpha_5 = 0$; no effect due to body-weight with treatment interaction, accepted at the 0.05 level of significance

H₀₂: $\alpha_4 = 0$; no effect due to treatment, accepted at the 0.05 level of significance

H₀₃: $\alpha_3 = 0$; no effect due to sex, rejected at the 0.012 level of significance

H₀₄: $\alpha_2 = 0$; no effect due to body weight, rejected at the 0.003 level of significance

TABLE 8

ANOVA TABLE FOR THE ANALYSIS OF GONAD WEIGHT DATA FOR MICE DUSTED WITH ALUMINA GEL CONTAINING 2.24 PPB OF TCDD

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Test Statistic -F _s -	Level of Significance P{F>F _s }
Observations	13	430,612.00			
Model	4	263,353.38			
Intercept	1	236,655.08			
Body Weight (B)	1	24,274.02	24,274.02	1.31	0.282
Treatment (T)	1	2,423.08	2,423.08	0.13	0.727
Interaction (BT)	1	1.61	1.61	0.00 [†]	0.993
Error	9	167,258.21	18,584.25		

Model: $G = \alpha_1 + \alpha_2 B + \alpha_3 T + \alpha_4 BT$

Sequential Conditional Hypotheses

$H_{01}: \alpha_4 = 0$; no effect due to body-weight with treatment interaction, accepted at the 0.05 level of significance

$H_{02}: \alpha_3 = 0$; no effect due to treatment, accepted at the 0.05 level of significance

$H_{03}: \alpha_2 = 0$; no effect due to body weight, accepted at the 0.05 level of significance

TABLE 9

ANOVA TABLE FOR THE ANALYSIS OF THYMUS WEIGHT FOR MICE DUSTED WITH ALUMINA GEL CONTAINING 2.24 PPB OF TCDD

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Test Statistic -F _s -	Level of Significance P{F>F _s }
Observations	22	9,229.0000			
Model	5	7,670.9750			
Intercept	1	7,236.4090			
Body Weight (B)	1	378.6052	378.6052	4.13	0.058
Sex (S)	1	0.0013	0.0013	<0.01	<0.922
Treatment (T)	1	47.8972	48.8972	0.52	0.481
Interaction (BT)	1	8.0623	8.0623	0.09	0.768
Error	17	1,558.0250	91.6485		

Model: $T = \alpha_1 + \alpha_2 B + \alpha_3 S + \alpha_4 T + \alpha_5 BT$

Sequential Conditional Hypotheses

$H_{01}: \alpha_5 = 0$; no effect due to body-weight with treatment interaction, accepted at the 0.05 level of significance

$H_{02}: \alpha_4 = 0$; no effect due to treatment, accepted at the 0.05 level of significance

$H_{03}: \alpha_3 = 0$; no effect due to sex, accepted at the 0.05 level of significance

$H_{04}: \alpha_2 = 0$; no effect due to body weight, accepted at the 0.05 level of significance

TABLE 10

ANOVA TABLE FOR THE ANALYSIS OF ADRENAL WEIGHT DATA FOR MICE DUSTED WITH ALUMINA GEL CONTAINING 2.24 PPB OF TCDD

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Test Statistic -F _s -	Level of Significance P F F _s
Observations	22	26,866.00			
Model	5	25,334.71			
Intercept	1	24,890.90			
Body Weight (B)	1	393.32	393.32	4.37	0.052
Sex (S)	1	7.80	7.80	0.09	0.768
Treatment (T)	1	1.99	1.99	0.02	0.889
Interaction (BT)	1	40.68	40.68	0.45	0.511
Error	17	1,531.28	90.07		

Model: $A = \alpha_1 + \alpha_2 B + \alpha_3 S + \alpha_4 T + \alpha_5 BT$

Sequential Conditional Hypotheses

$H_{01}: \alpha_5 = 0$; no effect due to body-weight with treatment interaction, accepted at the 0.05 level of significance

$H_{02}: \alpha_4 = 0$; no effect due to treatment, accepted at the 0.05 level of significance

$H_{03}: \alpha_3 = 0$; no effect due to sex, accepted at the 0.05 level of significance

$H_{04}: \alpha_2 = 0$; no effect due to body weight, accepted at the 0.052 level of significance