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ANALYSIS OF TOTAL 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

Mature mice were dusted every three days for 30 days with an alumina gel containing either 0.00 ppb or 2.24 ppb of TCDD. The control group (0.00 ppb) showed a slight weight gain while the test group (2.24 ppb) showed a slight weight loss. The final weight of the test group was 4.5 percent less than that projected on the basis of the weight change in the control group. However, this difference was not significant at the five percent level of significance.

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. 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 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.

Animals were weighed on the first day of the study, the day of the first application, and then on the 29th day of the study, the day after the last application.

The data so obtained are listed in Table I.

Analyses

Analyses were performed with respect to the absolute changes in weights over the 29 day test period. The average weight change for the control group, Group I, was +0.17 grams and for the test group -0.45 grams. Thus,

$$\hat{\mu}_1 = +0.17 \text{ grams,}$$

$$\hat{\mu}_2 = -0.45 \text{ grams.}$$

where μ_1 is expected weight change for the population of control subjects and μ_2 is the expected weight change for the population of test subjects.

Analysis of variance techniques were then utilized to test hypotheses concerning the significance of these estimates of μ_1 and μ_2 . The results of these analyses are summarized in Table II.

The first hypothesis to be evaluated was that the variance σ_1^2 of the observations represented by the data in Group I was the same as the variance σ_2^2 for the observations represented by the data of Group II. The F test statistic, F_S , so calculated for this test was 3.75 which corresponds to a level of significance α of 0.04 (4%).

That is:

$$\begin{array}{ll} H_0: \sigma_1^2 = \sigma_2^2 & F_S = 3.75 \\ H_A: \sigma_1^2 \neq \sigma_2^2 & \alpha = 0.04 \end{array}$$

For this type of test the level of significance should be less than 0.01 (1%). Since α was greater than 0.01 the plausibility of the hypothesis of equal variances was sufficiently high so as to tentatively permit the acceptance of the null hypothesis H_0 . This being the case, then a pooled estimate of variance was obtained which had 20 degrees of freedom.

That is:

$$\hat{\sigma}^2 = 0.9750.$$

The pooled estimate may thus be used to test the hypotheses concerning μ_1 and μ_2 .

The first of these hypotheses is that μ_1 and μ_2 are simultaneously equal to zero. That is that the mean weight changes in both cases did not individually and simultaneously differ significantly from zero. The hypothesis and the results of the test are as follows:

$$\begin{array}{ll} H_0: \mu_1 = 0 \wedge \mu_2 = 0 & F_S = 1.40 \\ H_A: \mu_1 \neq 0 \text{ or } \mu_2 \neq 0 & \alpha = 0.26 \end{array}$$

The level of significance for the test of this joint hypothesis is 0.26 (26%). Accordingly the plausibility of the hypothesis was sufficiently high so as to lead to the acceptance of H_0 .

The possibility remains though that while μ_1 and μ_2 may not differ significantly from zero their difference does. In this case this is an

important consideration since $\hat{\mu}_1$ was positive and $\hat{\mu}_2$ was negative. The results of the test of this hypothesis as given in Table II are as follows:

$$\begin{array}{ll} H_0: \mu_1 - \mu_2 = 0 & F_S = 2.16 \\ H_A: \mu_1 - \mu_2 \neq 0 & \alpha = 0.16 \end{array}$$

The level of significance for this test was thus 0.16 (16%). Again it was not sufficiently close to the 0.05 (5%) or 0.01 (1%) levels of significance so as to consider rejection of H_0 . Accordingly, the plausibility of the hypothesis was high and the hypothesis was accepted.

The third hypothesis is of little interest here. It merely states that the sum of μ_1 and μ_2 does not differ significantly from zero. Thus,

$$\begin{array}{ll} H_0: \mu_1 + \mu_2 = 0 & F_S = 0.65 \\ H_a: \mu_1 + \mu_2 \neq 0 & \alpha = 0.43. \end{array}$$

As before, the plausibility of this hypothesis was found to be high and hence was not rejected.

Summary

The short term effects of ingested TCDD on change in mouse weights over a thirty day period as evaluated from this study are such as to lead to an estimated weight loss of about 0.62 grams or 4.5% in the adult mouse. However, this change was not significant at the 0.05 (5%) level of significance; namely, it became significant at the 0.16 (16%) level of significance. Accordingly, there existed no basis for not accepting the null hypothesis of no effect due to TCDD as considered in the text of this study.

TABLE I

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

CONTROL GROUP WEIGHTS (GMS)			TEST GROUP WEIGHTS (GMS)		
Initial	Final	Difference	Initial	Final	Difference
17.06	17.55	+0.44	12.69	12.07	-0.62
13.50	16.80	+3.30	16.10	15.72	-0.38
11.00	11.43	+0.43	13.12	12.77	-0.35
13.40	12.60	-0.80	17.15	18.02	+0.87
15.25	14.23	-1.02	13.71	13.65	-0.06
12.50	12.72	+0.22	14.48	13.20	-1.28
14.01	14.38	+0.37	14.90	15.57	+0.67
13.12	13.10	-0.02	12.36	11.78	-0.58
14.10	13.26	-0.84	14.03	12.61	-1.42
13.40	12.97	-0.43	16.00	14.94	-1.01
			13.90	13.77	-0.13
			15.25	14.12	-1.13

TABLE II

ANALYSIS OF VARIANCE TABLE FOR TESTING HYPOTHESES OF NO EFFECTS DUE TO TCDD

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	Test Statistic F_s	Significance Level $P\{F > F_s\}$
Observations	22	22.2374			
Group I	10	14.0716			
Group II	12	8.1658			
Hypothesis-1 ($\mu_1 + 0 \wedge \mu_2 + 0$)	2	2.7370	1.3685	1.40	0.26
Hypothesis-2 ($\mu_1 \neq \mu_2$)	1	2.1080	2.1080	2.16	0.16
Hypothesis-3 ($\mu_1 + \mu_2 = 0$)	1	0.6290	0.6290	0.65	0.43
Error	20	19.5004	0.9750		
Group I	9	13.7826	1.9500	3.75	0.04*
Group II	11	5.7178	0.5198		

* Two tail