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MARCUS, 1998

OFFICE OF
RESEARCH AND DEVELOPMENT

December 28, 1998

MEMORANDUM

SUBJECT: Statistical Analyses of Standard Histopathological Measures of Thyroid Hypertrophy and Follicular Lumen Size Decrease in PND5 Rats

FROM: Allan H. Marcus, EMAG/NCEA-RTP (MD-52)

TO: Annie Jarabek, HPAG/NCEA-RTP (MD-52)

Attached is a set of statistical analyses of the histology data, provided as severity scores for both histology measures individual animals, that I received from you as a telefax from WPAFB (AFRL/HESD). A copy of these data is appended to the memo.

The construction of a data base for categorical data analyses from the raw data in the fax was straightforward, and is available as SYSTAT data sets for further analyses. I can also export the data to standard spreadsheets, if needed. They are shown as Tables 1 to 6 in the attached memo. I understand that these analyses are based on data in the Argus rat developmental neurotoxicology study (Argus, 1998a). The contingency table tests of association are straightforward and described in most elementary statistics texts. The logistic regression analyses in this version of SYSTAT used the iteratively reweighted least squares approach to maximum likelihood estimation described on p. 622 of the SYSTAT v. 5.0 manual (1995). These are very simple approaches, easily understood by most non-specialists. Further analyses using categorical regression methods may also be informative.

The sample sizes are on the small side for testing hypotheses. For that reason, the findings of marginal or statistically significant associations in the contingency table tests at 0.1 and 1 mg/kg-day are worrying, given that the study has small power to detect real effects of only modest magnitude. The logistic regression models are consistent with a steeper dose-response function at low doses than at high doses. The evidence as a whole leans toward a significant response at doses as low as 0.1 to 1 mg/kg-day. A larger study to look at these lower dose ranges would seem to be justified.

Attachment

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Statistical Analyses of Standard Histopathological Measures of Thyroid Hypertrophy and Follicular Lumen Size Decrease in PND5 Rats

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National Center for Environmental Assessment – RTP

1. DATA STRUCTURE AND PURPOSE OF THE ANALYSES

The purpose of the analyses was to provide an assessment of possible trends in toxicity data provided to me by Annie Jarabek, based on the rat neurodevelopmental study data for pups postnatal on day 5 (PND5), reported in (Argus, 1998a). There were two toxicity endpoints: (1) Follicular epithelial cell hypertrophy (denoted HYPER), and (2) decrease in follicular lumen size (denoted SIZE). Both were coded on a discrete scale of increasing seriousness, as 0, 1, 2 for HYPER and 0, 1, 2, 3 for SIZE. There were separate studies for females and for males, so SEX was also a discrete variable. Each set of experiments was done at 5 dose levels: control (0 mg/kg-day), 0.1, 1, 3, and 10 mg/kg-day. DOSE effects could be evaluated either as an ordered categorical scale or as a numeric scale. Including DOSE as an ordered categorical scale allowed use of contingency table methods, whereas use of DOSE or log(DOSE) as a numeric scale allowed use of logistic regression models. These provide different but complementary information about the relationship, using elementary analytical methods.

2. TESTING ASSOCIATION IN CATEGORICAL RESPONSE DATA

The individual rat data were combined into contingency tables and entered into the SYSTAT (1995) data analysis system. The basic data tables are shown below, along with the results for tests of association with DOSE in a table with r rows and c columns as shown. The first set of tests was done by likelihood ratio tests for each sex and for both sexes, for both endpoints. We use Fisher's symbols: * for 0.01<P<0.05, ** for 0.001<P<0.01, *** for P<0.001.

TABLE 1

HYPERTROPHY, FEMALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	4	1	1
0.1	3	2	1
1	1	2	3
3	3	2	1
10	0	4	1

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN FEMALES: 0.026*
DF=8

TABLE 2

HYPERTROPHY, MALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	5	1	0
0.1	1	4	1
1	2	3	1
3	1	4	1
10	0	2	4

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN MALES: 0.218 DF=8

TABLE 3

HYPERTROPHY, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	9	2	1
0.1	4	7	2
1	3	5	4
3	4	6	2
10	0	6	5

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION: 0.012*, DF=8

TABLE 4
SIZE, FEMALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, FEMALE / DOSE, mg/kg-day	LEVEL 0	1	2	3
0	2	3	1	0
0.1	1	3	2	0
1	1	4	1	0
3	1	1	2	2
10	0	2	3	1

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN FEMALES: 0.218, DF=12

TABLE 5
SIZE, MALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, MALE DOSE, mg/kg-day	LEVEL 0	1	2	3
0	4	1	1	0
0.1	1	3	2	0
1	1	1	4	0
3	0	2	4	0
10	0	0	3	3

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN MALES: 0.007**, DF=12

TABLE 6
SIZE, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, ALL DOSE, mg/kg-day	LEVEL 0	1	2	3
0	6	4	2	0
0.1	2	6	4	0
1	2	5	5	0
3	3	3	6	2
10	0	3	6	4

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN ALL SEXES: 0.008**, DF=12

Exact Fisher tests were performed on reduced 2 by 2 tables, using DOSE level 0.1 and 1 mg/kg-day vs. controls to see if there was a significant difference at low doses. Tests of the controls against the highest 2 doses were significant and are not shown here. The low-dose tests for HYPER used a combined HYPER score of 1+2 to combine the more serious effects These tables were then combined into single tables for the purpose of providing a concise display of the results. We have defined an additional symbol # for 0.05<P<0.10, or P < 0.05 one-tailed.

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TABLE 7
2 BY 2 CONTINENCY TABLE TESTS FOR HYPERTROPHY AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
HYPER LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	4	2	5	1	9	3
DOSE 0.1	3	3	1	5	4	8
P VALUE	1.0		0.080#		0.100#	

TABLE 8
2 BY 2 CONTINENCY TABLE TESTS FOR HYPERTROPHY AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
HYPER LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	4	2	5	1	9	3
DOSE 1.0	1	5	2	4	3	9
P VALUE	0.242		0.242		0.039*	

The 2 by 2 tests for SIZE effects required a more detailed level of the aggregated SIZE categories. We show separate results for category 0 vs. 1+2, and categories 0+1 vs. 2. Category 3 had no counts at dose levels 0, 0.1 and 1.

TABLE 9
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE EFFECT AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	2	4	4	2	6	2
DOSE 0.1	1	5	1	5	2	10
P VALUE	0.242		0.242		0.193	

TABLE 10
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0+1	2	0+1	2	0+1	2
DOSE 0	5	1	5	1	10	2
DOSE 0.1	4	2	4	2	8	4
P VALUE	0.242		0.242		0.640	

TABLE 11
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	2	4	4	2	6	6
DOSE 1	1	5	1	5	2	10
P VALUE	0.242		0.242		0.193	

TABLE 12
2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0+1	2	0+1	2	0+1	2
DOSE 0	5	1	5	1	10	2
DOSE 1	5	1	2	4	7	5
P VALUE	1.0		0.242		0.371	

3. LOGISTIC REGRESSION ANALYSIS

As a check on the overall relationship, we also carried out logistic regression analyses of response vs. dose and vs. log(dose), for males and females separately and for both sexes combined. The dose for controls was taken as 0, and log(dose) as log(0.01 mg/kg-day). The results are shown in the following tables.

TABLE 13
LOGISTIC REGRESSION COEFFICIENT OF HYPERTROPHY > 0 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.332	0.210	-16.90
MALE	0.614	0.397	-14.78
ALL	0.423*	0.192	-32.06

TABLE 14
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 0 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.335	0.313	-12.31
MALE	1.734	1.187	-10.68
ALL	0.614	0.378	-22.30

TABLE 15
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 1 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.198#	0.109	-18.34
MALE	0.635#	0.339	-15.15
ALL	0.279***	0.097	-35.66

TABLE 16
LOGISTIC REGRESSION COEFFICIENT OF HYPERTROPHY > 0 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.342*	0.174	-17.08
MALE	0.532**	0.207	-13.95
ALL	0.426***	0.132	-31.49

TABLE 17
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 0 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.269	0.205	-12.60
MALE	0.704**	0.284	-10.02
ALL	0.459***	0.166	-22.07

TABLE 18
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 1 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.330#	0.179	-18.20
MALE	0.572**	0.208	-15.20
ALL	0.430***	0.132	-34.86

The relationship between non-transformed dose and hypertrophy is statistically significant in both sexes combined, and positive but not significant in either sex separately. The relationship with the logarithm of dose is significant or very significant in all analyses. This suggests that the risk of a hypertrophic response increases as (roughly) the 0.3 to 0.5 power of dose. Since the dose-response function is nonlinear with a steeper slope near the origin, the possibility of significant responses at low doses is consistent with the contingency table tests.

The regression coefficients of any size > 0 vs. untransformed dose are positive but not significant, whereas after log-transformation, the effects for males and for both sexes are very significant. If the severity cutpoint for SIZE is taken as levels 2+3 vs. levels 0+1, then the relationship with dose is marginally significant in either sex and highly significant when sexes are combined. The effects for males and for both sexes combined are highly significant in the model for log of dose, which also suggests that the SIZE response probability at low doses increases as roughly the 0.3 to 0.5 power of dose.

Additional logistic regression models explored the possibility of a dose-sex interaction, with males having a steeper dose-response curve. No statistically significant gender effect was found, but it is unlikely that these small samples allow sufficient power to detect this effect.

4. SUMMARY

There appears to be strong evidence for a dose-response relationship between perchlorate dose and both endpoints, follicular epithelial cell hypertrophy and decrease in follicular lumen size. Even though the number of rats in each treatment group is smaller than is desirable to have substantial power against real effects of modest size at the two lowest dose levels, attention should be paid to the simple comparisons in Tables 7 and 8, which suggest a marginally significant increase in hypertrophy for males at 0.1 mg/kg-day, and an effect for both groups combined at both 0.1 (marginal) and 1 mg/kg-day (significant). Even here, one should note that the differences lie in the expected direction if there is a real dose-response relationship. Although there may be a dose-sex interaction, with males showing stronger effects than females, this was not significant, and combining the sexes gave evidence for an effect on follicular epithelial cell hypertrophy.

Similar analyses did not find a significant decrease in follicular lumen cell size at the lowest two levels using the very basic contingency table tests. More detailed evaluation is recommended, such as categorical regression analyses, or tests analogous to Williams' test. However, the logistic regression models suggested that there is a very significant dose response relationship overall, with a strong model-based suggestion of a steeper dose-response relationship for lumen cell size at lower doses.

Taking the small samples sizes and limited power of these data into account, there is an indication of increased effects at levels as low as 0.1 to 1 mg/kg-day, particularly for the follicular epithelial cell hypertrophy in males.

5. REFERENCES

1. Argus, 1998a. A neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to rats [report ammendment: July 27, 1998]. Argus Research Laboratories, Inc., Horsham, PA. Argus Protocol #1613-002,

2. Wilkinson, L. SYSTAT: The System for Statistics. SYSTAT Inc., Evanston, Il., 1995.

Appendix: Data as received by telefax.

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SUBJECT	AMMONIUM PERCHLORATE THYROID DATA

Comments:

Annie:

Hope this meets your needs. If not, get back with me so we can you what you need.

Bill Baker

TABLE 1 [illegible]

Male Data

Grp1 - 10.0 mg/kg								
A		Hypertrophy	Size					
57		2	3					
58		2	2					
60		1	3					
62		1	3					
63		2	2					
65		2	2					
Mean		1.67	2.5					
StDev		0.51639778	0.547723					
Grp 2 - 3.0 mg/kg								
A		Hypertrophy	Size					
67		1	2					
68		1	2					
69		1	1					
71		0	1					
72		1	2					
74		2	2					
Mean		1	1.67					
StDev		0.63245553	0.516398					
Grp 3 - 1.0 mg/kg								
A		Hypertrophy	Size					
75		2	2					
76		1	1					
77		0	2					
80		1	2					
82		1	2					
84		0	0					
Mean		0.83	1.5					
StDev		0.75277265	0.83666					
Grp 4 - 0.1 mg/kg								
A		Hypertrophy	Size					
85		0	0					
86		1	1					
87		1	1					
90		1	2					
91		2	2					
92		1	1					
Mean		1	1.17					
StDev		0.63245553	0.752773					
Grp - Control								
A		Hypertrophy	Size					
95		0	0					
96		0	0					
97		0	0					
98		0	0					
100		0	1					
101		1	2					
Mean		0.17	0.5					
StDev		0.40824829	0.83666					

Female Data

Grp1 - 10.0 mg/kg								
B		Hypertrophy	Size					
58		1	2					
59		1	1					
61		1	2					
64		2	2					
65		1	1					
66		1	3					
Mean		1.17	1.83					
StDev		0.40824829	0.752773					
Grp 2 - 3.0 mg/kg								
B		Hypertrophy	Size					
68		0	3					
70		0	0					
71		1	2					
72		0	3					
73		1	1					
74		2	2					
Mean		0.67	1.83					
StDev		0.81649658	1.169045					
Grp 3 - 1.0 mg/kg								
B		Hypertrophy	Size					
78		1	2					
79		1	1					
80		2	1					
81		2	1					
82		2	1					
83		0	0					
Mean		1.33	1					
StDev		0.81649658	0.632456					
Grp 4 - 0.1 mg/kg								
B		Hypertrophy	Size					
85		0	1					
87		0	0					
88		0	2					
89		1	1					
93		1	1					
94		2	2					
Mean		0.67	1.17					
StDev		0.81649658	0.752773					
Grp - Control								
B		Hypertrophy	Size					
96		2	2					
97		0	1					
98		1	1					
99		0	1					
101		0	0					
102		0	0					
Mean		0.5	0.83					
StDev		0.83666003	0.752773					