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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

005926

JUN - 4 1987

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCESMEMORANDUM

SUBJECT: Linuron Data Call-In, Statistical Reanalysis of Hematological Data from Rat Chronic Study; Caswell 528; EPA I.D. # 035506; Project 7-0509; Record No. 192059

TO: Mark Boodee, Review Manager  
Special Review Branch (TS-767C)  
and  
Robert Taylor, PM #25  
Registration Division (TS-767C)

FROM: James N. Rowe, Ph.D.  
Section V, Toxicology Branch  
Hazard Evaluation Division/HED (TS-769C) *James N. Rowe 6/2/87*

THRU: Quang Q. Bui, Ph.D., D.A.B.T. *Quang Bui 6/3/87*  
Acting Section Head, Section V  
Toxicology Branch/HED (TS-769C) *WBB 6/4/87*  
and  
Theodore M. Farber, Ph.D.  
Chief, Toxicology Branch/HED (TS-769C)

ACTION: Review of du Pont submission of statistical reanalysis of hematological data from previous chronic rat study with linuron; Caswell 528; EPA I.D. # 035506; Project 7-0509; Record no. 192059

RECOMMENDATIONS:

The NOEL for hematotoxicity, based on statistical analysis of percent Hb, is generally concluded to be 625 ppm for male rats and 50 ppm in female rats. These NOELs are in general agreement with hematotoxicity NOELs set for male and female rats based on data from a three generation reproductions study (see memo from J.Rowe to I. Surzenauer; Aug. 11, 1986; Acc. # 26053; EPA # I.D. 035506). In that review, a NOEL for hematological parameters of 625 ppm was determined (based on the lack of any significant effects) in F<sub>2b</sub> male rats. In F<sub>2b</sub> female rats, a NOEL of 25 ppm for hematotoxicity was determined (based on depressed RBC counts, Hb concentration, and hematocrit in the mid and high dose groups after 20 and 22 months treatment and a statistically significant depression in atypical lymphocytes in the mid and high dose groups at 22 months of treatment).

It is concluded that a NOEL for general hematotoxicity has been established in the 2-year rat chronic study and an additional two-year study is not required to further define these effects. The registrant has fulfilled the regulatory requirements for a chronic study in rodents.

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DATA EVALUATION RECORD

BACKGROUND:

A rat chronic study was previously submitted and reviewed by the Agency (J.W. Holder; Linuron Toxicology Chapter for Registration Standard; 9/15/82). Based on data gaps noted in the Registration Standard, the registrant submitted data regarding blood pigments and hematology data, in addition to the present submission. The hematology submission was meant to clarify the issue of a NOEL for general hematology effects observed in chronic exposure of rats to linuron in a three-generation reproduction test (see Accession #s 26053 and 259185). The blood pigment data (sulf- and methemoglobin following dietary exposure) were required in the Registration Standard for certain substituted phenyl urea compounds such as linuron. The original re-analysis was intended to show that a NOEL for general hematology had been established in the two-year rat study, and to support the Company's position that an additional chronic rat study was unnecessary.

The submitted re-analysis of the hematological data was initially evaluated by Ms. Bernice Fisher of the Biostatistics Team of the Toxicology Branch at this reviewer's request. It was determined that the Company's re-analysis failed to evaluate the total data set over the time of the study and an additional analysis was suggested to du Pont's statistician, Jay Graepl. Based upon the findings of the additional company analysis of the hematology data from the rat chronic study, a final EPA recommendation for the necessity of a repeat of the two-year rat study was to be made.

NEW SUBMISSION

At this reviewer's request, the Biostatistics team/Scientific Mission Support Staff (Toxicology Branch) has evaluated the latest company submission for the hematotoxicity of linuron in the rat chronic study using per cent hemoglobin (% Hb) as an indicator of any potential hematological effects (see attachment).

The findings of the Biostatistics team were as follows:

- 1) in males, the overall dose comparisons, as they relate to time, were not significantly different, nor were there statistically significant differences between controls and treated animals with pairwise comparisons.
- 2) in females, the time factor interaction was highly significant ( $p < 0.01$ ), and thus, the overall comparisons of dose were inappropriate; pairwise comparisons (Duncan's test) of the 6-month and 12-month data vs the controls indicated statistically significant differences for % Hb (6-mo: 15.22 g%/control vs 14.17 g%/625 ppm; 12-mo: 15.52 g%/control vs 14.93 g%/125 ppm and 625 ppm g%/625 ppm;  $p < 0.05$ ).

Thus, the NOEL, based on percent Hb, for hematotoxicity may be generally concluded to be 625 ppm for males and 50 ppm in females. These NOELs are in general agreement with hematotoxicity NOELs set for male and female rats based on data from a three generation reproductions study (see memo from J.Rowe to I. Sunzenauer; Aug. 11, 1986; Acc. # 26053; EPA # I.D. 035506). In that review, a NOEL for hematological parameters of 625 ppm was determined (based on the lack of any significant effects) in F<sub>2b</sub> male rats. In F<sub>2b</sub> female rats,

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a NOEL of 25 ppm for hematotoxicity was determined (based on depressed RBC counts, Hb concentration, and hematocrit in the mid and high dose groups after 20 and 22 months treatment and a statistically significant depression in atypical lymphocytes in the mid and high dose groups at 22 months of treatment).

It is concluded that a NOEL for general hematotoxicity has been established in the 2-year rat chronic study and an additional two-year study is not required to further define these effects. The registrant has fulfilled the regulatory requirements for a chronic study in rodents.

ATTACHMENT



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
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OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Linuron Rat Study - Evaluation of  
Hematological Measure - HB (g %)

Caswell No. 528

FROM: Bernice Fisher, Biostatistician  
Scientific Mission Support Staff  
Toxicology Branch  
Hazard Evaluation Division (TS-769C)

*Bernice Fisher 5/21/87*

TO: James N. Rowe, Ph.D.  
Section V, Toxicology Branch  
Hazard Evaluation Division (TS-769C)

THRU: Richard Levy, M.P.H., Team Leader - Biostatistics  
Scientific Mission Support Staff  
Toxicology Branch  
Hazard Evaluation Division (TS-769C)

*Richard Levy 5-21-87*

and

Reto Engler, Ph.D., Chief  
Scientific Mission Support Staff  
Toxicology Branch  
Hazard Evaluation Division (TS-769C)

*Reto Engler*

On the basis of the memorandum on Linuron, Comments on Statistical Reevaluation of Blood Data from the 2-year Rat Study (B. Fisher and B. Litt December 3, 1986), Dr. Rowe suggested that a reanalysis with time periods up to 12 months would be sufficient to establish any potential compound-related hemotoxicity. Thus, the previous problem of animal substitutions in the 2-year time period would be eliminated since during the first year, the hemotological readings were based on the same set of 10 animals at 3, 6, and 12-month time intervals. In addition, Dr. Rowe suggested that the reevaluation of the hemoglobin (g %) measures alone would be sufficient to identify hemotoxicity.

Consequently, this selected blood compound measure in both sexes was evaluated by means of a SAS-ANOVA computer program.

The structure of this model for ANOVA analysis assumed that the dose and time variables were fixed conditions, and that the number of animals (10) in each dose-time category represented repeat independent measurements (which were considered to be a nested effect sample).

It was also assumed that DuPont conducted this study by taking and measuring blood components for all dose levels at each time period in a random manner, so that a day-to-day time bias would not be inadvertently built into the values.

The results of the ANOVA analysis of hemoglobin measures (g %) in both sexes indicated that time was an important effect, regardless of dose levels (see Graph 1 and 2).

In males, the overall dose comparisons, confounded by time, were not significantly different. In addition, the pairwise comparisons of control with each of the dose levels, made by the use of the Least Significant Difference method, also showed no significant differences (see Table 1 for details).

In females, the time factor interaction with dose was highly significant ( $p < .01$ ). Thus the overall comparisons of dose were inappropriate and indicated that pairwise comparisons with control should be calculated separately for each time period. The statistical method, Duncan's test, showed that both in the 6-month and 12-month data, there was statistical significant ( $p < .05$ ) evidence of a difference between the controls and the higher dose (625 ppm) reading of hG (g %). while for the ~~5-month and~~ 12-month data there was a statistically significant ( $p < .05$ ) difference between the control and the mid-dose (125 ppm) group (see Table 2 for details).

In conclusion, only the pairwise comparisons of controls and the low dose (50 ppm) in both sexes for the aforementioned time periods showed no statistically significant differences.

Table 1

Linuron Rat Study - Males - Hemoglobin (g %); ANOVA Results

Hemoglobin (g %) - Means <sup>+</sup>				
Dose ppm	<u>Months</u>			Total Time (Average)
	3	6	12	
0	16.35	15.53	15.53	15.80
50	16.05	15.08	15.10	15.41
125	16.61	15.50	15.45	15.85
625	16.63	14.93	14.94	15.50

Table 2

Linuron Rat Study - Females - Hemoglobin (g %); ANOVA Results

Hemoglobin (g %) - Means <sup>+</sup>				
Dose ppm	<u>Months</u>			Total Time (Average)
	3	6	12	
0	15.55	15.22	15.52	15.07
50	15.82	14.94	15.34	15.37
125	15.19	14.95	14.66*	14.93
625	15.38	14.17*	13.74*	14.43

<sup>+</sup> Mean value based on 10 animals.

Significant differences among all dose groups denoted at Control. Significant differences in pairwise comparison denoted at Dose level.

\* p < .05

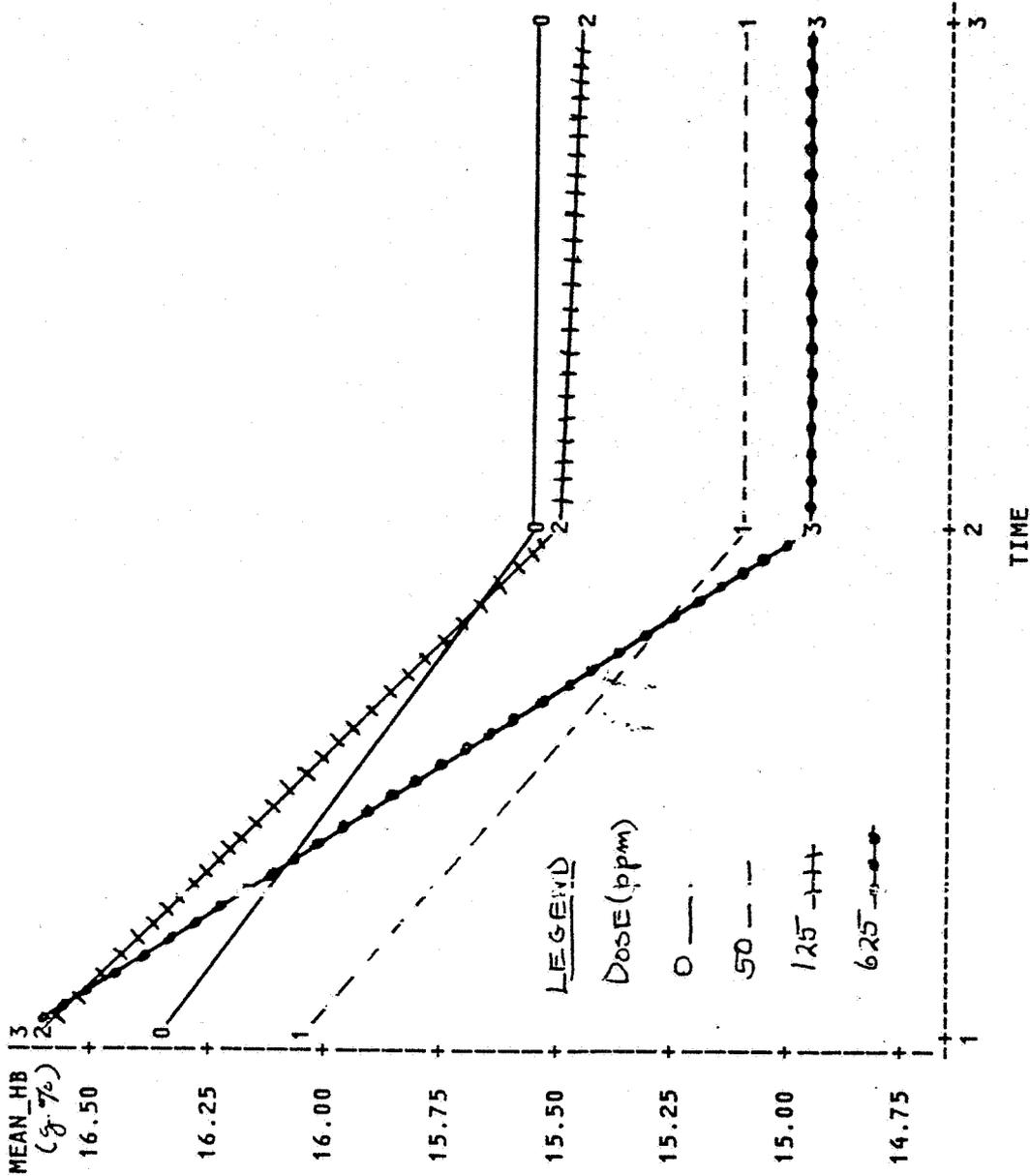
\*\* p < .01

References

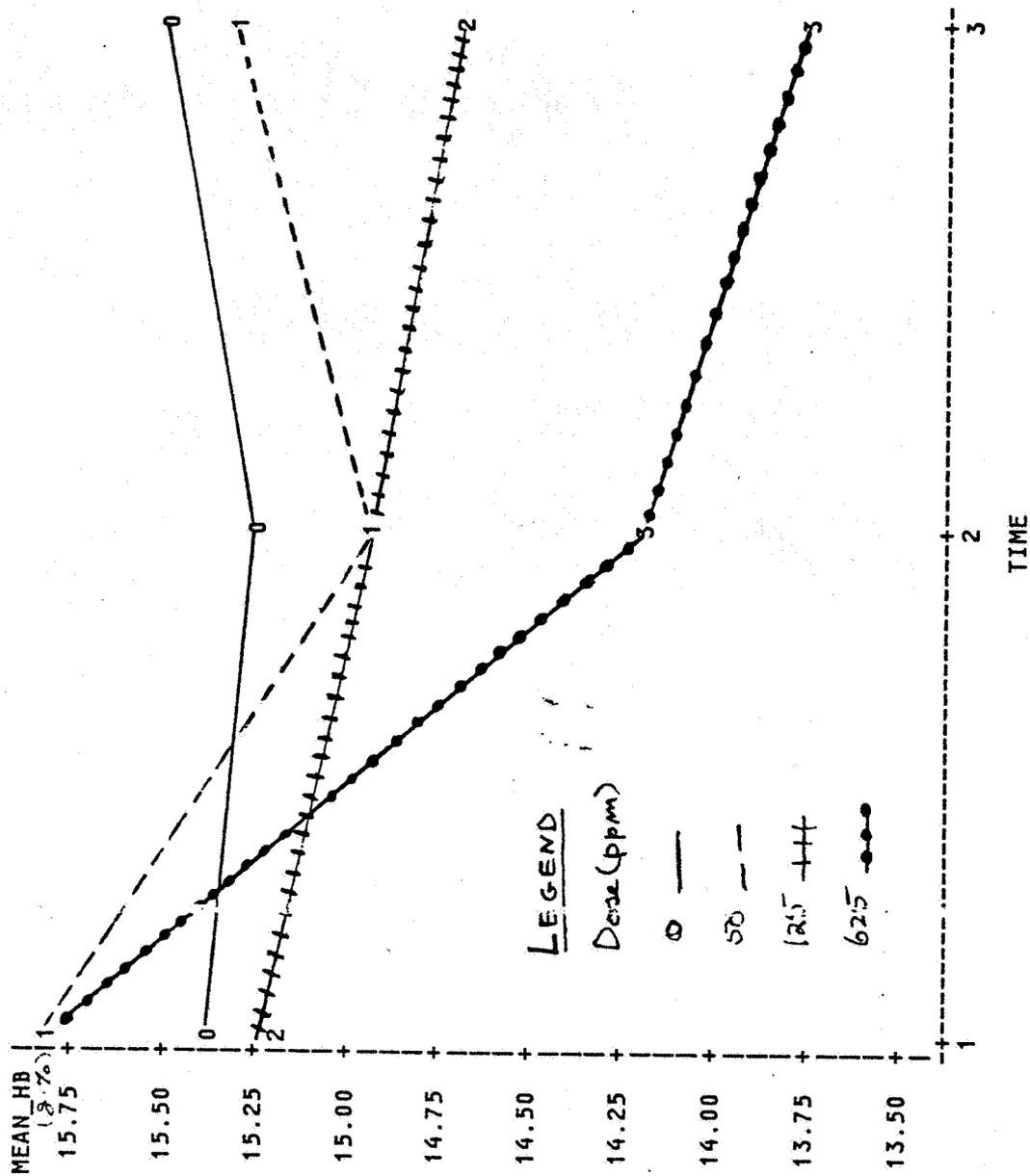
Fisher, R.A. (1942) The Design of Experiments, third edition,  
Edinburgh: Oliver and Boyd.

Snedecor, G.W.; Cochran, W.G. (1967) Statistical Methods,  
sixth edition, Ames, Iowa: The Iowa State University  
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Graph 1. — LINURON, CHRONIC RAT STUDY, MALE — HB MEASURES  
PLOT OF MEAN\_HBxTIME SYMBOL IS VALUE OF DOSE



Graph 2. - LINURON, CHRONIC RAT STUDY, FEMALES- HB MEASURES  
 PLOT OF MEAN\_HBxTIME SYMBOL IS VALUE OF DOSE



LEGEND  
 Dose (ppm)  
 0 —  
 50 —  
 205 +++  
 625 ●●●