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

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AUG 11 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Linuron erythrocyte precursor study performed in the rat. Haskell Laboratory Report No. 760-85, December 6, 1985; Acc. # 26053; EPA I.D. 035506; Caswell # 528; Proj. No. 1121

TO: Ingrid Sunzenauer, 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 7/31/86

THRU: Laurence D. Chitlik, D.A.B.T.
Section Head, Section V
Toxicology Branch/HED (TS-769C)

8/3/86

and

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch/HED (TS-769C)

ACTION: Review study on hematologic values in rats fed linuron for 20, 22, or 26 months; study required by Registration Standard for linuron: data derived from F_{1b} and F_{2b} litters from a 3-generation reproductive study; Accession # 035506, Caswell #528.

RECOMMENDATION:

It is concluded that there is a sex-related hematotoxicity in rats chronically exposed to linuron. Based on the lack of effects in the males at any dose level of linuron, a NOEL for hematological parameters of 625 mg/kg/day (HDT) is determined for the F_{2b} male rats. Based on depressed RBC counts, Hb concentration (statistically significant) and hematocrit in the mid and high dose groups of the female rats after treatment for 20 and 22 months and a statistically significant depression in atypical lymphocytes in the mid and high dose groups at 22 months of treatment, a NOEL for hematoxicity in F_{2b} female rats of 25 ppm is determined. The anemia produced in the F_{2b} female rats probably relates to a direct hemolytic effect of linuron on the RBCs through oxidation of the hemoglobin.

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Excerpts from data submitted by Lorbon on Linuron were released with this review (3 pgs)
These pages may be requested by writing Freedom of Information (A-101), EPA, Washington, D.C. 20460. Requestor will be asked to sign an Affirmation of Non-multi-
[illegible] (see MITRE)

This special blood study is considered scientifically acceptable. An acceptable study to establish a NOEL for potential blood pigment effects in the rat is still required.

BACKGROUND: The acceptable daily intake (ADI) for linuron has been based on a NOEL of 25 ppm from a chronic dog study (dose levels of 25, 125 and 625 ppm) and a safety factor of 100. Decreased RBC counts and a high incidence of abnormal blood pigments were observed at 125 ppm (Hodge, H.C. et al., 1963, EPA Accession #090386, MRID #00018374). This study was reviewed in the Toxicology Branch chapter of the Linuron Registration Standard by L. B. Dale). The NOEL established in the dog study was the basis of a recent risk assessment for dietary exposure of infants to linuron (C. Aldous, memo of 5/14/85). It was noted by Dr. Aldous that 2 out of 5 dogs fed 25 ppm linuron (the LDT) had an uncharacterized "abnormal pigment" in the blood, as did 4 out of 5 dogs at the 125 ppm level, and all dogs at the 625 ppm level. The NOEL employed in the ADI calculations for linuron was, nevertheless, given as 25 ppm, consistent with the observation that commonly measured hematological parameters, e.g., hematocrit, RBC counts, mean corpuscular hemoglobin, etc., were not found to be affected below 125 ppm.

In addition to the effects noted above in the dog study, a rat long term feeding study (Kaplan, A.M. et al., 1980, MRID #00029680; Everett, R.M. et al., 1980, MRID #000269679; reviewed by W. Dykstra of Toxicology Branch in a memo dated May 16, 1980) found hematological effects down to the LDT (50 ppm). At that dose, males had increased mean corpuscular hemoglobin in the absence of changes in hematocrit or hemoglobin concentration (per ml blood). This was taken by the investigators as an indication of reticulocytosis. Thus there was no NOEL established for hematological effects in the rat study. A "special dietary exposure" study was to be undertaken by the registrant to fill this data gap, as confirmed in a memo from R. Holt of du Pont to Robert Taylor (PM-25), dated Sept. 21, 1984. It was concluded from a review of a special study submitted by duPont, which examined the effects of subacute and chronic exposures of rats on met- and sulfhemoglobin concentrations, that the study was inadequate methodologically to determine any chemically-related effects on the blood pigments of concern (J. Rowe; review of 11/19/85). In a recent Data-Call-In Notice (May 19, 1986), EPA requested chronic testing in the rat and dog to determine a NOEL for blood dyscrasias.

DATA EVALUATION RECORD

CHEMICAL: Linuron
3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea(Lorox®, INZ-326);
Caswell no. 528.

TEST MATERIAL: Linuron; Haskell no. 14,703; purity not stated

STUDY IDENTIFICATION: Hematologic values in rats fed for 20, 22, or 26 months with 0, 25, 125 or 625 ppm INZ-326

Medical Research Project No. 4580
Haskell Laboratory Report No. 760-85
Date issued: December 6, 1985
Study Director: William C. Krauss, D.V.M.
Sponsor: E. I. du Pont de Nemours and Company
EPA Accession # 035506

CONCLUSIONS:

It is concluded that there is a sex-related hematotoxicity in rats chronically exposed to linuron. Based on the lack of effects in the males at any dose level of linuron, a NOEL for hematological parameters of 625 mg/kg/day (HDT) is determined for the F_{2b} male rats. Based on depressed RBC counts, Hb concentration (statistically significant) and hematocrit in the mid and high dose groups of the female rats after treatment for 20 and 22 months and a statistically significant depression in atypical lymphocytes in the mid and high dose groups at 22 months of treatment, a NOEL for hematoxicity in F_{2b} female rats of 25 ppm is determined.

This special blood study is considered scientifically acceptable.

METHODS: A photocopy of the methods section is attached. Comments are presented below:

1. The EPA Guidelines recommend blood sampling for chronic studies every six months versus the present study protocol in which sampling occurred at 20, 22 (F_{2b}) and 26 months (F_{1b}) in the rats.

2. No information regarding the purity, or chemical characterization was included, although the batch no. (Haskell no. 14,703) is given, which has been previously reported as 94.5% pure (see review on rat [illegible] and methemoglobin dated Nov. 19, 1985).

RESULTS:

A summary of hematology parameters is presented below (see pages 3, 4 of this review).

Regarding the F_{1b} rat data, the reviewer is in agreement with the authors of the study that the blood data are quite variable, particularly for control values for reticulocytes (males and females) and for RBC and Hb concentrations in the females which indicate anemia. This may relate to the advanced age of these animals, 26 months. Examination of selected blood smears (not presented in the summary table) in both the male and female F_{1b} rats indicates a high percentage

of the animals with abnormal RBC morphology,e.g., keratocytes, schistocytes, spherocytes(male= 80-100%; females= 67-88%; see pages 49-56 of the report). Therefore, it is not appropriate to consider this data for the setting of NOELs for the blood parameters studied.

Examination of the hematologic values for the male F_{2b} rats (on test for 20 months) does not indicate any significant alterations in any dose group for the RBC count, Hb concentration, hematocrit, WBC count including neutrophils, lymphocytes, atypical lymphocytes(statistically significant only at low dose), monocytes, eosinophils, absolute or relative reticulocytes, or any other parameter (see summary table below). The bone marrow smears (Appendix D of the report) indicate some reports of fatty marrow in the controls and all dose groups (4/5-controls, 5/5-low, 2/5mid, 5/8-high). It is not possible to determine the significance of this finding without historical control data, although this finding is probably normal for older rats, particularly since there is no dose-response effect.

RBC counts are decreased (not statistically significant) at 125 and 625 ppm in the female rats at both the 20 (6.79-control vs 6.17 and 6.09x10^6/uL, respectively) and 22 month (6.05-control vs 5.52 and 5.44x10^6/uL, respectively) sampling periods. This is reflected in the statistically significant decrease in Hb concentration observed at the mid and high doses in the 20 month blood samples (14.9-control vs 13.8 and 13.5x10^6/uL, respectively) as well as the moderate decrease observed (not statistically significant) at 22 months (14.0-control vs 12.4 and 12.6x10^6/uL, respectively). The hematocrit is depressed also at both of these doses for both sampling periods. The control females at 22 months appear somewhat anemic as evidenced by their RBC count (6.05x10^6/uL) and hematocrit (34%) as compared to the respective control values in the 20 month samples (6.79x10^6/uL, 38%). However, these control Hb values are in the normal range suggesting that the apparent modest anemia may not be a real effect.

An increase in the absolute number of reticulocytes is observed at 20 months in the high dose females (306 vs 201x10^3/uL in the controls) as well as a statistically significant increase in the relative number of reticulocytes (6.32 vs 2.97% in the controls). At 22 months the absolute number appears still elevated (105 vs 55x10^3/uL) as well as the relative reticulocyte counts at the mid and high doses--although there is considerable variability in high dose values (1.42(S.D.=.36)/mid and 1.90(S.D.=1.23)/high vs 0.94(S.D.=.40)/control). This is suggestive of a mild reticulocytosis in the females at the mid and high dose. Atypical lymphocyte counts were significantly depressed for the 22 month blood samples at the mid and high dosages (285%/control vs 30%/mid and 70% high. This parallels the observation of mild reticulocytosis noted in these animals

Summary Table on Selected Hematologic Values in F_{2b} and F_{1b} Rats

F_{2b}(20 mo) Males (ppm)	RBC x10^6/uL	Hb g/dL	Ht (%)	Plat x10^3/uL	WBC x10^3/uL	Neut WBCx%
0	6.83(1.00)[a]	14.1(2.1)	37(5)	1256(184)	15.7(4.6)	4564(2973)
25	7.37(0.66)	15.6(1.1)	39(3)	1107(144)	11.6(3.9)	2844(3052)
125	7.40(0.73)	15.2(1.2)	40(4)	1185(145)	15.7(5.9)	3542(1864)
625	7.19(0.81)	15.1(1.3)	39(3)	1103(190)	16.0(4.9)	3954(4085)
Females (ppm)						
0	6.79(0.49)	14.9(1.1)	38(4)	930(191)	8.0(4.3)	2030(1600)
25	6.25(1.25)	13.9(2.2)	35(6)	927(132)	6.3(2.9)	1673(1467)
125	6.17(0.65)	13.8(0.9)*	35(2)	977(160)	9.0(4.6)	2577(2446)
625	6.09(0.45)	13.5(0.7)*	35(3)	1137(191)	10.8(3.5)	2178(561)
(22 mo) Females (ppm)						
0	6.05(0.76)	14.0(1.1)	34(4)	1068(204)	12.1(8.6)	3435(3256)
25	6.63(0.73)	14.8(1.1)	37(4)	916(107)	6.9(2.3)	1049(627)*
125	5.52(1.05)	12.4(2.0)	31(5)	1149(446)	11.8(6.8)	4297(6487)
625	5.44(0.77)	12.6(1.3)	31(4)	1166(99)	10.8(3.0)	1425(420)
F_{1b}(26 mo) Males (ppm)						
0	6.95(1.87)	14.4(3.4)	37(8)	1296(311)	18.3(7.1)	6514(3813)
25	6.91(1.26)	14.1(2.5)	36(7)	1241(58)	13.8(3.5)	5465(1004)
125	6.97(0.60)	14.7(0.7)	36(2)	1327(223)	10.8(2.7)	2678(829)
625	6.13(0.76)	13.6(1.5)	34(4)	1539(232)	16.3(3.1)	4801(2944)
Females (ppm)						
0	5.49(1.41)	12.3(2.5)	32(6)	1109(166)	12.7(5.0)	4092(1902)
25	5.96(0.62)	13.2(1.0)	33(2)	1199(253)	14.1(6.7)	5164(3088)
125	5.77(1.37)	12.9(2.1)	33(7)	1179(235)	13.5(6.5)	[illegible]
625	5.17(0.88)	12.0(1.8)	29(4)	1235(260)	[illegible]	[illegible]

continued on next page

Summary Table on Selected Hematologic Values in F_{2b} and F_{1b} Rats (continued)

F_{2b}(20 mo) Males (ppm)	Lymph WBCx%	Alymph WBCx%	Mono WBCx%	Eosin WBCx%	Retic %	Absolute Retic x10^3/uL
0	9588(2075)[a]	228(289)	1114(710)	167(165)	3.51(3.45)	210(171)
25	7661(1355)*	93(145)	865(399)	85(85)	1.55(0.22)	114(16)
125	10671(4533)	146(226)	1045(712)	274(181)	1.72(0.99)	123(64)
625	10499(1878)	120(117)	1287(534)	141(131)	2.49(0.75)	175(42)
Females						
(ppm)						
0	5183(2529)	167(174)	583(415)	77(67)	2.97(1.30)	201(87)
25	4091(1767)	97(97)	376(192)	72(64)	4.95(4.23)	264(158)
125	5385(2084)	194(123)	688(518)	195(187)	4.38(5.62)	247(268)
625	7508(3477)	301(228)	636(348)	134(95)	6.32(3.12)*	306(105)
(22 mo)						
Females						
(ppm)						
0	7311(4635)	285(290)	917(731)	132(112)	0.94(0.40)	55(20)
25	5454(1700)	44(74)	280(226)	79(91)	0.68(0.30)	44(15)
125	7020(1868)	30(66)*	291(252)	123(83)	1.42(0.36)	79(28)
625	8820(2416)	72(70)*	310(165)	133(93)	1.90(1.23)	105(71)
F_{1b}(26 mo)						
Males						
(ppm)						
0	9002(3450)	237(176)	2458(1460)	130(89)	9.64(15.47)	442(516)
25	6135(2153)	212(156)	1841(1050)	187(308)	2.80(0.78)	187(38)
125	6460(1799)	178(82)	1359(597)	131(93)	3.00(1.54)	207(104)
625	8811(2082)	275(202)	2214(890)	198(221)	5.90(2.54)	347(104)
Females						
(ppm)						
0	6882(2745)	221(153)	1437(657)	97(102)	7.30(11.89)	281(319)
25	6401(2532)	407(419)	2014(1334)	98(200)	3.23(1.15)	190(57)
125	7157(2708)	266(243)	1431(879)	155(216)	5.24(4.68)	264(162)
625	5251(1819)	164(106)	701(372)	75(83)	4.45(2.33)	215(77)

[a] values represent the mean ± the standard deviation in parentheses. * Significantly different from control rats ($p<0.05$) by Mann-Whitney U criteria

Bone marrow smears indicate that generally all female test groups had findings of granulocytic hyperplasia and lymphoid hyperplasia of plasma cells (see table below):

FEMALES/F_{2b}-22 months

Controls(5 animals)	25 ppm (5 ")	125 ppm(5 ")	625 ppm (5 ")
granulocytic hyperplasia (3,3,3,3,3)	granulocytic hyperplasia (1,2,3,-,-)	granulocytic hyperplasia (1,1,1,2,3)	granulocytic hyperplasia (1,2,-,-,-)
lymphoid hyperplasia/plasma cells (3,3,3,3,4)	lymphoid hyperplasia/plasma cells (2,3,3,-,-)	lymphoid hyperplasia/plasma cells (2,3,3,4,-)	lymphoid hyperplasia/plasma cells (2,3,3,3,3)

*(evaluation grade of findings in parentheses: 1= very mild change or markedly cellular; 2= mild change or adequate cellularity; 3= moderate change or moderately decreased cellularity; 4= marked change or poorly cellular

As with the apparent increased incidence of fatty marrow noted previously in test groups (control included) of the F_{2b} males, this finding is probably an age-related but not compound-related finding since there is no dose-response effect evident. As noted by the authors, there was an apparent increase in erythropoiesis in the high dose group as evidenced by 3/5 females with mild to moderate changes in erythroid hyperplasia (compared with 0/5 in the controls). One of five intermediate rats also had moderate erythroid hyperplasia.

DISCUSSION

Blood samples and blood smears taken from animals on test in a three-generation reproduction study (F_{1b} and F_{2b} litter groups) indicate a toxic effect of linuron on the blood system in female but not male rats--based on the findings in F_{2b} litters--the F_{1b} litter data being uninterpretable due to variability in reticulocytes, RBC and Hb concentrations in the controls indicating anemia. There were essentially no changes in any of the hematology parameters measured in the F_{2b} males at any dose but several parameters were significantly affected in the female rats. These effects in the females included depressed RBC counts, Hb concentration (statistically significant) and hematocrit at mid and high dose [illegible] both 20 and 22 month periods, an increase in absolute reticulocytes and relative reticulocytes (statistically significant in the high dose at 20 months and [illegible] months (not statistically significant for either measurement at the later period) and a statistically significant depression in atypical lymphocytes in the mid and high dose groups at 22 months of treatment.

Bone marrow smears in the males indicate fatty marrow in the control and all treated dose groups in the F_{2b} males and findings of granulocytic hyperplasia and lymphoid hyperplasia of plasma cells in the F_{2b} females. These effects were not dose-related and are probably associated with aging of the experimental animals.

Due to the variability in the control hematology indices, F_{1b} blood data were not adequate for establishing NOELs for blood dyscrasias in the rat. This variability may well relate to the advanced age of the animals which was 26 months (as compared to 20-22 months in the F_{2b} rats) when blood samples were analyzed. However, the data from the F_{2b} animals appear acceptable for the determination of hematology NOELs. On the basis of the F_{2b} data, it is concluded that there is a sex-related hematotoxicity in rats chronically exposed to linuron. Since no effects were observed in the males at any dose level of linuron, a NOEL for hematological parameters of 625 mg/kg/day (HDT) is determined for the F_{2b} male rats. Based on depressed RBC counts, Hb concentration (statistically significant) and hematocrit in the mid and high dose groups of the female rats after treatment for 20 and 22 months and a statistically significant depression in atypical lymphocytes in the mid and high dose groups at 22 months of treatment, a NOEL for hematoxicity in F_{2b} female rats of 25 ppm is determined. The anemia produced in the female F_{2b} rats by linuron may relate to an effect on erythropoiesis, although it is more likely to be a direct compound-related hemolysis of the RBCs from oxidation of hemoglobin to methemoglobin and subsequently to sulfhemoglobin and other related denatured materials which induce RBC breakdown.

This special blood study is considered scientifically acceptable.