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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

NOV 19 1985

MEMORANDUM

SUBJECT: Review of Linuron sulf- and methemoglobin study performed in the rat. Haskell Laboratory Report No. 521-85, MR No. 7606-001 September 4, 1985: Caswell#528

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 11/8/85

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

W. Tieters for L. Chitlik 11-8-85
[illegible] 11/16/85

and

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

ACTION: Review study of the effects of linuron on methemoglobin and sulfhemoglobin blood concentrations in the rat. Accession #259185, Caswell #528

RECOMMENDATION: It is recommended that this study be designated as unacceptable for met- and sulfhemoglobin determinations with the possibility of upgrading to acceptable if additional information clarifying 1) the method for determination of total hemoglobin values and 2) the discrepancies in the reported theoretical methemoglobin, sulfhemoglobin, and hemoglobin values (for specifics, see Methods discussion, p. 4 and 5) are submitted. However, submission of these data alone will not be adequate to determine a NOEL for hemopoietic effects from linuron exposure since the registrant failed to examine red blood cell precursors such as reticulocytes, as originally requested by the Agency, or to relate the findings on blood pigments in the present study to the general blood picture, e.g., RBC count, hematocrit, etc, or to potential target tissues such as bone marrow.

Excerpts of data submitted by duPont on Linuron were included in this review. (9 pages). These pages may be requested by writing Freedom of Information (A-101), EPA, Washington, D.C. 20460. Requesters will be asked to sign an Affirmation of Non-multinational Status.

See D-7594B for data excerpts.

NOTE: In a recent draft Data Call In Notice, the Toxicology Branch recommended that a dog study (90 days in duration) be performed to establish a NOEL for blood effects from dietary exposure to linuron. For the purpose of economy and to avoid unnecessary duplication, if the registrant can establish that the dog is an equally or more sensitive species than the rat, the recommended dog study, provided it addresses all pertinent issues, would be acceptable for the purposes of establishing a NOEL for blood-related toxicity from linuron exposure and no additional test data in the rodent would be requested. Therefore, the registant is requested to present any information which might substantiate the dog as the more appropriate species for determination of the blood effects of linuron.

DATA EVALUATION RECORD

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

TEST MATERIAL: Linuron, DPX-Z326; Haskell no. 14,703; purity stated as 94.5%; material submitted by John C. Summers.

STUDY IDENTIFICATION: Effect of INZ-326 on methemoglobin and sulfhemoglobin concentrations in the rat.

Medical Research Project No. 7606-001
Haskell Laboratory Report No. 521-85
Date issued: September 4, 1985
Study Director: Timothy P. Pastoor, Ph.D.
Sponsor: E. I. du Pont de Nemours and Company
EPA Accession # 259185

CONCLUSIONS: The investigators concluded that "...single or multiple dosing of male rats with 200 mg INZ-326/kg body weight produced increases in SHb but not MetHb. This acute dosing regimen at high levels of INZ-326 demonstrated the capability of the compound to produce low concentrations of SHb. In rats chronically fed INZ-326, theoretical %MetHb was increased in female rats in the intermediate- and high-dose groups (125 and 625 ppm, respectively). %SHb was increased in male rats and actual %MetHb was increased in female rats in the high-dose groups". However, the data cannot be assessed intil discrepancies and questions that were raised during this review have been adequately explained. These are (for report page references, see methods discussion, pages 4 and 5):

1) The investigators make the statement that the method of adding cyanide to measure SHb may not have been sensitive enough to clearly distinguish levels of MetHb from levels of SHb(p. 19). Can this uncertainty be satisfactorily resolved?

2) Why do the hemoglobin values vary so markedly in the same control animals?

3) How can the %SHb be greater than the % theoretical MetHb, which supposedly contains actual MetHb + SHb. Similarly, there are several values in the tables for %TMetHb, SHb, and MetHb which do not appear to sum correctly.

4) The reported values for sulfhemoglobin are not equivalent to the values calculated by the reviewer using the total hemoglobin concentrations (± NaCN)(see summary table).

5) The reviewer wonders if there are any real biological effects shown in certain data reported herein, e.g., in the single dosing study, when the range of control values for SHb overlaps the range of treated values so significantly.

Furthermore, this study is of limited value in establishing a NOEL for possible blood effects in the rat because the study did not examine red blood

cell precursors such as reticulocytes, as originally requested in the EPA guidance document (6/29/84), nor did it attempt to relate the present findings on blood pigments to the general hematological picture presented by linuron exposure. Thus, even if the potential effect of linuron on blood pigments were adequately presented in the present study, one could not be confident that a true NOEL had been established. These problems could have been avoided if the registrant had submitted a protocol for EPA review, as originally promised (R. Holt, memorandum of 9/21/84).

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 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" 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.

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

1. It should be noted that, while not stated in the report, total hemoglobin was directly measured using the CO-oximeter and that theoretical, sulf- or methemoglobin are expressed as percentages of the total hemoglobin (personal communication, T. Pastoor, Study Director, 9/18/85). Additional information regarding the specifics for the use of the CO-oximeter should be provided, e.g., method of standardization for hemoglobin (total), number of replicates.

2. The investigators make the statement that the method of adding cyanide to measure SHb may not have been sensitive enough to clearly distinguish levels of data which bring into question not only the MetHb and SHb values but the entire methodology used in the study:

a. Why do the hemoglobin values vary so markedly in the same control animal, e.g.,

rat #392967: 4.5-14.5 within one hour; rat #392981: 10.0-14.6 also within one hour (Appendix D, p. 44)?

b. How can the %SHb be greater than the % theoretical MetHb, which supposedly contains actual MetHb + SHb (see summary table in review)? Similarly, there are several values in the tables for %TMetHb, SHb, and MetHb which do not appear to sum correctly.

c. Sulfhemoglobin value (%"SHb") (see summary table below) calculated by the reviewer from total hemoglobin concentrations before and after the addition of NaCN were often quite different (larger) from the values measured and presented as %SHb in the report. These values should be identical since both are a measurement of the blood pigment unconverted to the cyanmethemoglobin after adding excess quantities of NaCN.

d. What can be the biological significance (single dose expt.) of an increase of 1.1-1.8% of SHb in the treated group (p.22 of report) if the %SHb in the controls can vary from 0.2-1.5%?

3. Additional hematological data (blood cell precursors such as reticulocytes) should have been provided by the registrant in this study (as noted by C. Aldous in his memo of 11/9/84 on the linuron conference with du Pont held 9/19/84 and required by the the Guidance document for linuron of 6/29/84). Furthermore, du Pont originally stated (R. Holt, memo 9/21/84) that a protocol would be submitted outlining a study in the rat containing 3 dose levels and daily exposures up to 1 week. To the reviewer's knowledge, this protocol was never submitted for review by the Toxicology Branch.

4. It is unclear as to the meaning of the statement on the procedures for the analysis for whole blood(p. 32), i.e., "the difference between the mean "final" % metHb of the test rats and mean "final" % metHb of the control rats is the total % SHb."

5. There is an apparent discrepancy in the volume of blood sample (0.2ml vs 2.0ml) taken by distal amputation of the rat tail--compare p. 14 of methods section with p. 32 in the protocol.

RESULTS: The results of the study are presented in a summary table below. Due to the apparent discrepancies in the methemoglobin, hemoglobin and sulfhemoglobin values (outlined in methods section) it is not possible at this time to interpret the effect of linuron on the rat blood pigments following either the acute, repeated, or "chronic" dosing regimen. Discussion of the effects on sulf-/methemoglobin and hemoglobin will be presented when the apparent discrepancies have been clarified.

DISCUSSION: There are apparent discrepancies in 1) the sulfhemoglobin values presented in the report and those calculated from the total hemoglobin data (+ NaCN-added to convert all but the sulf- form of hemoglobin to cyanomethemoglobin) 2) in total hemoglobin concentrations measured in control rats and 3) in the quantitation between % theoretical MetHb, SHb and MetHb values in the data tables. These discrepancies must be clarified prior to a discussion of the study results.

In addition, the registrant omitted examining or relating the potential effects of linuron on these pigments to possible associated effects on red blood cell precursors such as reticulocytes, and to the general toxicological profile of linuron exposure to the hemopoietic system. Therefore, this study is of limited value in establishing a NOEL for linuron toxicity on the hemopoietic system.

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Summary Table

Group	%TMetHb	%SHb	%MetHb	Hb(g/dL) B	Hb(g/dL) A	%"SHb"(B-A/Bx100)
Single ds.(200mg/kg)						
Con. 1hr	0.9(0.1)[a]	1.4(1.1)	-0.5(1.2)	14.0(1.4)	14.0(1.8)	0.0
Trtd.	1.0(0.2)	0.4(0.3)	0.6(0.3)	12.8(1.1)	11.2(2.9)	12.5
Con. 2hr	1.3(0.7)	1.0(0.2)	0.3(1.0)	8.5(2.7)	9.8(0.2)	-15.3
Trtd.	0.4(0.3)	1.0(0.4)	-0.5(0.2)	8.7(1.9)	9.5(0.9)	-9.2
Con. 4hr	0.7(0.3)	0.6(0.2)	0.2(0.6)	11.1(1.5)	10.9(1.8)	1.8
Trtd.	1.5(1.8)	0.7(0.5)	1.1(2.1)	11.8(1.5)	11.0(1.3)	6.8
Con. 6hr	1.7(1.7)	1.5(1.2)	0.2(2.2)	11.6(3.1)	9.5(1.3)	18.1
Trtd.	0.9(0.5)	0.6(0.5)	0.4(0.9)	11.0(2.9)	10.5(3.9)	4.5
Con. 24hr	0.7(0.2)	0.2(0.4)	0.5(0.5)	11.2(0.8)	11.0(0.9)	1.8
Trtd.	1.8(0.7)	1.1(0.3)*	0.7(0.5)	10.9(1.4)	10.6(1.5)	2.8
Con. 48hr	0.9(0.3)	0.4(0.3)	0.6(0.4)	9.8(0.4)	9.5(0.4)	3.1
Trtd.	2.2(0.3)	1.8(0.7)*	0.4(0.7)	8.3(0.9)	7.7(1.1)	7.2
Con. 72hr	0.5(0.5)	1.1(1.3)	-0.6(1.5)	8.7(2.0)	8.3(2.0)	5.7
Trtd.	1.4(0.3)	2.5(1.0)	1.1(0.9)	6.1(2.3)	5.7(2.4)	6.6
Repeated(7x 200mg/kg)						
Con. 1hr	0.7(0.2)	1.1(0.6)	-0.4(0.6)	9.9(1.5)	11.2(1.4)	-13.1
Trtd.	4.3(0.2)*	3.4(0.6)*	0.9(0.6)	12.9(2.1)	12.0(2.3)	8.7
Con. 2hr	0.4(0.3)	1.8(0.8)	-1.5(0.5)	11.1(1.6)	10.5(1.8)	5.4
Trtd.	4.4(0.5)*	3.6(0.7)*	0.8(0.6)	11.3(1.3)	11.0(1.7)	2.7
Con. 4hr	0.4(0.2)	2.4(1.3)	-2.0(1.4)	10.3(1.3)	9.6(1.2)	6.8
Trtd.	4.3(0.3)*	3.9(0.2)*	0.4(0.3)	12.0(1.3)	10.9(1.2)	9.2
Con. 6hr	0.7(0.6)	0.9(0.9)	-0.2(1.3)	8.6(2.3)	8.4(2.2)	2.3
Trtd.	4.6(0.3)*	2.3(0.3)*	2.3(0.6)	10.6(1.3)	10.2(1.3)	3.8
Con. 24hr	0.5(0.1)	1.2(0.7)	-0.7(0.7)	9.4(0.9)	9.4(0.5)	0.0
Trtd.	4.6(0.4)*	2.9(0.6)*	1.8(0.9)	10.1(1.4)	9.8(1.5)	3.0
Con. 48hr	0.6(0.5)	0.7(0.9)	-0.1(1.3)	10.2(1.6)	9.9(1.7)	2.9
Trtd.	3.9(1.0)*	2.5(0.7)*	1.4(1.1)	9.2(1.4)	8.9(1.4)	3.2
Con. 72hr	0.7(0.2)	1.1(1.0)	-0.5(1.0)	11.0(1.3)	10.8(1.5)	1.8
Trtd.	3.4(0.7)*	3.9(2.0)*	-0.5(2.5)	11.4(1.1)	10.9(1.1)	4.4

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Group	%TMetHb	%SHb	%MetHb	Hb(g/dL) B	Hb(g/dL) A	%"SHb"(B-A/Bx100)
"Chronic"						
Males						
0 ppm(0mg/kg)†	0.9(0.4)	1.7(0.6)	-0.8(0.5)	10.3(2.1)	10.1(2.1)	1.9
25 ppm(1mg/kg)†	0.9(0.4)	1.2(0.8)	-0.4(0.9)	10.9(1.8)	10.5(1.8)	3.7
125 ppm(4mg/kg)†	1.2(0.5)	1.9(0.7)	-0.7(0.7)	11.3(0.7)	11.0(0.7)	2.7
625 ppm(22mg/kg)†	3.1(0.9)*	3.1(1.1)*	0.0(0.9)	11.9(1.2)	11.6(0.8)	2.5
Females						
0 ppm(0mg/kg)†	0.7(0.3)	1.1(1.0)	-0.4(1.1)	10.6(1.1)	10.3(1.2)	2.8
25 ppm(1mg/kg)†	1.0(0.4)	1.2(0.9)	-0.2(0.7)	11.1(0.6)	10.8(0.6)	2.7
125 ppm(6mg/kg)†	1.6(0.3)*	1.3(0.6)	0.3(0.7)	10.1(1.1)	9.6(1.0)	5.0
625 ppm(37mg/kg)†	2.8(0.7)*	1.7(1.1)	1.1(0.9)*	9.5(1.4)	9.3(1.1)	2.1

[a] value(S.D.); * significantly different from the control value ($p \leq 0.05$);
† mean daily intake of linuron during the week prior to blood sampling
%TMetHb = the theoretical %methemoglobin, measured before the addition of NaCN; %SHb = the sulfhemoglobin, measured after the addition of NaCN; %MetHb = the actual percent methemoglobin, calculated as the difference between the %TMetHb and %SHb measurements (%TMetHb-%SHb); Hb/B = total hemoglobin before the addition of NaCN (i.e., hemoglobin, oxyhemoglobin, carboxyhemoglobin, methemoglobin, sulfhemoglobin, and other minor forms of this pigment); Hb/A = after the addition of NaCN; %"SHb" = presumably the per cent not converted to cyanmethemoglobin would primarily represent SHb, which is not altered by NaCN addition