

US EPA ARCHIVE DOCUMENT



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

D/ 7506 / LINURON

2-3-87

005708
RELEASABLE

FEB 3 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Registrant's rebuttal comments on Linuron met- and sulfhemoglobin study in the rat; Caswell 528; EPA I.D. # 035506; Project 7-0129; original Accession # 259185; HLR 521-85

TO: Michael McDavit, 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*
11/20/87

THRU: Quang Q. Bui, Ph.D.
Acting Section Head, Section V *Quang Q. Bui*
Toxicology Branch/HED (TS-769C) *1-28-87*
and *M. J. W. P.*
Theodore M. Farber, Ph.D. *2/3/87*
Chief, Toxicology Branch/HED (TS-769C)

ACTION: Review of Du Pont's rebuttal comments on Linuron met- and sulfhemoglobin study in the rat; Caswell 528; EPA I.D. # 035506; Project 7-0129; original Accession # 259185; HLR 521-85

RECOMMENDATIONS:

It is recommended that study HLR 521-85 (Accession # 259185) be upgraded to classification: acceptable. Based on the statistically significant changes in blood pigments observed in mid and high dose female rats (125 and 625 ppm, respectively) and the statistically significant change in blood pigments observed in high dose male rats (625 ppm), NOELs of 25 and 125 ppm, respectively, for Linuron-related alterations (chronically administered in feed) in blood pigments in female and male rats are established. These NOELs are in general agreement with the findings for general hematotoxicity observed in a more recent study submitted by the registrant (Carakostas, 1986).

DATA EVALUATION RECORD

Issue # 1: Total Hemoglobin

REGISTRANT COMMENT:

Total Hb, MethHb, and SHb were determined spectrophotometrically by using a Model 282 CO-oximeter (Instrumentation Laboratory). MethHb and SHb were expressed as the percent of total Hb. This instrument was factory calibrated and was periodically checked for accuracy. The accuracy of this instrument was checked by comparing the value of a known Hb standard (Coulter Diagnostic) with the value obtained by the CO-oximeter. Based on these periodic checks, the CO-oximeter varied from the standard by less than 10%. The CO-oximeter was considered accurate and reliable for the measurement of total hemoglobin. No standards are available for MethHb or SHb.

EPA RESPONSE:

The method for the determination of Hb and relatively large changes in MethHb and SHb appears satisfactory. As noted by the registrant, the CO-oximeter measured the Hb standard within <10%, although the precise variation was not given. However small % changes (e.g., 1-2 %) would probably not be accurately measured. It appears that the sensitivity of the existing method for blood pigment measurements involving met- or sulfhemoglobin is limited.

Issue # 2: Hb, MethHb, and SHb Measurements

REGISTRANT COMMENT:

a. Sensitivity of the method to distinguish MethHb from SHb. Whereas we were able to clearly differentiate between SHb generated in vitro by hydrogen sulfide from normal amounts of MethHb, these values were much greater than the values determined in test animals. SHb values of greater than 50% were generated in vitro by hydrogen sulfide, whereas in vivo values of less than 2% were common. Therefore, we effectively demonstrated the technique of removing MethHb interference for SHb measurements, but the ability of this technique to differentiate between 1-2% MethHb or SHb is doubtful in practice and irrelevant in use.

The statement on page 19 of the report regarding the sensitivity of the assay was made to emphasize that the SHb and MethHb levels were so low that existing methodology could not clearly differentiate one from the other. Furthermore, the clinical significance of 1-2% MethHb or SHb is questionable. Therefore, higher resolution of these values by more sensitive methods (even if available) would not have helped discern biologically significant effects.

EPA RESPONSE:

As noted above, the analytical method utilized in this study (and apparently state-of-the-art) does not allow an accurate measurement of very small changes in red blood pigment of 1-2%. The issue of the clinical or biological significance of such changes is addressed below under the final EPA response to the registrant comments.

Issue # 3: Variations in total hemoglobin concentrations.

REGISTRANT COMMENT:

The reviewer noted that hemoglobin concentration varied considerably in some rats. Minor variations in hemoglobin concentration were expected since rats were being bled by tail cut and since there are normal fluctuations in erythropoiesis. However, the two large changes noted by the reviewer were indeed aberrant. Usually, large decreases in the hemoglobin concentration meant that some clotting of the sample had occurred. In such cases, as for rat #392907, the sample was unsuitable for SHb determinations. In the 2-3 other cases where large fluctuations in total occurred, yet SHb measurements were completed, the SHb and Methb were generally consistent with other SHb and Methb determinations in the same group and time point. In another case (rat #392984, page 43), the rat ceased to give a reliable blood sample and was therefore not used to determine total Hb, SHb or Methb. Thus, these fluctuations were relatively uncommon and did not change the SHb and Methb measurements or interpretation of the data.

EPA RESPONSE:

The fluctuations in Hb concentration, apparently due to clotting of the blood, could have been avoided by the addition of an anti-coagulant such as heparin or calcium oxalate. However, these blood determinations are not critical to the determination of the NOEL for the 18 month rat blood samples.

Issue # 4: Discrepancies in %SHb calculations

REGISTRANT COMMENT:

The reviewer noted that for several determinations the %SHb was greater than the % theoretical Methb (%TMethb). The reviewer also noted that several values in the Tables did not "appear to sum correctly."

With regard to the SHb determinations, at low levels of this pigment and low levels of Methb, it is possible (after addition of cyanide and subsequent measurements the %SHb) to obtain a spectrophotometric value similar to or greater than the % TMethb. This overlapping of values simply reflects the inherent variability in spectrophotometric determination of these values at low levels. The method used to determine % SHb is the only known method that will estimate %SHb (Howanitz, et al., 1979). Despite this being an established methodology, resolution between 1-2% Methb and SHb is doubtful. Note that in nearly all cases where the %SHb was greater than the %TMethb, the difference was less than 2%.

Discrepancies in the calculation of values reported in the tables can be resolved by reviewing the method used to calculate those values. Mean values were obtained for each group by

- ° subtracting %SHb from %TMethb for each rat, and
- ° computing the group means and standard deviations for %TMethb, %SHb, and %Methb.

Alternatively, if group means are first calculated and then the mean %SHb subtracted from the mean %TMethb, slightly different values can be obtained. We chose to calculate these values on an individual rat basis for two reasons:

005708

- the method statistically accounts for intra animal variation, and
- this method allows for the calculation of a standard deviation for %Met-Hb and thereby permits statistical evaluation. Subtracting the mean %SHb from the mean %MetHb does not give a standard deviation for the %MetHb.

EPA RESPONSE:

The lack of sensitivity of the CO-oximeter in measuring small changes in % MetHb or SHb has been previously noted. The reviewer agrees that the values for each rat should be calculated separately in order to allow the standard deviation to be determined. Examination of the data indicates that there was no discrepancy in the sums using the registrant's method of calculation of values.

Issue # 5: Method of calculation of %SHb

REGISTRANT COMMENT:

The reviewer's %SHb values do not match the %SHb values in the report. The reviewer used an incorrect method to calculate the %SHb. The %SHb is the measurement made on the CO-oximeter after addition of cyanide to the blood sample. %SHb is not calculated from the difference in total hemoglobin after addition of cyanide.

EPA RESPONSE:

The theoretical concept behind the %SHb calculation in the EPA review appears correct. The addition of NaCN to the total hemoglobin should convert all forms of hemoglobin (except sulphemoglobin) to cyanmethemoglobin (hemoglobincyanide), and thus theoretically measures total hemoglobin minus any sulphemoglobin originally present (J.B. Henry, 1979; Clinical Diagnosis and Management by Laboratory Methods, pgs. 863-868). The relationship of this latter value to the calculated SHb given in the study is unclear. However, due to the variability in these values as compared to the direct measurement of Met- and SHb using the spectrophotometer, and the low sensitivity of the latter method for measuring small % changes in these pigments, it is not correct to assume that these % SHb values would necessarily "add up".

Issue # 6: Biological relevance of reported SHb changes

REGISTRANT COMMENT:

e. The reviewer questioned "...if there are any real biological effects shown..." As for points "a" and "c", the significance of these results in demonstrating real biological effects depends on the analytical resolution of these effects and the magnitude of these effects in the animal. We showed that the methods used were effective in determining %SHb at levels much greater than observed in the test animals. The method used would have shown real effects if INZ-326 were capable of producing biologically significant amounts of SHb.

EPA RESPONSE:

The lack of sensitivity in the analytical method does not allow a resolution of the compound-related effects of linuron on subtle changes in the non-Hb

blood pigments. In this case, the overall blood picture must be considered in establishing a NOEL for blood-related effects of linuron administration. The issue of the biological or clinical significance of small amounts of SHb are addressed below.

Issue # 7: Clinical significance of SHb changes

REGISTRANT COMMENT:

The conclusions of this study need to be tempered in terms of the biological significance of SHb production, particularly at the levels detected in this study. The role of sulfur or sulfur-containing compounds is unclear in the production of SHb. Furthermore, SHb seldom exceeds 10%, even in patients receiving treatment with drugs known to induce the formation of SHb (Nelson, 1979). In discussions with clinical pathologists subsequent to the issuance of report #521-85, the measurement of SHb is considered difficult and the clinical significance at levels under 5-10% unclear. Had INZ-326 induced greater than 5-10% MetHb or SHb, then not only would the analytical method have clearly demonstrated this effect but also the clinical significance of these effects would have been discussed.

EPA RESPONSE:

The EPA recognizes that the toxicological significance of low levels of SHb in the blood of humans is uncertain. Changes in the blood to 15 to 20% methemoglobin or sulfhemoglobin could result in clinically significant effects such as cyanosis or anemia. However, smaller pigment levels (1-10%) may be deleterious to individuals in a compromised physiological state such as newborn infants where the activity of NADH-diaphorase is normally low, infants with congenital heart disease or pulmonary disorders like respiratory distress syndrome. It is important to note that sulfhemoglobinemia is an irreversible condition and there is no treatment for this phenomenon except to wait for the bone marrow and spleen to generate more normal hemoglobin pigment via de novo or salvage pathways.

Issue # 8: Relevance of data to hematological effects

REGISTRANT COMMENT:

The reviewer asked why the study was not designed to measure red cell precursors or relate the findings in this study to the general hematological picture presented by linuron exposure. Hematological parameters, red cell precursors, and bone marrow were examined in rats from the reproduction study. These data have been submitted to the Agency (Carakostas, 1985).

Furthermore, this study (HLR 521-85) was not designed to measure red cell precursors. The study was designed specifically to fulfill the generic data requirements for linuron outlined in the Guidance for the Reregistration of Pesticide Products Containing Linuron as the Active Ingredient (EPA Case Number 47 [035506]; June 29, 1984; page 54). The Agency requested data, "...relating levels of sulf- and methemoglobin following dietary exposure for [sic] certain substituted phenyl urea compounds such as linuron". This data requirement has been fulfilled with the submission of Haskell Laboratory Report Number 521-85.

These data generally coincide with the hematological picture described in previous reports. In this study, effects on blood pigments were observed in male

rats in the 625 ppm group and in female rats in the 125 and 625 ppm groups. In the study designed to examine red cells and red cell precursors (Carakostas, 1985), hematological effects were seen in female rats in the 125 and 625 ppm groups; there were no hematological effects in male rats. A clear NOEL was established in both of these studies. Although a connection between altered red cell pigments and the reduction in red cell mass is possible, a clear cause and effect relationship is uncertain.

EPA RESPONSE:

EPA has reviewed the Carakostas study (see memo: J. Rowe to I. Sunzenauer, August 11, 1986). It was concluded in that review, "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 (HVT) 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 hematotoxicity 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."

The reviewer would agree that the hematological picture in the rat red cell precursor study and the rat blood pigment study generally coincides, although the pigment changes appear to be a somewhat more sensitive parameter than general hematology parameter in males for blood toxicity based on the lower NOEL (125 ppm) estimated in the pigment study.