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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Diflubenzuron (Dimilin); EPA Reg. No. 37100-8. Company Response to Rat and Mouse Oncogenicity Data Reviews (Chen, 9/7/84 and 11/2/84). CASWELL No. 346A.

TO: Timothy Gardner, PM #17
Insecticide-Rodenticide Branch
Registration Division (TS-767)

FROM: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) RBJ 2/13/85

A. Recommendation

1. The Registrant has provided historical control data on the Sprague-Dawley rats which satisfies TB's questions concerning the incidences of thrombus of the heart, congestion/hemorrhage in the spleen, and chronic/active inflammation in the paranasal sinus and eyes observed in the Lifetime Oncogenic Study of Diflubenzuron in Rats. (J.H.S. Chen review, 9/7/84).

2. The Registrant has provided historical control data on the HC/CFLP mouse which satisfies TB's questions concerning the incidence of angiectasis in the ovaries, medullary hyperplasia and ceroid cells in the adrenals, and acanthosis in the stomach observed in the lifetime onogenic study of Diflubenzuron in Mice. (J.H.S. Chen review, 11/2/84).

3. The Registrant has provided data on % Hb transformed into either metHb or sulfHb for both rats and mice. Toxicology Branch considers this information adquately satisfies concerns addressed in our 9/7 and 11/2/84 reviews.

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B. Detailed Considerations and Conclusions

1. Lifetime Oncogenic Study of Diflubenzuron in Mice.

a. Data presented demonstrate that the observed incidences of angiectasis in the ovaries, medullary hyperplasia and prominent ceroid cells in the adrenals, and acanthosis in the stomach of treated mice are not different from historical controls from the same laboratory. Therefore, these are not considered compound related responses.

b. Evaluation of the met- and sulfhemoglobin data demonstrate a NOEL for both abnormal hemoglobin pigments resulting from dietary administration of diflubenzuron is equal to 16 ppm (2.4 mg/kg b.wt.)

c. Classification of Study:
Core - Guideline

NOEL = 16 ppm
LEL = 80 ppm (met- and sulfhemoglobin)

Not oncogenic at doses up to and including the highest dose tested of 10,000 ppm.

[Comment: Significance of the met- and sulfhemoglobin formation should be considered in light of the absence of any compound related effect on mortality, body weight, food consumption or urinalysis; no observable gross pathological changes except at doses $\geq$ 2000 ppm; no neoplastic changes at any dose; and non-neoplastic histopathological changes (e.g. extramedullary hemopoiesis, siderosis, pigment in liver/spleen cells, etc.) evident primarily at doses of $\geq$ 400 ppm.]

2. Lifetime Oncogenic Study of Diflubenzuron in Rats.

a. Data present demonstrate that the observed incidences of congestion/hemorrhage in the spleen, and chronic/active inflammation in the paranasal sinus and eyes of treated rats is not different from historical controls from the same laboratory. The increased incidences of atrial thrombus of the heart in treated rats was significantly different from historical control incidences at

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only one dose in one sex (625 ppm, males). Although incidences were increased in treated groups in comparison to controls in this study they do not increase in a dose related manner. There were no other adverse effects in the heart (e.g. degenerative cardiomyopathy) associated with compound ingestion, particularly at 2500 or 10,000 ppm. Therefore, the incidence of thrombus of the heart at 625 ppm in males is not considered related to treatment.

b. A NOEL has not been demonstrated in this study for met- and sulfhemoglobin formation. However, consideration of the data generated in the 1977 Hunter et al. 104 week rat feeding study permits the calculation of a NOEL for this response (see discussion in item 3. below).

c. Classification of Study:
Core - Minimum
NOEL is less than 156 ppm (lowest dose tested).

Not oncogenic at doses up to and including the highest dose tested.

[Comment: Study was originally designed to demonstrate oncogenic potential of diflubenzuron and permit calculation of methemoglobin and sulfhemoglobin responses.]

3. Comments on the Hunter, B., Colley, J. et al. study (1977) conducted at Huntingdon Research Centre, entitled "Effects of DU 112307 in Dietary Administration to Rats for 104 weeks".

This study was reviewed by S. Biscardi, Sept. 26, 1977 (PP #7F-1899, 6F-1773 and 6F-1832) and determined to be invalid. This study is reclassified, by this memo, to CORE - Supplementary on the basis that there is some useful information/data contained in the report. As noted by Mr. Biscardi, it provides little useful information on the oncogenic potential of diflubenzuron. However, some meaningful information contained in the report can contribute to our understanding of the non-neoplastic effects, namely, formation of met- and sulfhemoglobin.

Sulfhemoglobin was not evident according to the report because it was "below the level of detectability". However, this "level" was not indicated or stated in the report. Methemoglobin formation was determined but only in control, 40 and 160 ppm dose groups (dose groups excluded were 10 and 20 ppm). The following table is reconstructed from the data presented (average of 10 animals/group per sampling interval).

Table 1 - Incidence of Methemoglobin in Rats

	Weeks				
Males	13	26	52	78	102
0 ppm	0.1	0.2	0.2	0.1	0.2
40 ppm	-	0.3	0.1	0.1	0.1
160 ppm	0.2	-	0.3*	0.2*	0.1
Females					
0 ppm	0.1	0.1	0.1	0.1	0.1
40 ppm	-	0.2	0.1	0.1	0.2
160 ppm	0.2	-	0.2*	0.2*	0.2

[* $p < 0.05$]

It is difficult to evaluate these data since they were determined to only one significant figure. The report indicates significant differences (because of the standard deviation) only for high dose males and females at weeks 52 and 78. An investigation of individual animal measurements reveals that more high dose males and females had metHb values equal to or greater than 0.4 g% than did corresponding controls or 40 ppm animals. The "new" (1984) rat study contains much better data on met- and sulfhemoglobin formation. Nonetheless, data presented herein are consistent with those findings.

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Individual animal reports were re-examined for evidence of microscopic changes in the liver/spleen as a consequence of met- or sulfhemoglobin formation. Extramedullary hemopoiesis, siderocytes and green-black pigment in the red pulp of the spleen were reported, but no evidence of a dose response was noted. The following table is compiled from the incidences reported in all animals in the study..

Table 2 - Incidence of Extramedullary Hemopoiesis (EH), Siderocytes (S) and Green-Black Pigment (GB) in the Spleen

Dose (ppm)	Males	Females
0	3 - EH 1 - S	5 - EH
10	1 - Myeloid Leukemic cells (Significant structural changes)	6 - EH
20	1 - GB	4 - EH
40	1 - EH	7 - EH
160	1 - GB 1 - S	6 - EH 1 - S

[Evidence for such a response was not observed in the liver pathology].

Certainly, the histopathology reported demonstrates no clear evidence of an adverse hemopoietic effect. The severity of the lesions observed was also uniform among all groups.

Therefore, based on the "marginal" effects at 160 ppm for methemoglobin formulation (identified above), suported by a similar response at > 156 ppm in the rat study reviewed by Dr. Chen, (9/7/84), and the absence of any measurable effects at doses of $\leq$ 40 ppm in rats (1977, Hunter et al.) and $\leq$ 16 ppm in mice (Huntingdon Labs, 1984), TOX Branch considers a no observable effect level for methemoglobin and sulfhemoglobin formation to have been demonstrated at 40 ppm in the rat.

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4. Recalculation of the PADI

The PADI for diflubenzuron is recalculated based on the demonstration of a NOEL of 40 ppm for met- and sulfhemoglobin formation in the rat. The previous PADI was based on the same toxic response in the mouse but at a dose equivalent to 1.1 mg/kg b.wt. (determined by regression analysis in a 14 week study). The newly submitted studies in mice and rats (identified above in items B. 1. and 2.) are more definitive studies in many respects, particularly regarding met- and sulfhemoglobin formation. Collectively, the available data demonstrate a similar degree of sensitivity to diflubenzuron between rats and mice. Therefore, the data generated in the available lifetime feeding studies support the finding of a no observable effect level (NOEL) for met- and sulfhemoglobin formation, based on the rat, at 40 ppm in the diet. A safety factor of 100 is applied to the NOEL resulting in a provisional acceptable daily intake (PADI) of 0.02 mg/kg b.wt./day. The maximum permissible intake (MPI) for a 60-kg human is calculated to be 1.2 mg/day. A full ADI will be considered when the long-term non-rodent (dog) feeding study is submitted and evaluated. However, the demonstration of no oncogenic effects in 2 species at doses up to and including 10,000 ppm in the diet, alleviate previous concerns expressed for the use of dimilin (diflubenzuron).

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