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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Tim Gardner, PM #17
Registration Division (TS-767)

THRU: Robert B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

8/15/83

SUBJECT: Diflubenzuron 37100-8: Review of the Effect of Diflubenzuron Given by Oral Administration with the Feed on Toxicity and Tumour Development in HC/CFLP Mice (Interim Report 0-52 Weeks)
CASWELL#346A

Review and Evaluation of Lifetime Feeding Study in HC/CFLP mice with Diflubenzuron (Huntingdon Research Center PRD 360/821141, 56645/18/83, Cambridgeshire, England, Accession No. 250270, 250271, and 250272) - Interim 52-Week Report.

Procedure:

The method used to determine the toxic effects of the test compound, Diflubenzuron, when administered in the feed to mice is described below during the first 52 weeks of a lifetime study:

1. The study consisted of 728 HC/CFLP strain mice. The animals were acclimated to the laboratory for approximately two weeks, then divided into five dosage levels of diflubenzuron in diet (16, 80, 400, 2000, and 10,000 ppm) with a control group (52 males and 52 females in each treated group and 104 males and 104 females for the control group). An additional 72 males and 72 females for the control group and 36 males and 36 females for each treated group were used for clinical pathology and pathological investigations from interim sacrifices.

2. The Spratt's No. 2 rodent feed was provided throughout the study. Diet of 16, 80, 400, 2000, and 10,000 ppm of diflubenzuron were analyzed during weeks/ 1, 3, 6, 11, 18, 31, 40, and 50.

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3. All animals were examined for clinical signs each day during the first 4 weeks of the study. After this period of time, animals were checked twice weekly. The detailed observations for the clinical signs were carried out on a weekly basis from week 5 onwards. Any individual mouse showing signs of intoxication was killed and subjected to detailed macroscopic examination. Food consumption and body weight changes were recorded weekly throughout the test period for all groups of animals. Water consumption for each cage in the control and treated groups was measured for a 5-day period during weeks 23, and 49. Hematology, blood chemistry and urinalysis were determined after 6, and 12 months of treatment. At interim sacrifices (after 6, and 12 months of treatment), the brain, pituitary gland, heart, lungs, spleen, thyroid gland, liver, thymus, testes, ovaries, kidneys, pancreas and adrenals were weighed. All nodules, tissue masses and otherwise macroscopically abnormal tissues were routinely preserved for microscopic examination. The results of study were examined using Bartlett's test for heterogeneity of variance. A transformation leading to homogeneous variance was applied if necessary. Otherwise, the Kruskal-Wallis non-parametric analysis was used. All parameters were analysed on a cage mean basis. Each cage contained up to 4 mice of the same sex. Following the analysis of variance, treatments were compared by Williams' test (Biometrics 27; 103 and 28; 519., and 1971 and 1972) or its non-parametric equivalent (Shirley, Biometrics 33; 386., 1977).

4. The incidence of tumours was analysed by the method described by Peto (Br. J. Cancer 29; 101., 1974), modified where appropriate by Peto and Pike (Biometrics 29; 579., 1973).

Results:

1. Clinical Signs:

The only clinical sign of reaction to treatment was blue/gray discoloration of the extremities and dark eyes. These abnormal clinical signs were observed in mice treated with 10,000 ppm, all females and the majority of males treated with 2000 ppm, the majority of mice treated with 400 ppm, and a number of males treated with 80 ppm of diflubenzuron. Other incidental clinical signs were not related to the treatment of diflubenzuron.

2. Mortality:

	Incidence of Mortality (Week range 1-52)					
	Control	16 ppm	80 ppm	400 ppm	2000 ppm	10000 ppm
Males	19/176	5/88	10/88	12/88	5/88	10/88
Females	20/176	16/88	11/88	7/88	12/88	9/88
Total %	11	12	12	11	10	11

No treatment-related effect on mortality was observed during the first 52 weeks of study.

3. Growth Data:

a. Food consumption - The mean (group) feed consumption values were slightly higher in females at 10000 ppm group during the first 52 weeks of treatment. No marked differences in appetite were observed between treated and control group.

b. Body weight - No marked differences were observed in the mean (group) body weight values between the treated and control group after 52 weeks of treatment.

4. Water Consumption:

Based on the measurement of water intake during weeks 23 and 49, no treatment-related effects were observed among mice receiving diflubenzuron.

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5. Hematology:

Summarized Hematology - Group Mean Values

	Week 26		Week 52	
	Male	Female	Male	Female
Packed cell volume	N.D.	N.D.	N.D.	N.D.
*Hemoglobin	N.D.	XX Higher	N.D.	N.D.
*RBC	N.D.	N.D.	XXX Lower	XX Lower
*Mean Corpuscular Hemoglobin Conc.	XX Higher	X Higher	X Higher	X Higher
Mean Cell Volume	N.D.	N.D.	N.D.	N.D.
*WBC	X Higher	XX Higher	X Higher	XX Higher
*Platelet Count	XXX Higher	XXX Higher	XXX Higher	XXX Higher
*Methemoglobin	XXXX Higher	XXXX Higher	XXXXX Higher	XXXX Higher
*Sulphemoglobin	-	-	XXXXX Higher	XXXXX Higher
*Heinz Bodies	-	-	XXXX Higher	XXXX Higher

N.D. - No significant difference between the treated and control group.

X - Significant difference between the 10000 ppm and control group.

XX - Significant differences were noted when the 2000 ppm and 10000 ppm levels were compared to the control group.

XXX - Significant dosage-related effects were observed in the 400 ppm, 2000 ppm and 10000 ppm groups.

XXXX - Significant dosage-related effects were observed in the 80 ppm, 400 ppm, 2000 ppm, and 10000 ppm groups.

XXXXX - Significant dosage-related effects were observed in 16 ppm, 80 ppm, 400 ppm, 2000 ppm, and 10000 ppm groups.

6. Blood Chemistry/Urinalysis:

a. Blood Chemistry - Significantly higher cholesterol values were observed in week 24 for male mice treated with 10000 ppm of diflubenzuron. Marginal increase of the glutamic-pyruvic transaminase activity in males treated with 10000 ppm of the test compound was observed in weeks 24 and 50. Alkaline phosphatase levels were also marginally increased in male mice receiving 2000 ppm and 10000 ppm in week 24. No treatment-related effects on other parameters (glucose, protein, albumin, urea nitrogen, and glutamic-oxaloacetic transaminase activity) were observed.

b. Urinalysis - No significant differences in mean (group) urinalysis values were observed between the treated and control group in weeks 25 and 51 (volume, pH, specific gravity, protein, reducing substances, glucose, ketones, bile pigments, urobilinogen and hemoglobin).

7. Interim Sacrifice (after 26 weeks of treatment):

a. Macroscopic pathology - An increased incidence of splenic enlargement and dark discoloration were observed in all mice treated with 2000 ppm and 10000 ppm and female mice treated with 400 ppm of diflubenzuron. The hepatic surface irregularity was also increased in female mice treated with 10000 ppm of the test compound. Cyanosis of the cutis was observed in male and female mice receiving 2000 ppm and 10000 ppm of diflubenzuron. The incidence of all other findings in the following organs and tissues, "kidney, seminal vesicles, thymus, muzzle, tail, salivary gland, stomach, ovaries, uterus, adrenals, and subcutis", were not related to the treatment of diflubenzuron.

b. Organ weight - The group mean values of spleen weights for mice treated with 2000 ppm or 10000 ppm of diflubenzuron were higher than control weights and a slight increase was also observed in the spleen weights of females receiving 400 ppm of the test compound. The group mean values of liver weights for male mice treated with 2000 ppm and 10000 ppm of diflubenzuron were higher than those of the controls, and similar but less marked effect was observed in the females receiving 2000 ppm and 10000 ppm of the test compound were slightly higher than those of the controls. No treatment-related effect in the organ weight was noted for mice of the lower dosage groups. The weights of other organs (brain, pituitary, heart, lungs, kidneys, thymus, thyroid, testes/ovaries, and pancreas) were unaffected by the administration of diflubenzuron.

8. Interim Sacrifice (after 52 weeks of treatment):

a. Macroscopic pathology - An increased incidence of splenic enlargement in male mice treated with 2000 ppm and 10000 ppm and females treated with 400 ppm, 2000 ppm and 10000 ppm of diflubenzuron were observed. The hepatic enlargement was increased in male mice treated with 10000 ppm and female mice treated with 2000 ppm and 10000 ppm of the test compound. Cyanosis of the cutis was also observed in mice receiving 2000 ppm and 10000 ppm and male mice receiving 400 ppm of diflubenzuron. The incidence of all other findings in the following organs, and tissues "stomach, pancreas, lungs, urinary bladder, testes/ovaries, seminal vesicles, preputial gland, adrenals, lymph nodes, heart, fur, pinna, tail, abdominal cavity, hibernating gland and eye", were not related to the treatment of diflubenzuron .

b. Organ weight - The group mean values of spleen weights for mice treated with 2000 ppm and 10000 ppm of diflubenzuron were higher than control weights. The group mean values of liver weights for mice treated with 2000 ppm and 10000 ppm of the test compound were higher than those of the controls. The group mean values of thyroid weights for mice receiving 10000 ppm of diflubenzuron were also higher than control weights. The weights of other organs (brain, pituitary, heart, lungs, thymus, kidneys, adrenals, testes/ovaries, and pancreas) were unaffected by the administration of diflubenzuron.

8. Interim Sacrifice (after 52 weeks of treatment): Continued

c. Histopathology: Microscopic Examination

(i) Summarized Neoplasia Findings

	Control	16 ppm	80 ppm	400 ppm	2000 ppm	10000 ppm
Lymphosarcoma	11	6	7	5	7	7
Reticulum cell sarcoma	2	1	0	0	1	0
Pulmonary adenoma	2	0	0	0	1	0
Pulmonary adeno-carcinoma (F)	0	1	0	0	0	0
Benign liver tumour (M)	1	0	0	0	1	0
Malignant liver tumour (M)	0	0	1	0	0	0
Liver cell hemangioma (M)	1	0	0	2	1	0
*Epididymal fibrosarcoma (M)	0	0	0	1	0	0
Subcutaneous/intramuscular (M)						
Fibroma	0	0	0	1	0	0
*Hemangioma	0	1	0	1	0	0
Fibrosarcoma	1	0	0	0	0	0
*Uterine-Hemangioma (F)	0	1	0	0	0	0
*Ovarian-Granulosa cell tumor (F)	0	0	0	0	1	0
*Squamous cell carcinoma of the skin	0	0	0	1	0	0

According to the tumor profile of untreated male and female mice presented in Table A, the low incidence of tumor formation included the Epididymal fibrosarcoma, Subcutaneous tissue hemangioma, Uterine hemangioma, Ovarian granulosa cell tumor and Squamous cell carcinoma. Therefore, a careful examination of these tumors developing in mice treated with diflubenzuron for the next stage of study is critical. However, no treatment-related effect was detected in the incidence of the various tumor types encountered at this stage of study.

c. Histopathology: Continued

(ii) Summarized Non-Neoplasia Findings (% Remarkable Incidence)

	Control	16 ppm	80 ppm	400 ppm	2000 ppm	10000 ppm
Lungs	29%	22%	19%	19%	18%	10%
Thymus	3%	12%	.5%	0	5%	5%
Lymph nodes	13%	11%	5%	10%	0	0
*Spleen	29%	33%	29%	62%	86%	0
*Liver	71%	67%	81%	86%	86%	95%
Kidneys	3%	11%	10%	5%	9%	9%
Urinary bladder	0%	0	0	0	5%	0%
Uterus (F)	17%	0	30%	18%	55%	18%
Cervix (F)	0	12%	0	0	9%	0
Ovaries (F)	0	0	20%	9%	8%	0
Testes (M)	10%	0	0	0	9%	10%
Seminal vesicles (M)	25%	10%	27%	20%	18%	0
Thyroids	0	6%	0	0	0	0
Adrenals	5%	0	5%	0	5%	5%
Pituitary	0	0	5%	0	0	0
Stomach	26%	17%	19%	29%	18%	14%
Salivary gland	0	0	5%	0	0	0
Skin (M)	0	0	0	0	0	0
Eyes	11%	0	5%	10%	5%	0
Head	0	0	0	0	0	0

*Spleen: An increased incidence of extramedullary hemopoiesis in mice treated with 2000 and 10000 ppm and male mice treated with 400 ppm of diflubenzuron were observed. Numbers of siderocytes were increased in mice receiving 2000 ppm and 10000 ppm and male mice receiving 400 ppm of the test compound. These significant findings were related to the hematological changes and the increased spleen weight recorded in the higher levels of treatment groups.

*Liver: An increased incidence of hepatocyte enlargement in mice treated with 2000 ppm and 10000 ppm of the test compound were observed. These findings were related to the increased liver weight recorded at the higher levels of treatment.

The incidence and distribution of the other morphological changes recorded in the spleen, liver and other tissues in this study did not demonstrate any treatment-related effects.

Conclusions:

1. An average of 10-12% mortality was experienced in all groups (treatment and control) during the 52-weeks of study. Treatment-related effects were observed in all groups treated with 16 to 10000 ppm of dilfubenzuron at this stage of study.

2. Based on the data obtained from the study, particularly clinical signs, diflubenzuron causes blue/gray discoloration and dark eyes in mice. Hematology findings in the mice confirms the elicitation of such discoloration as being caused by the abnormally high methemoglobin (greenish brown to black in color), sulphemoglobin (abnormal pigments), and heinz bodies (denatured hemoglobin) related to treatment with diflubenzuron.

3. The liver and spleen effects are related to the treatment with diflubenzuron in the higher treatment groups.

4. The evidence of tumorigenicity related to treatment with diflubenzuron in mice is incomplete at this stage of study. Further examination of the development of tumor types identified in the first 52 weeks of study and those occurring in low incidence or not seen in controls must be carefully pursued in the final stage of the study.

5. Classification: Study Incomplete - Interim Report

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