PP-689
TOX-147P

001474

May 13, 1968

410

Mr. Kenneth B. Nash
Pesticides Regulation Division
Agricultural Research Service
U. S. Department of Agriculture
Washington, D. C. 20250

Reg. No. 1022-00

Referral Date: 4/26/68

Dear Mr. Nash:

We have received the toxicological data on Chapman Weed Free D-8P (Diuron) Reg. No. 1022-00.

We have no objection to the registration of this label.

Sincerely yours,

Lamar B. Dale, Ph.D.
Pharmacologist
Registration Section
Pesticides Program

LBDale:reb

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LBDale:reb
June 18, 1968

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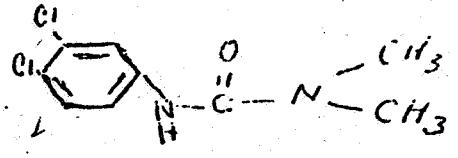
NAME: Diuron

CHEMICAL NAME:

001474

3-(3,4-dichlorophenol)-1,1-dimethylurea

STRUCTURE:



EMPIRICAL FORMULA:

$C_9H_{10}C_2N_2O$

SOLUBILITY: Solubility in water at 25°C = 42 ppm.

Very low solubility in hydrocarbon solvents.

USE: Pre-emergence herbicide

ACUTE ORAL TOXICITY (Rat)

LD_{50} = 3.4 g/kg.

10-Dose Subacute (RAt)

1060 mg/kg/day - 6/6 survived 10 treatments.

Discomfort, pallor, no weight gain

Hemosiderosis of spleen, with compensatory red blood cell formation;

spleens enlarged and congested.

90-Day Feeding Toxicity (Rats)

50ppm: - 0/10, no growth effect, no clinical signs of toxicity

CBC normal - no pathology.

500 ppm: 0/10; no effect on growth in males, slight effect in females. No clinical signs of toxicity in males; cyanosis in females. CBC in males normal, lower in females. No pathology in either sex.

5000 ppm

0/10, retardation of growth, pallor, cyanosis, CBC lowered, chronic methemogloginemia; enlargement of spleen, increased blood formation.

Acute Dermal Irritation (Rabbits)

50% Water Paste - Non-irritating to intact skin, moderatley irritating to abraded skin.

DATA SUMMARY

Two Year Chronic Feeding Study in Rats

Pilot feeding studies (40-42 days) using groups of 5 male and 5 female rats were carried out with the following dietary levels: 0 (control) 200, 400, 2000, 4000 and 8000 ppm. No significant growth retardation was observed in male or female rats fed dietary levels of 2000 ppm or less. Marked inhibition of growth was observed in male and female rats fed dietary levels of 4000 or 8000 ppm. A significant increase in mortality was noted only in rats fed 8000 ppm dietary level. After six weeks decreased red cell counts and hemoglobin values were observed in male rats fed dietary levels of 4000 ppm or greater and in female rats fed dietary levels of 2000 ppm or greater. Hematological findings in male and female rats fed dietary levels of 200 or 400 ppm Diuron were within normal limits. An abnormal blood pigment, not methemoglobin, was found in rats given dietary levels of 2000 ppm or greater.

The histological appearance of the liver, kidney and spleen of the rats fed the various dietary levels of Diuron was not different from the appearance of these organs in the control rats. Deviations observed in the weights of these organs were not attributable to Diuron fed at 4000 ppm and below.

A 24 month ingestion study was conducted, in which groups of 35 male and 35 female rats were maintained on diets containing the following concentrations of Diuron; 0 (25 ppm, 125 ppm, 250 ppm and 2500 ppm).

Dietary levels of 125 ppm or less had no effect on growth. A dietary level of 250 ppm resulted in a slight reduction in growth and a more marked depression was observed when rats were fed at the highest (2500 ppm) dietary levels of Diuron. Increased mortality was noticed in male rats fed dietary levels of 250 or 2500 ppm within the first 14 weeks of the study. Male rats fed diets containing 25 or 125 ppm showed no greater mortality than did the male controls. The mortality among the female rats fed dietary levels as high as 2500 ppm was not greater than that observed among the female control rats. The deaths may be attributed to respiratory and other infections. Urine chemistry values showed normal levels of sugar and protein. Hematological findings in the male rats fed dietary levels as high as 2500 ppm were within normal limits. No evidence of hematological alteration was observed in female rats fed dietary levels of 25 or 125 ppm. Female rats fed dietary levels of 250 or 2500 ppm showed on occasion reduced hemaglobin values during the first year of the study only and this change was associated with slight decrease in the red blood cell count. An abnormal blood pigment was detected by spectophotometric techniques in male and female rats at dietary levels of 125 ppm of Diuron or greater. Oxyhemoglobin bands were normal in both control and treated rats. Organ weights and organ weight-body weight ratios were within normal limits.

Changes noted microscopically in the bone marrow and spleen of male and female rats fed the 2500 ppm dietary level suggested increased turnover of red blood cells. At the lower dietary levels, these changes were in constant; they were not observed in male rats, but were noted in female rats,

except at 25 ppm. No alteration that could be attributed to the ingestion of Diuron at dietary levels as high as 2500 ppm, was observed in the brain, lungs, heart, pancreas, kidneys, urinary bladder, adrenals, gonads, stomach, small and large intestine, muscle or thyroid gland. Hemosiderin pigment was found in the Kupffer cells; otherwise the liver cells were normal. A typical epithelial hyperplasia of the urinary bladder was observed in 4 rats, (3 male and 1 female) fed the 2500 ppm dietary level. A similar alteration was also noted in one male control rat and in one male rat fed the 125 ppm dietary level. This change was attributed to an enzootic bladder infestation by the parisite Trichosomoides Crassicauda which has been reported as a cause of hyperplasia of the bladder epithelium.

* In the supplementary study, groups of 20 male and 20 female rats were placed on diets containing 0 (control), 250 or 2500 ppm Diuron for periods ranging from 15 months to 23 months. These studies indicated a slight growth depression in rats ingesting diets containing 2500 ppm of Diuron. No growth depression was noted in the rats maintained on the 250 ppm dietary level. Increased mortality due to respiratory and other infectious diseases was observed among female rats fed 2500 ppm Diuron in one study and among male rats in the second study and the 250 and 2500 ppm dietary levels. Significant decreases in the red blood cell count were noted in male rats fed 2500 ppm after 2 and 9 months on diet, but not after 17 months on diet. Red blood cell counts among the female rats fed 2500 ppm were not significantly different from the controls at 2 and 9 months, but were at 17 months.

Significantly lower hemaglobin values were observed in both sexes fed the 2500 ppm dietary level after 2, 9 or 17 months on diet. The female rats fed the 250 ppm dietary level showed a significant decrease in hemaglobin content after 2 months on diet, but the mean hemoglobin value at 9 months, while lower than controls, was not significantly different. An abnormal blood pigment was detected in the male and the female rats fed the 2500 ppm dietary level for periods of 9 or 16 months. No abnormal pigment was detected at the 250 ppm diet level. Organ wights and organ weight-body weight ratios were normal except for spleen weights of the rats fed on the 2500 ppm dietary level of Diuron for 17 months. The spleen weights of female rats were significantly greater than the spleen weights of the control rats.

Histological examinations of tissues taken from the rats fed the 2500 ppm Diuron diets for the 9 or 17 months showed changes in bone marrow and spleen that were related to an increased turnover of the red blood cell. At 250 ppm, there was evidence of similar reactions of lesser degree in females only. There were no other histologic changes attributable to Diuron.

Reproduction studies were performed over 3 generations and involved the production of 2 liters in each generation. Reproductive performance was comparable for the rats maintained on the control diet and for those fed the diet containing 125 ppm of Diuron. The growth of the male rats in the second liters (F_1b) and the first (F_1) generation control and Diuron fed was comparable.

Female rats from the F_1b liters and fed 125 ppm Diuron diet showed a slight weight depression after 10 weeks (but not at 4 weeks) on the diet as compared to control female rats. The male and female weaned rats born in the second liters (F_2b) of the second generation and fed the 125 ppm Diuron diet did not grow as well as the F_2b control rats. The male and female rats born in the first liters (F_3a) and the third (F_3) generation and fed the 125 ppm dietary level showed a significant weight depression. (Males at 10 weeks; females at 4 and 10 weeks) as compared to the control rats. After 10 weeks the rats fed the 125 ppm Diuron diet were given the control diet. The weight depression observed during the period of Diuron ingestion persisted even though the rats were then receiving the basal diet. At weaning, rats from the second liters (F_3b) of the third generation were sacrificed. Hematology disclosed no changes attributable to Diuron. Organ weights and organ - body weight ratios were comparable for control rats and for the Diuron fed rats. Histological study of tissues sections of these rats revealed no change that could be attributable to the administration of Diuron. The remaining F_3b rats were placed on their respective diets for an 11 week period. These male and female rats fed the 125 ppm Diuron diet grew as well as the controls.

A second, 3 generation reproductive study was conducted at 125 ppm Diuron in view of the unexplainable growth differences observed in the F_2b and F_3a liters of the first reproductive study. In the second study reproductive performance was normal and compared favorably with the first study. All liters (except F_2a) of the second study were maintained on their respective diets for 12 or 13 weeks. No growth retardation was observed in any of these rats.

LBDale,Jr.:mw
June 21, 1968

DIURON

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2 Year Feeding Study in Dogs

In pilot dog feeding studies, 2 dogs were given various daily doses of Diuron for short periods of time. One dog given 8 mg/Kg body weight (16.0 ppm in the diet) for 4 weeks maintained body weight, showed normal urinary and hematological findings, and normal organ weights at the time of sacrifice. A thorough histological study revealed only normal tissues. A second dog was first given a daily dose 20 mg/Kg thereafter increased to 40 and 80 mg/Kg, which doses were tolerated, and then to a dose of 200 mg/Kg, which was accompanied by food refusal and vomiting. The dog was then replaced on the basial diet for a brief period, and finally was maintained for 2 weeks at 80 mg/Kg and then for 2 weeks at 120 mg/Kg. Body weight declined a little in the last 2 weeks of this study. Urinary analyses gave normal values. Hematological studies revealed a decrease in red blood cell counts, hemoglobin values and hematocrit percentages. Organ weights were normal. The histological study of a large number of tissues showed normal tissues except for a slight abnormality in the liver unrelated to Diuron feeding. No histological changes were attributed to the administration of Diuron in either dog.

In a 2 year feeding study, groups of 6 beagle dogs each, 3 males and 3 females per group, were maintained on diets containing 0, 25, 125, 250, and 1250 ppm of Diuron. Body weights were maintained for all dogs except those on the 1250 ppm which lost a little weight. 2 dogs were sacrificed in moribund condition during the 2 year period, one (25 ppm after only 8 weeks apparently of heart disease, the other (1250 ppm) after about 75 weeks from a strangulated hernia. (In neither case was the death related to the Diuron feeding). In the 2 year study urine samples collected periodically gave normal values for sugar/pro-

tein. Hematological samples taken 11 times at roughly 3 month intervals were normal for dogs given the 25 and 125 ppm diets. A trend toward decrease in values in the dogs given 250 ppm was significant only in the lower red blood cell counts of the male dogs at the end of the study. The dogs given the 1250 ppm diet exhibited depressed red blood cell counts, hemoglobin values and hematocrit percentages after 3 months; the lower values persisted during the remainder of the study. Spectral analyses of the blood pigments showed normal oxyhemoglobin for all dogs. No abnormal pigment occured in male or female dogs at 25 ppm, nor in female dogs at 125 ppm; however, traces were found in 2 of the 3 male dogs at 125 ppm and in all of the dogs at 250 ppm. This abnormal pigment (probably a sulfhemoglobin, not methemoglobin) was present in identifiable amounts in the blood of 2 of the female dogs given 1250 ppm in the diet and traces were found in the 4 remaining dogs. Organ weights and organ weight-body weight ratios were normal in every case except one: the liver weight-body weight ratios were elevated for male and female dogs given the1250 ppm diet. Urine and feces contained roughly equivalent concentrations of Diuron and a count for roughly equal amounts excreted. The Diuron residues in the muscle, fat, liver, kidney, spleen, and blood of these dogs increased roughly in proportion to the dietary levels. The tissue levels of Diuron reflected the daily intakes and showed no evidence of accumulation. Thorough histological studies revealed no changes that were attributed to the ingestion of Diuron in dogs given the 250 ppm or less. In the dogs given the 1250 ppm diet, (a)an increased erythrogenic activity was evident in the bone marrow counts, and (b) an increased pigment deposition (presumably hemosiderin) had occured in certain liver cells. These changes probably reflect an increased red blood cell destruction (low grade anemia was recorded) which evoked an increase in erythrogenic activity. There was no evidence of carcinogenic activity.

CONCLUSIONS

Diuron, 3-(3,4-dichlorophenol)-1,1-dimethylurea is a pre-emergence herbicide with tolerances established on a number of food crops (see attached). Diuron is relatively non-toxic having an LD_{50} in rats of 3.4 gm/kg.

Chronic, two-year feeding studies and reproductive studies have been carried out. The only significant effects were the appearance of traces of an abnormal blood pigment and a hemolytic effect in large doses.

It is known from previous studies on urea herbicides that these compounds, in sufficient dosage, exert a hemolytic effect. This is evident in the chronic administration of Diuron from the hematology of rats and dogs fed for two years at dietary levels of 2500 ppm and 1250 ppm, respectively. Histology of the blood forming tissues also disclosed and increased erythrogenic activity at these levels. In addition, a darkening of the blood has been observed in rats fed high doses of compounds of this class.

The abnormal blood pigment was identified as sulfhemoglobin. It is difficult to assess the importance of the presence of trace amounts of sulhemoglobin pigment in the blood. If it is assumed that low concentrations of Diuron in the blood convert extremely small amounts of hemoglobin to sulhemoglobin and that more is converted with increasing Diuron levels in the blood, and assumption which fits the results reasonably, then a coherent hypothesis can be made to explain the overall response.

Sulfhemoglobin is an irrevisible combination, unlike methemoglobin, the mollicule is permanently altered. Sulfhemoglobin is in some ways more active physiologically then is methemoglobin, e.g., to produce a cynically apparent cyanosis in humans, the following amounts of pigment are required: of reduced hemoglobin, 5 gm/100 ml; of methemoglobin, 1.5 gm/100 ml; of sulfhemoglobin, 0.5 gm/100 ml. Evidence that the red blood cell life span is for shortened by the presence of sulfhemoglobin is contradictory. Wintrobe believes the evidence gained from examining the effects of a variety of chemical agents is most consistent with a presumption of two separate events: (a) the production of sulfhemoglobin and (b) a hemolytic action. Chemicals differ in their ability to cause these changes. Regardless of the relative sulfhemoglobin forming potency and the hemolytic potency of Diuron, the likely processes and their sequelae can be listed as follows: 1. Diuron is absorbed and appears in the circulating blood in concentrations roughly proportional to the dietary levels. 2. Diuron induces (a) sulfhemoglobin formation, and (b) a hemolytic effect; both increased with increasing blood levels. 3. Despite the disadvantages of irreversible reaction and possibly related hemolytic effect, sulfhemoglobin functions as a somewhat inefficient oxygen carrier. Traces of sulfhemoglobin thus should be of no greater concern than the low levels of carboxyhemoglobin or methemoglobin usually found in human blood.

Diuron poses no undue human health hazards.