10-5-66

October 5, 1966

003308

Petitions General Branch

Dr. O. C. Pittsburgh
Deputy Director
Division of Toxicological Evaluation

Bromacil (5-bromo-3-sec-butyl-6-methyluracil) on pineapple and citrus fruit.

[illegible] (No. 6F049)

E. I. DuPont de Nemours Company
Wilmington, Delaware
(AF 4-408)

The data in this petition establishes a "no effect" level of at least 50 ppm for the dog and the rat. This is a conservative figure and according to our usual evaluation 250 ppm would be considered a "no effect" level. Therefore, there is sufficient data on acute, subacute, and chronic toxicity and on reproduction to show the safety of the requested tolerance of 1 ppm on pineapples and citrus fruits.

CONCLUSION:

The requested tolerance of 1 ppm bromacil on pineapples and citrus fruits is safe.

cc:

FDA
[illegible]

Concurrence: 10-5-66

Evaluation of toxicologic and pharmacologic data for "Hyvac" X Bromacil Weed Killer

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E. I. Dupont de Nemours Co.
Wilmington, Delaware

The petitioners request that a residue tolerance of 1 ppm for this herbicide be established for pineapples and citrus fruits. To the knowledge of this reviewer no other tolerance has been established for this material.

The active ingredient in this herbicide is 5-bromo-3-sec-butyl-6-metnyl-uracil (Bromacil). The weed killer is a wettable powder containing 80% of the active ingredient.

Acute Toxicity (Oral, in Dogs):

The material tested was the 80% wettable powder. It was not possible to obtain a lethal dose or LD_{50} in dogs because of emesis. The doses ranged from 5.0 g to 100 mg/kg. Besides emesis, the material elicited salivation, mydriasis and incoordination.

Acute Toxicity (Oral, in Rats):

The material was administered by intubation as a 30% aqueous suspension of 80% wettable powder. There were 10 animals per dose level and a 14 day test period. The LD_{50} was 5200 mg/kg. Toxic effects observed were rapid respiration, prostration and weight loss.

Subacute Toxicity (Oral in Rats):

The material was a 10% aqueous suspension of the 80% wettable powder. It was administered by intubation 5 times a week for 2 weeks. There was 1 mortality; 5 of 6 animals survived 10 daily doses of 1333 mg/kg. There were disturbances to the gastrointestinal tract, CNS and incoordination noted.

90-Day Feeding Study (Rats):

There were 10 males and 10 females at each dose level. The levels were 0, 50, 500, 2500 ppm in the diet. After 6 weeks, the upper level was raised to 5000 ppm. After 10 weeks half of these animals were placed on a diet of 6000 ppm for 1 week and then to 7500 ppm for 2 weeks.

Results:

There were no deaths. There was a lower growth rate at the 2500 ppm level and up. Hematology showed low erythrocyte counts for males after

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30 days at the highest level. This improved at 90 days. Urinalysis was normal. There was no microscopic pathology or other effects at 50 or 500 ppm. Microscopic changes were observed at 5000 ppm or more for the thyroid and liver (increased thyroid activity; enlargement of centrolobular cells of the liver).

Dermal Toxicity:

Skin Absorption (Rabbits):

At 5000 mg/kg there was no indication of toxicity nor gross pathology.

Sensitization:

A 50% suspension of the material caused a mild skin irritation for young guinea pigs in 24 hrs.

Inhalation Test: (Rats):

At a concentration of 4.8 mg/liter and 2.1 mg/liter of atmosphere, and given 4 hours of exposure, the material caused rapid respiration. There was dried blood around the mouth and nose of 1 out of 4 rats. There were no deaths.

Eye Irritation (Rabbits):

Direct application to the eye surface caused a temporary conjunctivitis. There was no corneal injury.

Mutagenic Studies:

The possibility of the compound being incorporated into nucleic acids was studied because of the similarity of the compound to certain precursors of DNA. Studies were with the C^{14} labelled material. From the publications presented, in which the suspected DNA is isolated in several instances and examined, the indications are that such incorporation does not occur.

Metabolism and Degradation:

The principal compound isolated from urine (rat) was 5-bromo-6-hydroxymethyl-3-sec-butyl-uracil. The metabolite was identified by thin-layer chromatography, infrared spectra, NMR and mass spectrophotometer. Traces of 2 other metabolites were not identified. There was also a trace of the administered compound present.

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Soil studies with the labelled herbicide showed that after 1 year, 75% of the material is degraded to CO_2. Only 23.5% remained in the soil.

In studies of the uptake and metabolism of the material in orange trees, it was found that the compound was metabolized to the same compound identified in the urine of rats (see above). Less than 4% of the applied radioactive compound was found in orange plants, the root system of which was exposed to 10 ppm of the material. A minor plant metabolite was not identified. Approximately 10% of the applied radioactivity was metabolized to CO_2 by the plant, or otherwise degraded.

No toxicity data are presented for the metabolites.

Two Year Feeding Study in Rats:

Initially, 259 males and 256 females were housed in pairs (sexes separated). The pre-test period was 14 days. The animals were placed on test in 5 equal weight groups of 36 males and 36 females each (total, 180 males and 180 females). There were 2 control groups and 3 test levels: 0.005, 0.025 and 0.125% of the compound.

Results:

Weight Gain and Food Efficiency:

There was a statistically significant weight retardation for the 0.125% female group at the 1st and 2nd years. The other groups were not affected. The food efficiency for the female 0.125% group was also slightly less than normal.

Clinical Observations and Mortality:

There were no noticable clinical signs of toxicity. Mortalities were not greatly different from the control rats. There were 3 deaths in the control groups and 3 in the treated groups during the 1st year. Mortalities, or animals killed in extremis was greater in the second year. The total mortality, 58% in males and 49% in the females seems quite high.

Hematology:

The following were studied at various times during the 2 year study; differential WBC, RBC, WBC, hemoglobin concentration, hematocrit and cell size. All were in normal range and there was no evidence that their values were altered by the comp and at any feeding level.

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Urinalysis:

The following were noted at various times during the study: volume, color, appearance, osmolality, blood, sugar, pH and protein of the urine. The values were not markedly different for control or treated rats, except for osmolality in some instances. The difference in osmolality for the 0.125 male group from controls was slightly significant at 2 test periods.

Biochemistry:

No effect of the test material on alk. phosphatase activity was found at any test period. Results for the protein bound iron (PBI) tests, discussed in the Summary, could not be found in the tables presented. It is stated that "there was no difference between control group and test group fed 0.025%" of the compound for 9 months. It is not stated if the protein bound iron tests at higher levels, or after a longer time on the compound were affected. (Reviewer's note: PBI tests are of little value in any case without concurrent determinations of the iron-binding capacity.)

Gross Pathology:

There was no great difference between test and control organ weights.

Histopathology:

There was perhaps, a dose-related effect to the thyroids. Hyperplasia was noticed at the highest level. Also, there was one follicular cell adenoma in a female rat at the highest level which could be compound-related. Examination of rats which died during the study showed nothing related to ingestion of the compound.

Tissue Residue Analysis:

There were detectable amounts of the compound in tissues but there was no evidence of excessive accumulation. The liver and kidneys had the largest amounts (about 2 ppm).

Reproduction Studies (Rats):

The animals used for the study were taken from the main feeding (2-yr.) study (previously described) after approximately 12 weeks of feeding the compound. Animals from only 1 dietary level (0.025%) and a control group were taken. There were 12 males and 24 females per group. These animals constituted the F_0 generation. The 1st litter was designated F_{1a}; the 2nd litter as F_{1b}. The F_{1b} litter was maintained on the

same diet. At 110 days they were mated to yield F_{2a} and F_{2b} litters. The same procedure was followed yielding F_{3a} and F_{3b} litters.

Results:

Fertility, gestation, viability lactation indices were noted.

There were no marked differences between control and treated groups. There were no pathological changes, gross or microscopic, attributable to the compound. There was no mention of any deformities in the offsprings.

Two Year Study in Dogs:

There were 3 males and 3 females to each of 4 groups (1 control group; 3 treated). Dietary levels of the compound were 0.005, 0.025 and 0.125%.

Results:

Body weight:

There was some decline in body weight for males and females at the 0.125% level at the start of the experiment. The growth rate stabilized thereafter. Food consumption was not affected by the presence of the compound.

Clinical Observations:

Appearance, rectal temperature, pulse, and respiration were normal. One animal (0.005% level) was sacrificed in extremis. Illness was stated as not dose-related. All other dogs survived the 2 year study.

Hematology:

Erythrocytes, hemoglobin, hematocrit, leucocytes and differential count were not markedly altered or affected over the 2 years by the presence of the compound.

Urinalysis:

It was not affected over the 2 year study.

Biochemistry:

Sugar, urea nitrogen, cholesterol, alkaline phosphatase values were not affected by the compound.

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Pathology:

Organ weights were not markedly different from the controls. There were no histopathologic findings to note.

Tissue Residue:

There was no evidence of excessive accumulation of the compound in the tissues.

Comments and Evaluation:

The reproduction study in rats used only 1 dose level. From the data supplied in the petition, this probably was not a toxic dose. The small number of animals initially on test (12 females and 12 males) may be questioned for a study of this kind.

The 2-year dog study had but 6 ~~rats~~ dogs per group. In view of the uneventful findings, however, I would consider the study sufficient.

Metabolite toxicity apparently was not attempted. Acute toxicity of the herbicide is presented only in rats. Short term toxicity was also only in rats. Further studies along these lines in rabbits, perhaps, would be desirable. At least, acute toxicity for the metabolites should be determined.

The data for protein bound iron determinations could not be found, although it is discussed in the rat study Summary. Significance of these tests can not be evaluated.

From the data, it would appear that 0.005% (50 ppm) is a reasonable "no effect" level to consider. USDA figures show per capita consumption in the U. S. of approximately 113.3 lbs of citrus fruit and pineapple per year, or 51200 g. If residue of herbicide of 1 ppm were on these products, per capita intake would be 51.2 mg capita/year or approximately .002-mg/kg/day. Considering 50 ppm as a no effect level in dogs, a dog would consume 3.75 mg/kg/day. Thus, there is a safety factor of 1500-fold which suggests no particular hazard. Granting of the tolerance however, should await correction of deficiencies noted in this evaluation.

Junius M. Webb