4/29/63

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OPTIONAL FORM NO. 10
5010-104

UNITED STATES GOVERNMENT

Memorandum

TO : MR. F. J. McFARLAND
PESTICIDE BRANCH

DATE: April 29, 1963

FROM : Dr. M. L. Quaife
Division of Pharmacology, Toxicology Branch

SUBJECT: Evaluation of pharmacological data, submitted in petition to establish residue tolerances on corn, fodder or forage (including field corn, sweet corn, and popcorn); soybeans (dry and succulent forms); and meat fat, meat, and meat by-products from cattle for 3-(3,4-dichlorophenyl)-1-methoxy-1-methyl urea, commonly called linuron.

PESTICIDE PETITION No. 356

E. I. du Pont de Nemours and Co., Inc.
Wilmington, Delaware
(AF 4-408)

A petition for the above herbicide was first presented by du Pont on April 12, 1962. It requested that a residue tolerance of 1 ppm be established on the products listed above. It was assigned Pesticide Petition No. 356, but, because of inadequate toxicity data, was not filed.

By letter of November 27, 1962, du Pont asked that Pesticide Petition No. 356 be accepted for filing-if necessary because of incompleteness-as provided by Regulation No. 120.7(d)(1), without further notice to du Pont. Petitioner would then file the report on chronic toxicity studies on dog (which will be ready about Jan. 15, 1963) as a substantive amendment in accordance with Regulation No. 120.9.

The following data on toxicity were provided in detailed reports, submitted by Dr. H. C. Hodge of Rochester, New York, on Oct. 17, 1961, and on November 12, 1962, as well as by the original petition.

Acute Oral Toxicity - Rats:

LD_{50} not given. The approximate LD is variously given as between 1.0 and 2.25 g/kg.

Subacute Oral Toxicity - Rats:

Six of six rats survived ten doses at 200 mg/kg/day. Animals showed signs of discomfort and depression of the central nervous system, and weight gains were less than those of the controls. However, organs of animals, which were killed on the last day of treatment or ten days thereafter, were grossly and microscopically normal (data from du Pont Haskell laboratory).

In a study, [illegible] Dr. [illegible] Hodge, groups, consisting each of five male and five female rats, were fed for one month the following dietary levels of linuron: Zero (control), 30 ppm, 60 ppm, 300 ppm 600 ppm, 1,200 ppm, and 3,000 ppm.

Males on diets with more than 60 ppm and females, fed more than 600 ppm, gained more slowly, graded effects being shown. Growth was severely retarded in animals on the 3,000-ppm diet, and significant mortality occurred in this group (3/5 M and 3/5 F rats).

No hematological abnormalities were found at the end of the experiment.

Acute congestion of the spleen was found in rats in all groups, possibly to a greater extent in rats from high-level groups; the pathologist attributed the spleen congestion to the ether anesthesia used (Cf. Hodge report of November 12, 1962, p. 34).

An abnormal pigment, of hemoglobin, occurred in rats given the 3,000-ppm diet. There was a suggestion of an abnormal band in spectra of blood from the 1,200-ppm rats.

Liver weight-body weight ratios of male and female rats, fed the 3,000-ppm diet, and male rats, fed the 1,200-ppm diet, were higher than were those of controls.

Average spleen weight-body weight ratios were elevated in male rats, fed the 3,000-ppm diet, and, irregularly but considerably (+25%) in all female samples fed rats (Cf. Table 3, p. 28 of report of November 12, 1962, by Dr. Hodge.)

That histological studies of livers, kidneys, and spleens gave negative results is stated in Dr. Hodge's report. However, the detailed "microscopic notes" (Hodge report of November 12, 1962, p. 36) lists acute liver congestion for the rats, which received 3,000 ppm of linuron whereas all other rats are stated to have normal livers.

A 90-day feeding test was carried out in the Haskell laboratory on groups of rats, each containing eight male and eight female rats. Results are tabulated.

Results

	DIETARY LEVEL		
	80 ppm	400 ppm	3,000 ppm
Mortality	0/16	0/16	0/16
Effect on growth	None	None	Marked retardation
Clinical Toxicity	None	None	None
Hematology			
Red blood cells	Normal	Sl. Lower	Lower
White blood cells	Normal	Normal	Increased (Male)
Pathology	No injury	Minor Effects	Moderate Effects*

Chronic Oral Toxicity - Rats:

Chronic feeding studies (2-year) have been started at Dr. Hodge's laboratory, using both rats and dogs. The rat study is completed, and that on dogs will be finished shortly.

Groups of 35 male and 35 female weanling rats were given diets, to which the following amounts of linuron were added. Zero (control), 25 ppm, 125 ppm, and 625 ppm. Criteria used and results found are given below.

Body weights: Growth curves were essentially the same for controls and the two groups given lesser supplements. The 625-ppm rats weighed about 15-25 g less than the others at 4 weeks and about 20-35 g less at 6 months, and these latter values were statistically (by t test; p=0.01) significantly less than corresponding values for controls. At 20 months, the 625-ppm female rats averaged in body weight about 50 g less than controls; while the male rats averaged about the same as the controls.

Mortality: Although male rats on the 625-ppm supplement showed higher mortality early in the study, at 24 months, mortality was the same (25-27/35) for all four groups. Female rats had similar mortality for the three supplemented groups (22-24/35), all of which exceeded mortality of the female controls, which was 16/35 at 24 months.

Most of the deaths were attributed to respiratory infection.

*Moderate changes in bone marrow, spleen, and Kupffer cells of the liver, reflecting bodily response to destruction of blood cells; slight to moderate changes in the liver, kidneys, pancreas, testes, lungs, adrenals, and thyroid.

That of only four rats which died with tumors present (total deaths =183), one was a female control rat, two were 625-ppm female rats, and one was a 125-ppm male, suggests that linuron is not tumorigenic.

Urine analyses: Urinalyses for sugar (Benedict's method) and protein (turbidometric method of Kingsley et al.) were carried out on pooled samples at intervals throughout the two years. Urines were kept at 0° without added preservative (period of time not stated) prior to assay. Usually, only trace amounts of sugar were found. Protein was excreted in high amounts (greater than 0.1%-criterion of the Hodge report) as frequently by the controls as by the experimental rats.

Food Consumption: On one occasion, after the rats had been on the experimental regime for about one month, food consumptions of 10 control rats and of 10 rats on the 625 ppm-diet were measured; this was done for 3 consecutive days.

Both actual weights of food consumed and food consumed-body weight ratios were comparable (means agreeing within 10-12%) for male and female controls and their corresponding supplemented rats.

This being true, food consumption was not again determined.

Hematology: Observations made include total red blood cell counts, hemoglobin values, microhematocrit determinations, total white cell counts, differential counts, and observations on certain characteristics of the red blood cell. These were made pre-experimentally, at monthly intervals during the first 6 months, every two months during the second 6 months, and every 3 months during the second year.

No indication was found in all these data to show that addition of linuron to the diet affected the hematological variables studied.

Blood chemistry: Spectra of diluted blood samples were run before and after addition of potassium cyanide and of potassium ferricyanide (using separate blood samples for each chemical). Bloods from control and 625-ppm rats, at 7 weeks, and from control and from 25-ppm, 125-ppm, and 625-ppm rats, at the end of the experiment, were so examined.

The object was to see whether any abnormal blood pigment would be present, as was the case for rats on the highest supplement-level in the pilot study.

No abnormal spectra of blood from any of the rats tested were observed.

Tissue assay: Tissues were obtained at the end of the experiment from rats at all supplement levels, as well as from controls. These were pooled and analyzed for linuron content by a presumably specific assay procedure. Values are given for the following pooled samples: Muscle, fat, liver, kidney, spleen, blood, urine, and feces.

As was to be expected, samples from control animals had no detectable residues of linuron except for 1 gamma/g in urine and 0.6 gamma/g in feces. For the 25-ppm rats, tissue contents of linuron ranged from 1.8 gamma/g (muscle) to 14 gamma/g (kidney), with 11 gamma/g in fat. The 125-ppm rats had 1.2 gamma/g in blood and 1.8 gamma/g in muscle-with a high of 40 gamma/g in fat. Rats on the 625-ppm supplement had tissue levels ranging from 19 gamma/g in blood and 26 gamma/g in muscle up to 215 gamma/g in fat.

Evidently, of the tissues tested, linuron is preferentially deposited in body fat.

Organ weight: Weights of organs, determined chiefly at end of or in latter part of the experiment, were recorded for liver, kidney, testes, lung, brain, stomach, heart, and spleen.

There were no marked differences, in the average organ weights or in organ weight - body weight ratios of the rats, fed the various dietary levels of linuron, when compared with control values.

Pathology: Histological studies were made on rats which died during the 15 months when autolysis was not advanced and when cause of death was not apparent. Samples from these rats of kidney, lung, liver, sometimes heart, spleen, and pancreas were studied.

Results were negative, regarding tissue changes attributable to linuron. Cause of death in most instances was attributed to some infection, probably respiratory.

Rats, still alive after 24 months on the experimental regime and which comprised 9% rats, were killed, and the following tissues were studied for gross and microscopic pathology: Brain, lungs, heart, liver, spleen, kidneys, adrenals, gonads, stomach, large and small intestine, urinary bladder, femoral bone marrow, muscle, and thyroid gland.

Results: No toxic effects, that could be attributed to the linuron in the diet, were found in male or female rats, supplemented for 24 months at 25 or 125 ppm.

In both males and females, supplemented at 625 ppm, hemosiderin content of the spleen was slightly elevated. Marrow fat was reduced in 625-ppm females. The M/E ratio (ratio of myeloid to erythroid precursors) was reduced in the males at the 625-ppm supplement-level. An abnormally high incidence of endometrial hyperplasia was found in the 625-ppm females, while associated with considerable acute inflammation.

The pathologist concluded that linuron, administered orally for 17 to 24 months at a 625-ppm level, has a probable toxic effect, apparently directed at the red blood cells. Also, at this level, linuron may increase the incidence of endometrial hyperplasia in female rats.

Tumors, found at autopsy (24 months), were noted and classified. According to the Hodge report (of November 12, 1962, p. 88); they were all of the type observed in the rat colony. The thyroid lesions were focal in nature and probably adenomas.

The pathologist's report does not indicate that any carcinogenic effect of linuron was shown in this study, and the conclusion in the Hodge report (see reference in preceding paragraph) that linuron is not carcinogenic under conditions of their study can be accepted as probably true.

Rat reproduction study: Reproduction studies were carried out over three generations. Rats on the basal ration (controls) and rats, supplemented with 125 ppm linuron, were used. The latter level of supplement was chosen because it was the highest one that failed to retard growth in 90 days in the chronic oral toxicity study.

The proportion of successful matings, average number of pups per litter, mortality of the pups (both during the first 5 days and during the 6th to 21st day), and average weight of pups at weaning were nearly the same for both groups.

Growth of the second litters (F_{1b}) of the F_1 generation, controls and linuron-fed, was comparable. However, weanling rats of the second generation (F_{2b}), fed the 125-ppm diet, did not grow as well as the F_{2b} control rats. Growth of these rats was followed for as long as 19 weeks. At the end of this time, female rats, fed linuron, weighted less than female controls; while linuron-fed male rats weighed on the average 24 g. less than the male controls (mean body weights 349 g vs. 373 g).

Rats of the F_{3a} generation were studied for growth (although usually discarded at weaning) to gain more information on the above-noted growth depression in linuron-fed rats.

At 10 weeks, male treated rats were significantly ($p=0.05$) lighter in weight than the male controls. Female supplemented rats showed lesser growth depression, averaging about 10 g in weight difference.

At 10 weeks, all F_{3a} rats were given the basal diet, without linuron, to see whether the supplemented rats could "catch up" in body weight. They did not. At 22 weeks, male supplemented rats averaged 39 g less than their controls, and the females 6 g less.

Whereas F_{3b} rats are usually discarded at weaning in a reproduction study of the kind, some of them were kept on their respective diets (control and linuron-supplemented) for a further 13 weeks. This time, the linuron-fed rats grew faster than the controls. At 11 weeks, males averaged 7 g heavier and female rats 21 g more than their respective controls.

Rats (F_{3b}), which were sacrificed at weaning, showed no significant differences in organ weights or in organ weight-body weight ratios between the control and the 125-ppm rats.

Histological study of these rats gave no evidence of any tissue changes that could be attributed to the linuron in the diet. Lack of increased amounts of hemosiderin in the spleens of linuron-fed animals, as compared to their controls, is singled out by the pathologist as histological evidence that no hemolysis was caused by ingested or stored linuron.

Hematological studies of these rats using blood samples, drawn at 40 days of age, showed no significant differences between supplemented and control rats.

Eye Irritation - Rabbits: Ten mg of the fry powder or 0.1 ml of a 10% suspension of linuron in "Nujol" produced only faint irritation of the conjunctiva of rabbits' eye, and the irritation lasted two days. Microscopic examination of the eyes, 7 days after exposure, showed there was no abnormality. This conjunctival irritation was the same as that exposure, showed no abnormality. This conjunctival irritation was the same as the expected to be caused by an inert foreign particle.

Skin Irritation and Sensitization - Guinea Pigs: In tests on intact and abraded skin of guinea pigs, a 10% aqueous suspension of the 50% wettable powder was mildly to moderately irritating, but it did not produce skin sensitization.

DISCUSSION:

The chronic rat oral toxicity study showed no toxic effect of linuron, fed at 125 ppm for 2 years. (The retarded growth in rats of the F_{2b} and F_{3a} generations of the reproduction study was offset by normal growth of the F_{3b} generation.)

In the 90-day test rats fed 400 ppm linuron, showed slight toxic effects (lower R.B.C.'s and "minor" pathological effects). Rats, supplemented at 80 ppm, had no ill-effects therefrom.

The only recorded ill-effects of linuron at 100 ppm or below is the slight growth retardation for male (but not female) rats, fed 60 ppm for a month.

Evidently the no-effect level is of the order of 100 ppm for the rat, and the 125-ppm level, as massively documented by the rat chronic toxicity study, can be taken as the no-effect level, pending results of the chronic study in dogs.

7

The petitioner has filed the chronic toxicity study for dogs as an amendment. However, certain experimental results on poultry studies were filed after that. The "time clock" starts again when the last of the amendment is submitted, so that the time period for final review is again extended.

Following are results of the chronic dog study:

Subacute Oral Toxicity - Dogs - Preliminary Study:

One dog received 1.5 mg/kg of linuron for 2 weeks and 6 mg/kg for four weeks. The other dog received, initially, 15 mg/kg, then 150 mg/kg; as the dog could not tolerate this dose (vomiting and lessened food intake), it was reduced to 60 mg/kg for 1 month. Both dogs were then killed.

One dog had gained about 0.2 kg. The other one gained at first, then lost sharply at a dose level of 150 mg/kg., then gained again as the dose level was decreased to 120 and then 60 mg/kg.

Urinanalyses for protein and sugar gave normal results for both dogs. Hematology values were normal in the first dog; while for the second dog R.B.C.'s, Hb values, and hematocrit percentages also decreased.

Organ weights were normal for both dogs, and there was no gross or histopathology.

Chronic Oral Toxicity - Dogs:

Beagles (three male and three female per group) received, daily for 2 years, dog chow, containing either 0 (controls), 25, 125, or 625 ppm of linuron.

Criteria of response were: Body weight, results of urine analyses for sugar and protein, hematological values, results of assay for blood pigment and for oxyhemoglobin, organ weights, and results of both gross and histopathological examination of many body tissues in each dog. Results follow:

Body Weights: All dogs gained weight except three-two females on 625 ppm and one female, fed 25 ppm of linuron. All males on 625 ppm linuron gained some weight. Body weight data provided no clear information of a possible effect of linuron on body weight.

Urinalyses: Urine samples were collected before the experiment began and once a month thereafter. They were kept at 0° without preservative till analyzed. It seems possible that lack of preservative might have caused loss or oxidation of any sugar present, as happens in blood samples, refrigerated without preservative.

However, taking results as valid, one would conclude that ingestion of linuron caused no abnormal excretion of protein or sugar by these

8

Hematology: Blood samples were taken twice in the pre-experimental period. They were also obtained at 2 weeks, and at 3, 6, 9, 12, 16, 20, 22, and 24 months.

Results for dogs after 2 years on experiment follow:

Dogs on 25 ppm of linuron showed no significant alteration in red blood cell counts, hemoglobin values, or hematocrit percentages. Female dogs, fed 125 ppm linuron for two years, had a statistically significant decrease in their mean red blood cell count; while similarly-fed male dogs had a slight, but not significant, decrease in the count.

On a supplement level of 625 ppm, male dogs had an average red blood cell count significantly below that of controls; similarly-fed female dogs showed only a slight decrease.

The petitioner cannot explain why a dose-response relationship developed for male dogs and not for females except, possibly, because of small size of the test groups.

Blood pigments: Analyses of bloods, sampled at 48 weeks, revealed an abnormal blood pigment in blood from 3 dogs on 625 ppm of linuron and normal blood spectra for 2 controls. The oxyhemoglobin band was normal for all dogs. ((The abnormal pigment was characterized by a band in the 618-to 620-millimicron region, following addition of KCN to the blood.)

Bloods of all surviving dogs were tested at 108 weeks. Results follow:

Dietary Level of Linuron	Numbers of Dogs and Sex	Findings
Controls	3♂	No abnormal spectra
	3♀	No abnormal spectra
25 ppm	3♂	No abnormal spectra
	1♀	No abnormal spectra
	2♀	"Somewhat abnormal"
125 ppm	2♂	"Somewhat abnormal"
	1♀	No abnormal spectra
	2♀	"Somewhat abnormal"
625 ppm	1♂	"Somewhat abnormal"
	2♂	"Abnormal"
	2♀	"Somewhat abnormal"
	1♀	"Abnormal"

Oxyhemoglobin spectra were normal for all the dogs.

Organ Weights: At sacrifice, the liver, kidneys, testes, lungs, spleen, brain, and heart of each dog were weighed. Organ weight-body weight ratios were calculated. All were found to be unaffected by dietary supplementation of the dogs, excepting liver weight-body weight ratios. These were slightly, but not significantly (by t-test), greater than those of controls.

Linuron in Tissues: Tissues (composites from one female and one male each) from dogs on each dietary level were assayed for content of linuron. The method of Bleidner (Agr. Food Chem. 2, 476 (1954)) or modifications of it were used.

Controls showed no detectable residues in any tissues analyzed. For supplemented dogs, tissue contents varied from 0.50 ppm in muscle of dogs, fed 25 ppm of linuron, to 160 ppm in fat of dogs on the 625-ppm supplement.

Linuron was excreted in the urine and, to a somewhat greater extent, in feces.

In fat of dogs quantities of linuron present were: 25-ppm supplement level, 3.8 ppm; 125 ppm-level, 22 ppm; 625 ppm-level, 160 ppm.

By way of comparison, values for DDT in perirental fat of rats after 2 years' supplementation are given. (Cf. O. G. Fitzhugh, Ind. Eng. Chem. 40, 704-5 (1948)). They were: 100 ppm, 103 and 109 ppm; 400 ppm, 966 and 1,091 ppm; and 800 ppm, 4,220 ppm. Evidently, storage of linuron in body fat of dogs was light, as compared to rat storage of DDT.

Pathology: After 2 years, animals were killed and observed for growth or histopathology of various organs and tissues.

One dog, a male on 125 ppm, had developed paralysis of the hindquarters, and he was sacrificed at about 10 months on experiment. He showed no gross or microscopic abnormality except for a dilated, inflamed bladder and digestive changes in the sciatic nerve. Neither of these effects were ascribed to dietary linuron.

For the other animals, also, no gross pathology was seen which was ascribed to ingestion of the test compound.

The following tissues were examined histologically: Brain, heart, lungs, liver, kidneys, adrenals, gonads, stomach, large and small intestine, urinary bladder, muscle, and thyroid.

10

Bone marrow smears showed average figures for erythroid precursors per 100 total precursor cells, as follows: Controls, 427; 25-ppm dogs, 397; 125-ppm animals, 45[illegible]; and 625-ppm dogs, 544. The differences betwee mean values for control and for the 25-and 125-ppm dogs are not statistically significant. However, the difference between mean values for controls and for 625-ppm animals is significant at p=0.001.

10

It is clear that animals fed the 625-ppm showed unequivocal erythroid hyperplasia.

Histological study confirmed this. Marrow sections showed reduced fat in the 625-ppm-fed animals, probably due to replacement of the marrow fat by hyperplastic erythroid marrow. Also, brown pigment, probably hemosiderin, accumulated in hepatic Kupffer cells of these animals.

No such changes were seen in the 25-ppm and 125-ppm fed animals.

The pathologist attributed both the above changes to effects of linuron. The observed changes suggest that red cells were being broken down more rapidly than normal, with resultant anemia and secondary stimulation of erythrogenic activity.

No other histological abnormalities, ascribed to effects of linuron, were seen.

For these dogs, 25 ppm is the "no effect" level. At this level, dogs showed normal body weight gains, no sugar or protein in the urine, and normal hematological values, as well as normal oxyhemoglobin spectra. Further, organ weights were normal, and no gross or microscopic pathology was seen. The only possible detrimental effect was the occurrence of an abnormal blood pigment (with a spectral band at 618-20 millimicrons) in two of six dogs in the group. However, taking this slightly negative effect in conjunction with all the above positive effects, we believe that 25 ppm is the no-effect level for the dog.

The petitioner requests that 1 ppm of linuron be allowed for products, listed in the title.

These comprise approximately 7.7% of the average American diet (Cf. A. J. Lehman's compilation).

Thus, a maximum of 0.08 ppm of linuron would be present in the whole diet, assuming its presence on all foods for which a tolerance is requested at 1 ppm.

Since the no-effect level for the rat is approximately 100 ppm and that for the dog 25 ppm there is a margin of safety of more than 200 to 1.

CONCLUSION:

A tolerance of linuron at 1 ppm is safe for the food commodities for which it is requested in this petition. The margin of safety is greater than 200.

INIT:OGFitzhugh

11